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Colonization history and human translocations explain the population genetic structure of the noble crayfish (*Astacus astacus*) in Fennoscandia: Implications for the management of a critically endangered species

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Abstract

1. The noble crayfish (*Astacus astacus*) is an endangered freshwater species in Europe. The main threat is from lethal crayfish plague, caused by the oomycete *Aphanomyces astaci* that has been spread over Europe by introduced North American crayfish species, acting as chronic carriers of the disease.

2. Most of the remaining noble crayfish populations are found in the Baltic Sea area, and there is an urgent need to implement conservation actions to slow down or halt the extinction rate in this region. However, limited knowledge about the genetic structure of populations in this area has so far precluded the development of conservation strategies that take genetic aspects into consideration.

3. Key objectives of this large-scale genetic study, covering 77 locations mainly from northern Europe, were to describe the contemporary population genetic structure of the noble crayfish in the Fennoscandian peninsula (Sweden, Norway, and Finland), taking postglacial colonization history into account, and to evaluate how human activities such as stocking have affected the genetic structure of the populations.

4. Analyses of 15 microsatellite markers revealed three main genetic clusters corresponding to populations in northern, middle, and southern Fennoscandia, with measures of genetic diversity being markedly higher within populations in the southern cluster. The observed genetic structure probably mirrors two main colonizations of the Baltic Sea basin after the last glaciation period. At the same time, several deviations from this pattern were observed, reflecting past human translocations of noble crayfish.

5. The results are discussed in relation to the conservation and management of this critically endangered species. In particular, we recommend increased efforts to protect the few remaining noble crayfish populations in southern Fennoscandia and the use of genetic information when planning stocking activities, such as reintroductions following local extinctions.

1 | INTRODUCTION

The loss of biodiversity is a global threat that has received much attention (e.g. The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), 2019). Biological diversity is found at all levels in nature: from ecosystems, communities, and species, to intraspecific genetic diversity. Genetic variation within a species is essential to maintain fitness and evolutionary potential in a changing environment (Allendorf, Luikart, & Aitken, 2013; Frankham, 1995; Frankham et al., 1999; Hedrick & Kalinowski, 2000). Threatened species that are simultaneously exploited pose particular problems for management. In such cases, knowledge about population structuring is important to avoid the overexploitation of vulnerable populations and to prevent loss of genetic variation (Allendorf, England, Luikart, Ritchie, & Ryman, 2008; Laikre & Ryman, 1996).

It is not always evident at which level of biological organization conservation measures should be applied to preserve the genetic diversity and integrity of threatened species and populations (Casacci, Barbero, & Balletto, 2014; Fraser & Bernatchez, 2001; Weeks, Stoklosa, & Hoffmann, 2016). In addition to continuing microevolutionary processes, the genetic architecture of any species is to a large extent the product of past events, such as colonization history and historical changes in climate and geomorphology (Avise, 2000). In addition, more recent human impacts such as translocations, fishing exploitation, and selective harvest may have affected the genetic structure and effective population sizes (Allendorf et al., 2008; Kitada, 2018; Kuparinen, Hutchings, & Waples, 2016). Although detailed knowledge about all of these processes may be needed when planning conservation programmes, such information is typically missing (Laikre, 2010).

The noble crayfish (*Astacus astacus* L.) is a threatened freshwater species in Europe, with a natural distribution ranging between Russia in the east, Fennoscandia in the north (Sweden, Norway, and Finland), France in the west, and Greece in the south. The conservation status of the species differs among regions. Owing to declining abundance, the noble crayfish is listed as Vulnerable at the international level by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Edsman, Füreder, Gherardi, & Souty-Grosset, 2010), although recent information suggests that a classification as Endangered may be more appropriate (Richman et al., 2015). In Sweden, the noble crayfish is listed as Critically Endangered by the Swedish Species Information Centre (SLU Artdatabanken, 2020) because only 2% of pristine populations are estimated to remain (Bohman, 2019). In Norway and Finland the status of the noble crayfish is similarly unfavourable (Ruokonen et al., 2018; Vrålstad, Johnsen, Fristad, Edsman, & Strand, 2011), and noble crayfish is classified as Endangered in these countries

(Henriksen & Hilmo, 2015; Hyvärinen, Juslén, Kemppainen, Uddström, & Liukko, 2019; Strand et al., 2014; Vrålstad et al., 2011). The noble crayfish is also listed in Annex V of the European Habitats Directive (Council of the European Communities, 1992). Species on Annex V are those 'whose taking in the wild and exploitation may be subject to management measures'. However, in Fennoscandian countries, one of the major measures for the conservation of noble crayfish has been to promote a local small-scale subsistence fishery to enhance the will to protect the species (Edsman & Schröder, 2009; Taugbøl, 2004; see below).

Habitat loss and acidification have contributed to the sharp decline, but the main threat to the species has been, and still is, the crayfish plague caused by the oomycete *Aphanomyces astaci*, spread over Europe by introduced North American crayfish species (Holdich, Reynolds, Souty-Grosset, & Sibley, 2009; Martin-Torrijos, Kokko, Makkonen, Jussila, & Dieguez-Uribeondo, 2019; Perdikaris, Kozak, Kouba, Konstantinidis, & Paschos, 2012). In Fennoscandian countries, introductions of crayfish species into new waters require a permit from the relevant management authorities (Skurdal et al., 1999). Despite this legislation, the illegal introduction of the signal crayfish (*Pacifastacus leniusculus*), causing continuous losses of noble crayfish populations resulting from the spread of crayfish plague, is considered the main reason for extinction in these countries (Bohman, Degerman, Edsman, & Sers, 2011; Bohman, Nordwall, & Edsman, 2006; Edsman, 2016; Ruokonen et al., 2018; Strand et al., 2019).

The noble crayfish has a significant cultural, social, and economic value in Fennoscandia, particularly in Sweden, which has the highest per capita consumption of freshwater crayfish worldwide (Gren, Campos, Edsman, & Bohman, 2009). The species is mainly exploited by the owners of private fishery rights in lakes and streams for their own consumption and for sale in the local market. Despite the critical conservation status of the species, the current fishery of remaining local populations is not regarded as a problem but rather as beneficial: the high social and traditional value of the fishery most likely increases the incitement to manage noble crayfish populations instead of illegally releasing signal crayfish (Edsman & Schröder, 2009; Edsman & Smietana, 2004; Taugbøl, 2004).

Measures to halt the decline of the noble crayfish in Fennoscandia have been in operation for decades, including massive information campaigns to increase public awareness. In the 'Action plan for noble crayfish' established by the Swedish authorities (Edsman & Schröder, 2009), reintroduction programmes of noble crayfish in lakes and rivers where the species has been extirpated are listed as an important conservation tool. In addition to conservation measures, the supplemental release of noble crayfish into existing populations has also been carried out to support local fisheries. Thus, stocking activities in Fennoscandia have a dual purpose of improving

the status of the critically endangered noble crayfish and improving a sustainable fishery.

Releases of noble crayfish and other species into the wild ideally require knowledge about the population genetic structure, especially regarding the choice of stocking material (Laikre & Ryman, 1996). However, although the general recommendation in Fennoscandian countries and elsewhere has been to use stocking material of local origin and with sufficiently high genetic diversity (Kozak, Fureder, Kouba, Reynolds, & Souty-Grosset, 2011; Schrimpf et al., 2017), in practice genetic aspects have rarely been considered in previous attempts to reintroduce the species, simply because such information has not been available.

The first genetic studies of noble crayfish were hampered to a varying degree by a lack of variable genetic markers with known inheritance (Agerberg, 1990; Fevolden, Taugbøl, & Skurdal, 1994). Edsman, Farris, Kallersjo, and Prestegaard (2002) reported microsatellite-like genetic variation within the rDNA–ITS1 region and found genetic differences between populations, which have had some management use (Alaranta et al., 2006; Edsman et al., 2002). However, the ITS-linked microsatellites are part of a multicopy gene family and therefore cannot be treated as discrete codominant Mendelian markers (Harris & Crandall, 2000), which has precluded standard genetic analyses. Gross et al. (2013) presented the first genetic study on noble crayfish based on variable nuclear microsatellite markers. They reported clear genetic differentiation between populations from the Baltic Sea and the Black Sea catchments. However, their sampling did not allow a complete understanding of the colonization history and population genetic structure within Fennoscandia, where most of the remaining noble crayfish populations occur. In particular, samples from southern Sweden were not included (except one sample from the island of Gotland).

Subsequent studies have focused mainly on intraspecific genetic variation in continental Europe (but see Makkonen, Kokko, & Jussila, 2015). Schrimpf et al. (2014) presented evidence for two separate areas in south-eastern Europe used as refugia by noble crayfish during the last glaciation period and have suggested that the North Sea and the Baltic Sea basins were probably recolonized independently from one of these refugia, the Eastern Black Sea basin, via different colonization routes. In a subsequent study, focused mainly on Western Europe, Schrimpf et al. (2017) proposed four management units for the preservation of genetic diversity and integrity within that part of Europe. Similar questions on phylogeographical origin and genetic structure of noble crayfish in the southern Balkan Peninsula have also been addressed by Laggis et al. (2017). The geographical coverage of previous studies on noble crayfish do not allow a conclusive analysis of either the recolonization history of the Baltic Sea region or the effects of postglacial microevolutionary processes on the population genetic structure of this species within Fennoscandia. The main objectives of the present microsatellite study, covering 70 locations in the Fennoscandian Peninsula and an additional seven samples from more southern parts of the distribution range, were to: (i) describe the

contemporary genetic population structure; (ii) reconstruct the postglacial colonization history into the Baltic Sea basin; (iii) evaluate how human translocations of noble crayfish have affected the genetic structure; and (iv) suggest conservation strategies for preserving the genetic diversity and integrity of this critically endangered species.

2 | METHODS

2.1 | Samples

Noble crayfish were collected over a 20-year period (from the mid-1990s to the present day) from lakes, streams, and ponds, mainly in Sweden, Norway, and Finland (Figure 1). Tissue samples typically consisted of a small (3–4 mm) piece of limb stored in 95% ethanol. Samples were mainly from wild populations but were also taken from a few crayfish farms. Some of the sampled wild populations are now extinct owing to crayfish plague (Appendix S1). Minor parts of the material have been included in previous genetic studies (Edsman et al., 2002; Gross et al., 2013).

The material initially included 3,523 noble crayfish individuals, representing 124 combinations of a specific sampling site and year. However, many of the samples comprised only a few crayfish (e.g. 22 samples with $n < 10$). Therefore, genetically similar samples collected from the same lake or water system in the same or different years were pooled. Decisions on whether to pool samples were made based on initial tests for local spatiotemporal genetic differences; samples from the same water body lacking statistically significant differences in allele frequencies or with significant but small heterogeneity (fixation index, $F_{ST} < 0.01$) were pooled. Moreover, only samples with $n \geq 15$ (some of which included pooled material) were included in further analyses. This procedure resulted in the remaining total material comprising 3,347 individuals across 77 samples, of which a majority (3,190 individuals, 70 samples) originated from Sweden, Norway, and Finland (Appendix S1; Figure 1). Most analyses shown here were performed on the latter subset, as the main objective was to study population structure in the Fennoscandian Peninsula. The seven samples from more southern areas (Denmark, Poland, Czech Republic, and Montenegro) were only used in a few comparative analyses.

2.2 | Microsatellite and statistical analyses

Total DNA was extracted using a protocol based on Chelex (Walsh, Metzger, & Higuchi, 1991). A set of 15 microsatellite loci (Koiv, Gross, Paaver, Hurt, & Kuehn, 2009; Koiv, Gross, Paaver, & Kuehn, 2008; Appendix S2) were analysed in one multiplex polymerase chain reaction (PCR). For the PCR, 4 µl of Type-it polymerase (Qiagen, Hilden, Germany), 4 µl of primer mix, and 0.5 µl of template, with approximately 100 ng of DNA, were used. The PCR was run using an initial step of 5 min at 95°C followed by 29 cycles of 30 s at 95°C, 90 s at 61°C, 30 s at 72°C, and a final step of 15 min at 60°C. Electrophoresis was run on an ABI Prism 310 Genetic Analyzer (Applied

FIGURE 1 Geographical locations for the genetic samples analysed (1–70, Fennoscandian Peninsula; 71–77, mainland Europe). All samples were from wild, self-reproducing populations except for samples 28, 53, 63, and 66, which were collected at noble crayfish farms. For further details about samples and locations, see Appendix S1
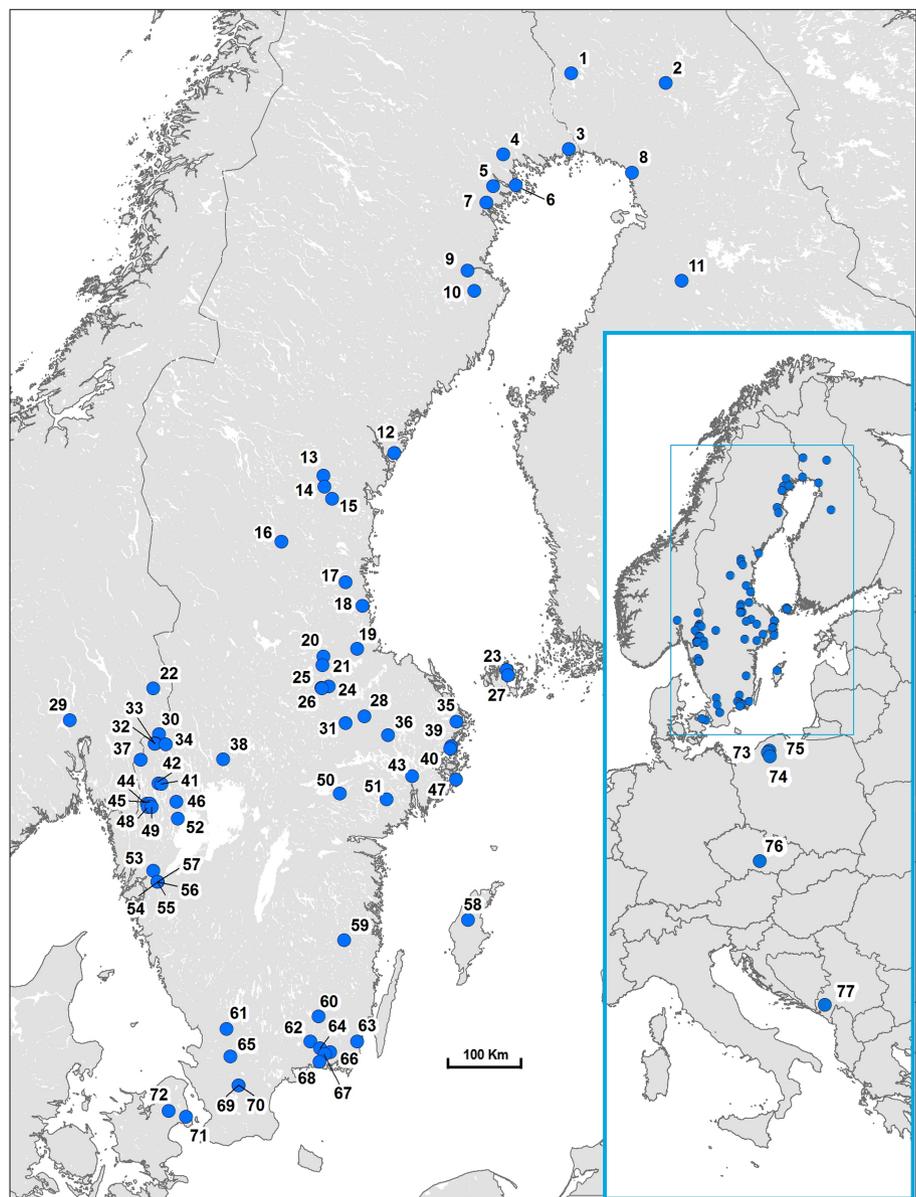
Biosystems, Foster City, CA, USA) with the Liz 600 sizer. Allele sizes were determined using the ABI GENOTYPER 3.7 (Applied Biosystems). Potential problems with stuttering, large allele dropouts, and null alleles were evaluated using the permutation procedure in MICROCHECKER 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). Tests for putative outlier (non-neutral) loci were performed with ARLEQUIN 3.5 (Excoffier, Laval, & Schneider, 2005; Excoffier & Lischer, 2010) and BAYESCAN (Foll, Beaumont, & Gaggiotti, 2008), with details described in Appendix S3. ARLEQUIN 3.5 was also used to estimate hierarchical F-statistics and the Garza and Williamson (2001) diversity index was used to search for genetic indications of past population bottlenecks.

FSTAT 2.9.3.2 (Goudet, 2001) was used to test for genotypic disequilibrium, to compute unbiased estimates of gene diversity, allelic richness, and F-statistics, and for evaluations of deviations from Hardy–Weinberg proportions and post-hoc comparisons of genetic diversity within the distinct groups of samples identified. All statistical tests with FSTAT were based on permutations (10,000 randomizations). Genetic relationships among samples were visualized using principal component analysis (PCA) with PCA-GEN 1.2 (Goudet, 1999). A neighbour-joining tree (Saitou & Nei, 1987) based on the pairwise Cavalli-Sforza and Edwards (1967) chord distance, as recommended for microsatellites by Takezaki and Nei (1996), was constructed with PHYLIP (Felsenstein, 2005) and visualized using FIGTREE 1.4.3 (http://tree.bio.ed.ac.uk/).

The Bayesian method in STRUCTURE 2.3.4 (Falush, Stephens, & Pritchard, 2003; Pritchard, Stephens, & Donnelly, 2000) was used to identify larger geographical groups (clusters) in the total material, consisting of genetically similar genotypes with minimum overall levels of linkage and Hardy–Weinberg disequilibrium. A model with no prior information about sampling locations was used, assuming admixture and correlated allele frequencies between

clusters (with a burn-in of 50,000 steps, followed by 100,000 Markov chain Monte Carlo replicates). The number of clusters (K) in the analyses was increased from 1 to 15, using 10 replicate runs per K. The most likely value for K was inferred following Evanno, Regnaut, and Goudet (2005), as implemented in STRUCTURE HARVESTER (Earl & Vonholdt, 2012), followed by subsequent analysis with CLUMPP (Jakobsson & Rosenberg, 2007) to identify shared modes among replicate runs. DISTRUCT (Rosenberg, 2004) was used for producing a graphic visualization of the final results at the individual level. Model-based approximate Bayesian computation (ABC), as implemented in DIYABC 2.1.0 (Cornuet et al., 2014), was used to evaluate how differentiation observed among genetically similar groups (clusters) of populations is related to postglacial recolonization. An important aim was to investigate whether postglacial immigration to different parts of Northern Europe occurred from one or several multiple glacial refugia. Details on ABC analyses are provided in Appendix S4.

3 | RESULTS

The total number of observed alleles across all 77 samples was 159, yielding an average number of alleles per locus of 10.6 (range 3–26). In the 70 samples from Norway, Sweden, and Finland, the corresponding total number of alleles was 101, with an average of 6.7 alleles per locus (range 2–21). Statistically significant ($P < 0.05$) deviations from Hardy–Weinberg proportions were observed in 72 out of 797 tests (9%), which is slightly above the level expected by chance alone (5%). Among 1,000 pairwise comparisons of genotypic proportions at variable loci, restricted to the 19 largest samples ($n \geq 40$), 72 (7.2%) were significant at the 5% level, which is just marginally higher than the proportion expected by chance alone. In addition, the deviations from random association of genotypes were distributed across a large number of locus pairs. Hence, no clear signs of linkage among particular loci were observed.

Analyses with MICROCHECKER restricted to the same 19 large samples indicated the possible existence of null alleles or problems with stuttering in 11 out of 201 tests (i.e. combinations of locus and sample). Notably, seven of these 11 deviations included locus *Aas3950* in samples originating from southern and middle Sweden. However, comparisons of estimates computed with and without *Aas3950* (i.e. 15 versus 14 loci) of the average heterozygosity, F_{IS} (inbreeding coefficient of an individual relative to the subpopulation; Appendix S1), and global and pairwise F_{ST} (not shown) were essentially identical, and this locus was therefore retained.

Analyses with ARLEQUIN and BAYESCAN aimed at identifying candidate 'outlier loci' (i.e. markers potentially affected by natural selection) included the 70 samples from different geographical locations in Fennoscandia. As detailed in Appendix S3, several significant differences were obtained when analysing the data under a non-hierarchical island model. However, after removing genetically admixed samples, and after accounting for hierarchical population structuring, no outlier loci could be identified unambiguously. Thus,

significances in the non-hierarchical analysis most likely represented false positives (cf. Excoffier, Hofer, & Foll, 2009), and the whole set of 15 microsatellite markers was therefore regarded as selectively neutral and used in further analyses.

The average expected heterozygosity across loci was 0.29 within the 70 samples from Fennoscandia, ranging from 0.10 to 0.45. The global F_{ST} (Weir & Cockerham, 1984) value estimated across all locations and loci was 0.21 ($P < 0.001$), with the pairwise F_{ST} value varying from 0.00 to 0.71. A dendrogram based on all 77 samples (Figure 2) showed two distinct groups of locations representing the northern regions of Finland and Sweden and the middle regions of Sweden and Norway, respectively. In contrast, the remaining samples, mainly from southern Sweden and continental Europe, formed a more diverse group with less clear geographical patterns. The analyses with STRUCTURE, aimed at identifying more large-scale geographical relationships, divided the 3,190 noble crayfish from Sweden, Norway, and Finland into three main genetic clusters (K), largely corresponding to locations in northern, middle, and southern Fennoscandia (Figures 3 and 4). However, a number of deviations from the general pattern of three geographically delineated groups exist, mainly seen as genetically southern samples occurring in middle Sweden (Figure 4). Notably, a majority of the deviating samples represent noble crayfish farms or wild populations with a known stocking history, where individuals from hatcheries have been released to enhance weak populations or to reintroduce the species following local extinctions (Appendix S1; Figure 3). Among the apparently misplaced samples, there is a gradient with a varying degree of genetic admixture among the three main genetic clusters identified (Figures 3 and 4).

To enable genetic comparisons of seemingly unaffected populations, 'pure' samples were arbitrarily defined as having the largest average individual ancestry coefficient identified by STRUCTURE (q_x), exceeding 0.9. Seemingly admixed samples from wild and hatchery populations ($n = 17$), where none of the three clusters was clearly dominant ($q_x \leq 0.9$), were removed. A hierarchical analysis of molecular variance (AMOVA) based on the remaining 53 samples revealed the presence of highly significant differences in allele frequency among the three clusters (fixation index between groups, $F_{CT} = 0.20$; $P < 0.001$) when accounting for population differentiation within the same clusters (fixation index among populations within groups, $F_{SC} = 0.11$; $P < 0.001$).

In line with the above results, a PCA based on pairwise F_{ST} estimates between Fennoscandian samples also indicated the presence of three distinct main groups associated with geographical origin (Figure 5). Combining the PCA with results from STRUCTURE further illustrates that three out of four hatchery stocks have a clear southern genetic origin, whereas the fourth one represents a mixture of all genetic clusters. Moreover, a number of wild samples display various degrees of genetic admixture among the three main population groups (Figure 5).

As shown in Figure 6, the average expected heterozygosity and allelic richness across the 53 pure samples were markedly higher within the southern Fennoscandian cluster, whereas the middle and

FIGURE 2 Neighbour-joining dendrogram based on pairwise chord distances. The small dendrogram depicts all branches with original lengths. For details on samples (geographical location, sample size, etc.), see Figure 1 and Appendix S1
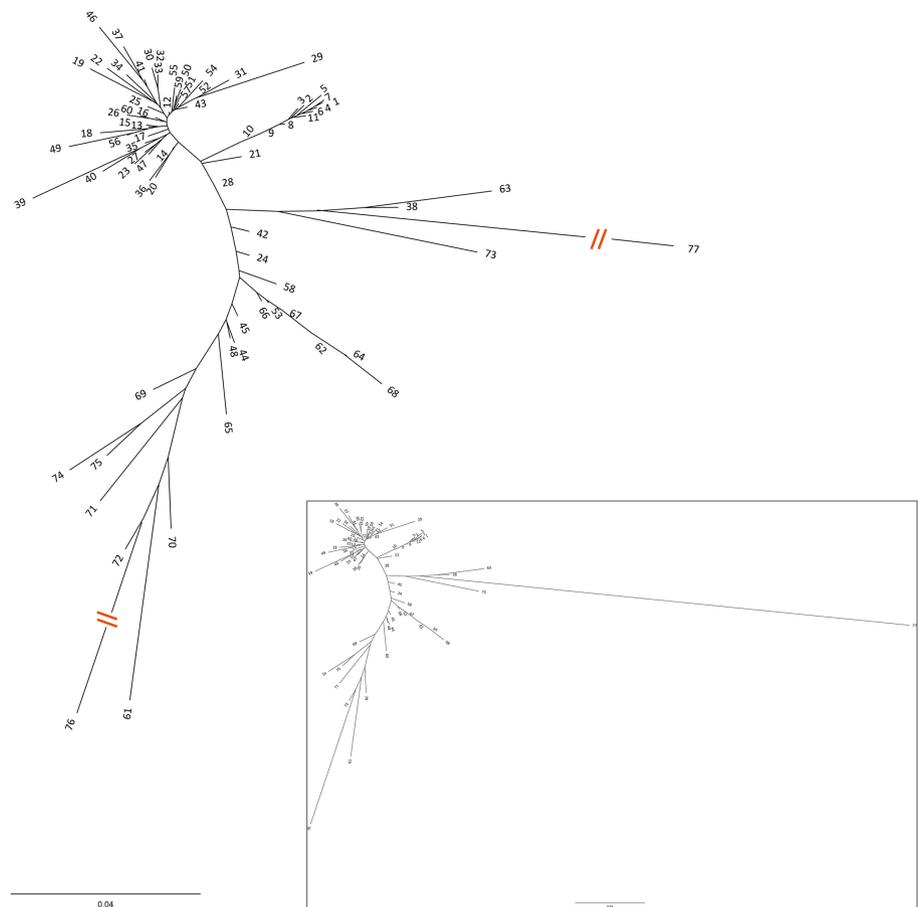

northern clusters displayed lower levels of genetic diversity, with a tendency towards slightly higher values in the north. Similarly, the Garza–Williamson index was clearly lower within the middle and northern clusters, again with a somewhat higher value in the north, indicating that populations in these clusters have been affected by historical bottlenecks to a larger extent than populations in the southern cluster. Moreover, the levels of genetic diversity in southern Fennoscandia were similar to those observed in samples from mainland Europe (Figure 6).

The analysis with DIYABC included comparisons of four postglacial immigration scenarios that may explain the observed genetic structure in Fennoscandia (Appendix S4). These scenarios differed with respect to: (i) the time since the divergence between the southern and the combined middle–northern clusters (after or before the last glaciation, i.e. corresponding to one or two glacial refugia); and (ii) the possibility for secondary contact (yes or no) in southern Sweden between ancestors of the southern and the combined middle–northern clusters. As detailed in Appendix S4, the highest statistical support was obtained for a scenario with a single refugium, where the contemporary genetic structure reflects different immigration routes and past bottlenecks after the last glaciation period without extensive secondary contact. Under this most likely scenario, the median time since divergence was estimated to be approximately 2,300 generations (95% PI 1,300–3,000) for the split between the ancestors of the southern versus the combined middle–northern clusters.

Similarly, the time since divergence between the more genetically similar middle and northern clusters was estimated to be approximately 1,200 generations (95% PI 1,000–2,200). Assuming an average generation interval of about 7.5 years (Edsman et al., 2010), these time estimates correspond to about 17,000 and 9,000 years before present, respectively.

4 | DISCUSSION

In this large-scale microsatellite study of the endangered noble crayfish, strong evidence was found for three genetically distinct population groups, largely corresponding to locations in northern, middle, and southern Fennoscandia (Figures 3–5). Among these groups, the southern genetic population cluster clearly expressed the highest level of genetic diversity, comparable with noble crayfish from mainland Europe (Figure 6). The southern cluster was also genetically more similar to the noble crayfish from the European mainland (Figure 2). The results suggest that the postglacial recolonization of Fennoscandia probably involved two independent colonization events following separate routes from a common refugium in south-eastern Europe. The data obtained further indicate a history of extensive movements and stocking of noble crayfish in Fennoscandia, especially releases of crayfish with a southern genetic origin in lakes and catchments in middle Sweden (Figure 4).
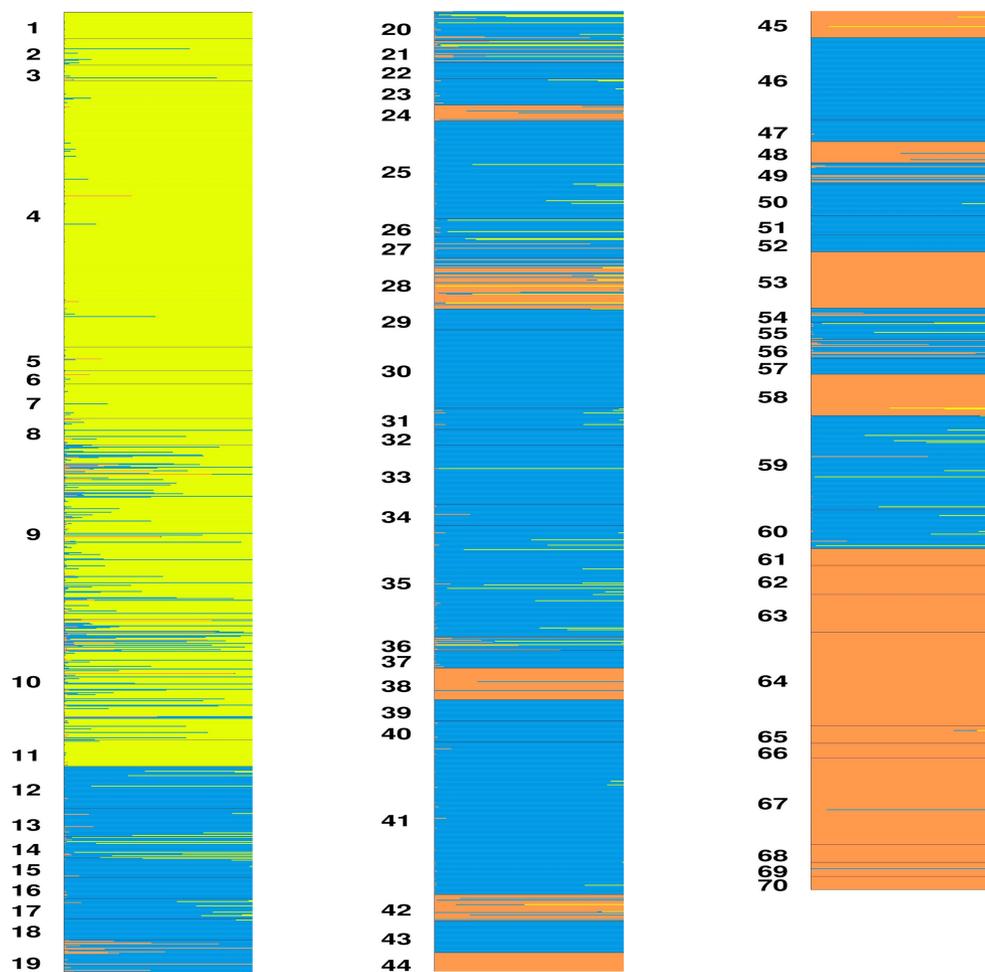
FIGURE 3 Results from analyses with STRUCTURE. Coloured bars illustrate inferred ancestry at $K = 3$ for single individuals ($n = 3,190$) in 70 samples representing northern (1–11), middle (12–60), and southern (61–70) Fennoscandia (cf. Figure 1). Note that some samples from locations in middle Fennoscandia are dominated by genetic material originating from the southern cluster

4.1 | Postglacial colonization

Although microsatellites evolve comparably quickly, and are therefore less often used for phylogenetic analyses, the results for populations unaffected by stocking seem to reflect recolonization events following the last glaciation that are in line with previous studies. The genetic affinity between the southern Fennoscandian cluster and populations from the European mainland indicates that these parts of southern Sweden were colonized from the south. The middle and northern Fennoscandian clusters, on the other hand, are most likely from the same postglacial origin as the noble crayfish found east of the Baltic Sea. In agreement with these findings, Gross et al. (2013) reported markedly lower heterozygosity and allelic richness in noble crayfish from Estonia, Finland, and Sweden (middle and northern regions), compared with samples from Germany and the Czech Republic. Although the northern Finnish samples and the more southern ones included in Gross et al. (2013) do not overlap geographically, it seems likely that the distinct Finnish population group that they identified is of the same origin as the northern genetic cluster. Together with increasing genetic distances observed in mainland Europe for populations in Estonia, Finland, and Sweden, respectively (Gross et al., 2013), this suggests a south-eastern colonization path to the

Baltic Sea area associated with episodes of genetic drift caused by population bottlenecks or founder effects.

Further indirect support for two independent colonization events to the Baltic Sea area comes from the phylogeographical study by Schrimpf et al. (2014). Combining mitochondrial DNA (mtDNA) sequences and microsatellite data, they concluded that the North Sea and Baltic Sea basins in Northern Europe were likely to have been colonized independently via different paths from a common refugium in the eastern Black Sea basin, although only two Fennoscandian samples (both from Finland, with no samples from southern Sweden) were analysed. In a comprehensive follow-up study of mtDNA variation (cytochrome oxidase I gene, COI) in Finland and Estonia, Makkonen et al. (2015) found fixation for the same haplotype as observed by Schrimpf et al. (2014) in their Finnish samples. These results are not in conflict with our suggestion of two independent colonization events to the Baltic Sea basin. Rather, we suggest that the southern Fennoscandian cluster may belong to the same phylogenetic group that according to Schrimpf et al. (2014) colonized the North Sea basin. Supplementary mtDNA data for noble crayfish in southern Sweden (and preferably other parts of Fennoscandia) will be needed, though, to validate this hypothesis.

FIGURE 4 Geographical distribution of the three main genetic clusters in Fennoscandia identified by STRUCTURE (red = southern, blue = middle, yellow = northern; cf. Figure 3), shown as averages per sample location (sampling site numbers refer to the sites indicated in Figure 1, which are described in more detail in Appendix S1). Note that samples in middle Fennoscandia (12–57) with a substantial element of genes from the southern cluster (red) represent crayfish farms or wild populations with a presumed stocking history (Appendix S1)
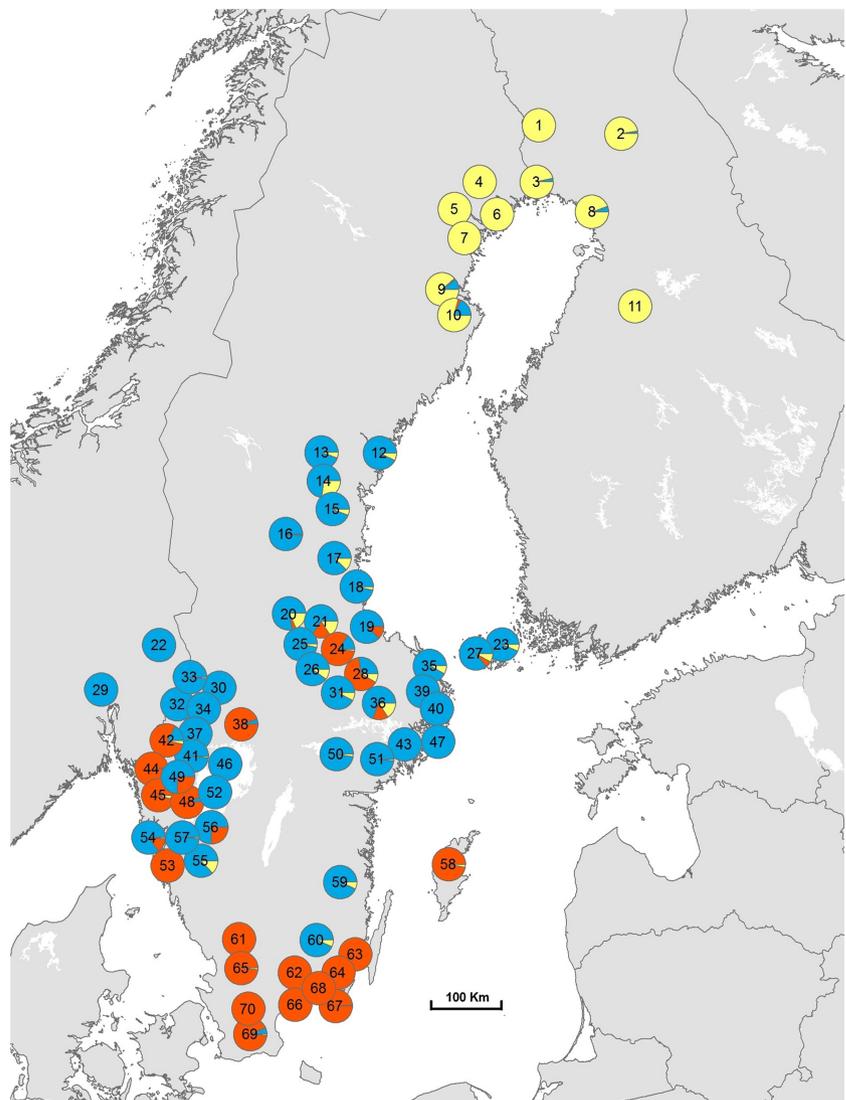
Any further conclusions on how and when the genetically distinct clusters colonized Fennoscandia appear difficult to draw with the information available. However, in connection with the retreating ice (approximately 13,000–9,000 years before present) the Baltic Sea area experienced a climatically dynamic period with alternating brackish and fresh water conditions (Björck, 1995), where the stages of low salinity may have facilitated long-distance colonization by noble crayfish. Phylogeographical studies of several other freshwater species have found similar genetic evidence for multiple colonizations of the Fennoscandian Peninsula (e.g. Delling, Palm, Palkopoulou, & Prestegaard, 2014; Koskinen et al., 2002; Nesbø, Fossheim, Vøllestad, & Jakobsen, 1999; Nilsson et al., 2001).

4.2 | Releases of noble crayfish

As observed in other studies on crayfish species in mainland Europe (Schrimpf et al., 2017), the results of this study suggest extensive movement and stocking of noble crayfish with deviating genetic origin.

Most of the translocations identified genetically in this study represent releases of noble crayfish with southern genetic origin into lakes and catchments in middle Sweden. Among 44 samples analysed from wild populations in middle Fennoscandia (defined as the geographical area encompassing samples 12–57; see Figure 1), 12 (27%) displayed varying degrees of admixture (including complete dominance) with the southern genetic cluster according to analyses with STRUCTURE ($q_{Southern} \geq 0.10$). For nine out of 12 (75%) of these admixed localities, there are more or less precise documentations of historical releases, including information on the origin of the stocking material (Appendix S1), supporting the genetic results. If the selection of population samples is reasonably representative for all noble crayfish populations in middle Fennoscandia, the movement and stocking of crayfish with a southern genetic origin has been very common in this area.

According to existing documentation (Appendix S1), the large-scale stocking of noble crayfish has also been carried out in northern Fennoscandia. In Finland, extensive movements and releases of noble crayfish have taken place historically, especially from locations in the south to northern parts of the country (Jussila & Mannonen, 2004). In
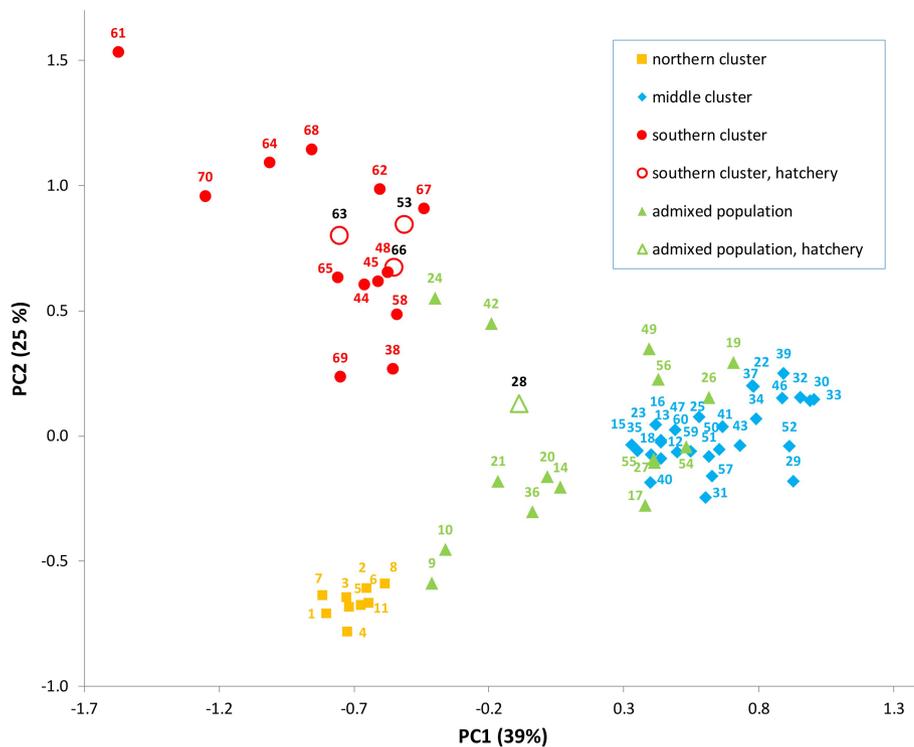
FIGURE 5 Principal component analysis (70 Fennoscandian samples, 15 loci) based on pairwise F_{ST} estimates. The two most informative components (PC1 and PC2) explain approximately 65% of the total variation between samples. Colours refer to the three main clusters and cases of putative genetic admixture identified using STRUCTURE (see details in the text). Small numbers refer to samples of wild and hatchery populations shown in Figure 1, with details provided in Appendix S1
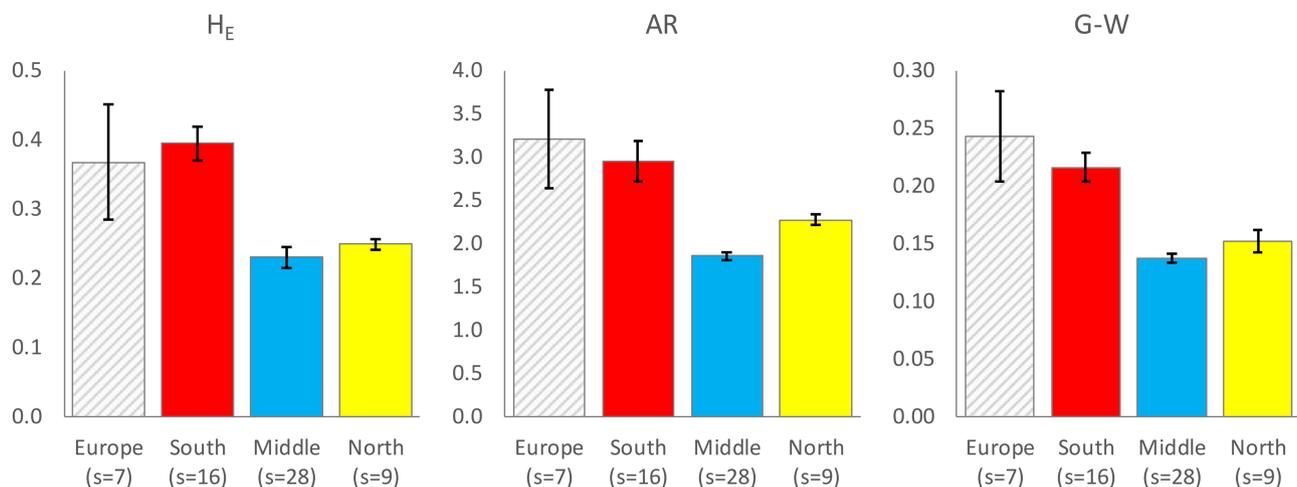
FIGURE 6 Average (± 1.96 SE) expected heterozygosity (H_E), allelic richness (AR) and Garza–Williamson diversity index (G–W) calculated across loci for 'pure' (i.e. $q_x > 0.9$) samples from the three genetic clusters in Fennoscandia (south, middle, and north) identified by STRUCTURE (cf. Figures 3 and 4). The number of population samples (s) per cluster is shown in parenthesis. For comparison, corresponding estimates for seven samples from mainland Europe (Figure 1) are also included

northern Sweden, large numbers of noble crayfish from northern Finland were released during the 1950s–60s (Gydemo & Gydemo, 1990). The genetic homogeneity of the northern cluster observed here (see samples 1–10 in Figure 2) is in line with a common origin resulting from the massive and recent stocking of Finnish noble crayfish in northern Sweden. In contrast, and in line with the present results (Figure 4), there seems to be no documentation on releases in northern Sweden of noble crayfish originating from the southern cluster.

It is not known how far north the natural distribution of noble crayfish in Fennoscandia went before large-scale releases of the

species began in this area. If at all present, the species was probably rare in northern Sweden (Alm, 1929) and in northern Finland (Skurdal et al., 1999). Thus, it is unclear whether the northern genetic cluster existed at all in northern Sweden and Finland before large-scale movements of noble crayfish within Finland, and later from Finland to Sweden, were initiated. In a few of the northern Swedish samples there are indications of admixture, including genetic elements from the middle cluster, but it remains unclear whether this admixture represents a natural contact zone or whether crayfish belonging to the middle genetic cluster previously existed

naturally in the north but were swamped by large-scale releases of Finnish crayfish.

The possibility that the genetically distinct population cluster in southern Sweden originates from human translocations from mainland Europe cannot be excluded, but natural colonization from the south at some stage following the retreat of the ice appears more likely for several reasons. First, the interest in crayfish as a common food source (an incitement for moving the species) dates back only about 150 years in this part of Europe (Swahn, 2004), and we are not aware of any historical documentation of human crayfish movements from the continent. Second, the southern genetic cluster dominates completely in southern Sweden without any clear genetic elements from the middle cluster. Thus, if noble crayfish were deliberately introduced to southern Sweden (from mainland Europe), the species must have been completely absent in this area before the introduction. Third, there are pronounced allele frequency differences between populations in southern Sweden (Figure 2) and comparably high within-population genetic diversity (Figure 6). Thus, if populations in this area originated from recent stocking, several founding continental populations must have been used.

4.3 | Implications for conservation and management

The genetic diversity observed in the samples from middle and northern Fennoscandia is markedly lower compared with more southern populations in Fennoscandia and mainland Europe. Similar results were reported by Gross et al. (2013) and Schrimpf et al. (2014), showing lower genetic variability in the Baltic region compared with populations in central and south-eastern Europe. Makkonen et al. (2015) also reported very low genetic variability among Finnish noble crayfish populations. Nevertheless, the peripheral populations in the Baltic Sea area arguably have a particular conservation value because they form genetically distinct groups (cf. Gross et al., 2013) that represent a majority of the noble crayfish populations that remain globally (Holdich et al., 2009).

The preservation of the intraspecific diversity and integrity of noble crayfish in Fennoscandia requires that conservation and management strategies take account of the clear genetic differentiation observed between the three population clusters identified in this study. These distinct groups have evolved during a long time period when the noble crayfish recolonized Fennoscandia, probably following different colonization routes. We recommend, therefore, that the main population groups identified here should receive status as three distinct management units (MUs; cf. Moritz, 1994) to preserve the genetic diversity and integrity of the species in Fennoscandia.

With the possible exception of the island of Gotland, where only one out of many local populations have been analysed genetically (and multiple historical introductions are known; R. Gydemo, pers. comm.), approximately 90% of all current populations in Sweden (Swedish Crayfish Database; Bohman, 2019) occur in counties dominated by the middle genetic population cluster. The additional populations (approx. 10%) are roughly equally distributed among counties dominated by the southern and northern clusters. In the past two decades, about half of the populations in southern and middle Sweden have become extinct. In contrast, almost no extinctions have occurred in northern Sweden where illegal introductions of signal crayfish have been very rare (Bohman, 2019). Consequently, the southern population cluster is perhaps the most threatened, as the extinction rate is high and only few populations remain. In addition, the southern cluster is the most genetically distinct and variable cluster, indicating that focusing on halting the extinction of populations in this region should be given the highest priority.

Genetic differentiation also exists between local populations within the three genetic clusters, although less pronounced and limited mainly to frequency differences for the same alleles. Such small-scale population sub-structuring is expected because of genetic drift in isolated populations. It is unknown to what extent allele frequency differences at presumably neutral microsatellites mirror adaptive divergence. Local adaptation may occur at different geographical scales, and its detection requires controlled breeding experiments (Rogell et al., 2012) or genomic surveys (Funk, McKay, Hohenlohe, & Allendorf, 2012). Nevertheless, it appears likely that adaptive genetic differences exist, at least among the population clusters identified, as these are likely to have been reproductively isolated for thousands of years across different environments in the Baltic Sea area. In a continuing common garden experiment, noble crayfish populations from all three genetic population clusters have been compared with respect to important fitness traits, and preliminary results indicate the presence of heritable trait differences that may reflect local adaptations (J. Dannewitz, S. Palm, L. Edsman, unpubl. data).

The results of this study have implications for the practice of stocking noble crayfish. Although rare to our knowledge, supplementary releases of noble crayfish of local origin to support existing wild populations (so-called 'supportive breeding') may lead to a reduction in the genetically effective population size, which in turn may result in elevated inbreeding and the loss of future adaptive potential (Ryman, Jorde, & Laikre, 1995; Ryman & Laikre, 1991; Wang & Ryman, 2001). Releases of noble crayfish of non-local genetic origin to enhance wild populations may also have severe consequences and appear to be far more common than supportive breeding. First, released individuals may have low fitness because they are not adapted to the local environment into which they are stocked (Tallmon, Luikart, & Waples, 2004), which may imperil the aim of the action. Second, releases of individuals of foreign genetic origin into an existing population may result in introgression of exogenous genes and a breakdown of locally adapted gene complexes, with a reduction in fitness as a result (so-called 'outbreeding depression'; Allendorf et al., 2013). Third, the stocking of non-local crayfish involves a risk of spreading diseases (e.g. Kozak et al., 2011).

As a result of the risks mentioned above, releases of noble crayfish (of local or non-local genetic origin) to enhance existing wild populations should be avoided unless regarded as necessary: for example, owing to a high risk of population extinction. The highest priority should instead be to identify reasons for the decline in natural production, and to focus on actions to solve those issues. To ensure genetic similarity, the reintroduction of the species in areas where it has become extinct should

be based on stocking material from genetically mapped populations with sufficient genetic variation from the same cluster as the extinct population (i.e. the southern, middle, or northern population groups identified in this study), collected from a geographically close location.

Although the movement of noble crayfish between areas is not recommended in general, the historical stocking of southern noble crayfish into lakes and catchments in middle Sweden may have a conservation value as living gene banks for the southern genetic cluster. If the extinction rate in southern Fennoscandia continues, risking the southern genetic cluster becoming extinct within its natural distribution range, movements of noble crayfish of southern origin to other areas to establish additional living gene banks may be justified. However, such measures need to be carefully planned and monitored by responsible management and conservation authorities.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest associated with this work.

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REFERENCES

Agerberg, A. (1990). Genetic variation in three species of freshwater crayfish; *Astacus astacus* L., *Astacus leptodactylus* Aesch., and *Pacifastacus leniusculus* (Dana), revealed by isozyme electrophoresis. *Hereditas*, 113, 101–108. https://doi.org/10.1111/j.1601-5223.1990.tb00073.x

Alaranta, A., Henttonen, P., Jussila, J., Kokko, H., Prestegaard, T., Edsman, L., & Halmekyto, M. (2006). Genetic differences among noble crayfish (*Astacus astacus*) stocks in Finland, Sweden and Estonia based on the ITS1 region. *Bulletin Francais de la Peche et de la Pisciculture*, 380-81, 965–975. https://doi.org/10.1051/kmae:2006035

Allendorf, F. W., England, P. R., Luikart, G., Ritchie, P. A., & Ryman, N. (2008). Genetic effects of harvest on wild animal populations. *Trends in Ecology & Evolution*, 23, 327–337. https://doi.org/10.1016/j.tree.2008.02.008

Allendorf, F. W., Luikart, G., & Aitken, S. N. (2013). *Genetics and the conservation of populations* (2nd ed.). Oxford, UK: Wiley-Blackwell Publishing.

Alm, G. (1929). Der Krebs und die Krebspest in Schweden. *Zeitschrift für Fisherei*, 27, 123–138 (in German).

Avise, J. C. (2000). *Phylogeography: The history and formation of species*. Cambridge, Massachusetts: Harvard University Press.

Björck, S. (1995). A review of the history of the Baltic Sea, 13.0-8.0 ka BP. *Quaternary International*, 27, 19–40. https://doi.org/10.1016/1040-6182(94)00057-c

Bohman, P. (2019). Swedish crayfish database. Swedish University of Agricultural Sciences, Department of Aquatic Resources. Retrieved from http://www.slu.se/kraftdatabasen

Bohman, P., Degerman, E., Edsman, L., & Sers, B. (2011). Exponential increase of signal crayfish in running waters in Sweden—Due to illegal introductions? *Knowledge and Management of Aquatic Ecosystems*, 401, 23. https://doi.org/10.1051/kmae/2011040

Bohman, P., Nordwall, F., & Edsman, L. (2006). The effect of the large-scale introduction of signal crayfish on the spread of crayfish plague in Sweden. *Bulletin Francais de la Peche et de la Pisciculture*, 380-81, 1291–1302. https://doi.org/10.1051/kmae:2006026

Casacci, L. P., Barbero, F., & Balletto, E. (2014). The 'Evolutionarily Significant Unit' concept and its applicability in biological conservation. *The Italian Journal of Zoology*, 81, 182–193. https://doi.org/10.1080/11250003.2013.870240

Cavalli-Sforza, L. L., & Edwards, A. W. F. (1967). Phylogenetic analysis models and estimation procedures. *American Journal of Human Genetics*, 19, 233–257. https://doi.org/10.1111/j.1558-5646.1967.tb03411.x

Cornuet, J. M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., ... Estoup, A. (2014). DIYABC v2.0: A software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics*, 30, 1187–1189. https://doi.org/10.1093/bioinformatics/btt763

Council of the European Communities. (1992). Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Communities*, L206, 7–50.

Delling, B., Palm, S., Palkopoulou, E., & Prestegaard, T. (2014). Genetic signs of multiple colonization events in Baltic ciscoes with radiation into sympatric spring- and autumn-spawners confined to early postglacial arrival. *Ecology and Evolution*, 4, 4346–4360. https://doi.org/10.1002/ece3.1299

Earl, D. A., & Vonholdt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Edsman, L. (2016). Utvärdering av åtgärdsprogram flodkräfta 2008-2014. Aqua Reports 2016:19 (in Swedish with English summary). Retrieved from https://pub.epsilon.slu.se/14122/

Edsman, L., Farris, J. S., Kallersjo, M., & Prestegaard, T. (2002). Genetic differentiation between noble crayfish, *Astacus astacus* (L.), populations detected by microsatellite length variation in the rDNA ITS1 region. *Bulletin Francais de la Peche et de la Pisciculture*, 367, 691–706. https://doi.org/10.1051/kmae:2002082

Edsman, L., Füreder, L., Gherardi, F., & Souty-Grosset, C. (2010). Astacus astacus. The IUCN Red List of Threatened Species 2010: E. T2191A9338388. https://doi.org/10.2305/IUCN.UK.2010-3.RLTS.T2191A9338388.en

Edsman, L., & Schröder, S. (2009). *Åtgärdsprogram för flodkräfta 2008–2013 (Astacus astacus)*. Fiskeriverket Och Naturvårdsverket, Rapport 5955 (in Swedish with English summary).

Edsman, L., & Smietana, P. (2004). Roundtable—Session 2—Exploitation, conservation and legislation. *Bulletin Francais de la Peche et de la Pisciculture*, 372-73, 457–464. https://doi.org/10.1051/kmae:2004019

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study.

Molecular Ecology, 14, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Hofer, T., & Foll, M. (2009). Detecting loci under selection in a hierarchically structured population. *Heredity*, 103, 285–298. https://doi.org/10.1038/hdy.2009.74

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, 1, 47–50. https://doi.org/10.1177/117693430500100003

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. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, 164, 1567–1587.

Felsenstein, J. (2005). PHYLIP (phylogeny inference package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Fevolden, S. E., Taugbøl, T., & Skurdal, J. (1994). Allozymic variation among populations of noble crayfish, *Astacus astacus* L., in southern Norway: Implications for management. *Aquaculture and Fisheries Management*, 25, 927–935. https://doi.org/10.1111/j.1365-2109.1994.tb01354.x

Foll, M., Beaumont, M. A., & Gaggiotti, O. (2008). An approximate Bayesian computation approach to overcome biases that arise when using amplified fragment length polymorphism markers to study population structure. *Genetics*, 179, 927–939. https://doi.org/10.1534/genetics.107.084541

Frankham, R. (1995). Inbreeding and extinction—A threshold effect. *Conservation Biology*, 9, 792–799. https://doi.org/10.1046/j.1523-1739.1995.09040792.x

Frankham, R., Lees, K., Montgomery, M. E., England, P. R., Lowe, E. H., & Briscoe, D. A. (1999). Do population size bottlenecks reduce evolutionary potential? *Animal Conservation*, 2, 255–260. https://doi.org/10.1111/j.1469-1795.1999.tb00071.x

Fraser, D. J., & Bernatchez, L. (2001). Adaptive evolutionary conservation: Towards a unified concept for defining conservation units. *Molecular Ecology*, 10, 2741–2752. https://doi.org/10.1046/j.0962-1083.2001.01411.x

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, 27, 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Garza, J. C., & Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, 10, 305–318. https://doi.org/10.1046/j.1365-294x.2001.01190.x

Goudet, J. (1999). *PCA-GEN, Version 1.2*. Institute of Ecology, Biology Building, UNIL, Lausanne, Switzerland. Retrieved from https://www2.unil.ch/popgen/softwares/pcagen.htm

Goudet, J. (2001). FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Retrieved from http://www2.unil.ch/popgen/softwares/fstat.htm

Gren, I. M., Campos, M., Edsman, L., & Bohman, P. (2009). Incomes, attitudes, and occurrences of invasive species: An application to signal crayfish in Sweden. *Environmental Management*, 43, 210–220. https://doi.org/10.1007/s00267-008-9210-7

Gross, R., Palm, S., Koiv, K., Prestegaard, T., Jussila, J., Paaver, T., … Edsman, L. (2013). Microsatellite markers reveal clear geographic structuring among threatened noble crayfish (*Astacus astacus*) populations in Northern and Central Europe. *Conservation Genetics*, 14, 809–821. https://doi.org/10.1007/s10592-013-0476-9

Gydemo, R., & Gydemo, R. (1990). Utsättningar av flodkräfta i Västerbottens län. Rapport Swedish University of Agricultural Sciences, Department of Aquaculture, 4 (In Swedish).

Harris, D. J., & Crandall, K. A. (2000). Intragenomic variation within ITS1 and ITS2 of freshwater crayfishes (Decapoda: Cambaridae): Implications for phylogenetic and microsatellite studies. *Molecular Biology and Evolution*, 17, 284–291. https://doi.org/10.1093/oxfordjournals.molbev.a026308

Hedrick, P. W., & Kalinowski, S. T. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, 31, 139–162. https://doi.org/10.1146/annurev.ecolsys.31.1.139

Henriksen, S., & Hilmo, O. (2015). *Norsk rødliste for arter 2015*. Norge: Artsdatabanken.

Holdich, D. M., Reynolds, J. D., Souty-Grosset, C., & Sibley, P. J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, 394-395, 11. https://doi.org/10.1051/kmae/2009025

Hyvärinen, E., Juslén, A., Kemppainen, E., Uddström, A., & Liukko, U.-M. E. (2019). The 2019 red list of Finnish species. Ympäristöministeriö & Suomen ympäristökeskus, Helsinki.

Intergovernmental Science–Policy Platform on Biodiversity and Ecosystem Services (IPBES). (2019). In E. S. Brondizio, J. Settele, S. Díaz, & H. T. Ngo (Eds.), *Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science - Policy Platform on Biodiversity and Ecosystem Services*. Bonn, Germany: IPBES Secretariat.

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23, 1801–1806. https://doi.org/10.1093/bioinformatics/btm233

Jussila, J., & Mannonen, A. (2004). Crayfisheries in Finland, a short overview. *Bulletin Francais de la Peche et de la Pisciculture*, 372-73, 263–273. https://doi.org/10.1051/kmae:2004001

Kitada, S. (2018). Economic, ecological and genetic impacts of marine stock enhancement and sea ranching: A systematic review. *Fish and Fisheries*, 19, 511–532. https://doi.org/10.1111/faf.12271

Koiv, K., Gross, R., Paaver, T., Hurt, M., & Kuehn, R. (2009). Isolation and characterization of 11 novel microsatellite DNA markers in the noble crayfish, *Astacus astacus*. *Animal Genetics*, 40, 124–124. https://doi.org/10.1111/j.1365-2052.2008.01781.x

Koiv, K., Gross, R., Paaver, T., & Kuehn, R. (2008). Isolation and characterization of first microsatellite markers for the noble crayfish, *Astacus astacus*. *Conservation Genetics*, 9, 1703–1706. https://doi.org/10.1007/s10592-008-9539-8

Koskinen, M. T., Nilsson, J., Veselov, A. J., Potutkin, A. G., Ranta, E., & Primmer, C. R. (2002). Microsatellite data resolve phylogeographic patterns in European grayling, *Thymallus thymallus*, Salmonidae. *Heredity*, 88, 391–401. https://doi.org/10.1038/sj/hdy/6800072

Kozak, P., Fureder, L., Kouba, A., Reynolds, J., & Souty-Grosset, C. (2011). Current conservation strategies for European crayfish. *Knowledge and Management of Aquatic Ecosystems*, 401, 01. https://doi.org/10.1051/kmae/2011018

Kuparinen, A., Hutchings, J. A., & Waples, R. S. (2016). Harvest-induced evolution and effective population size. *Evolutionary Applications*, 9, 658–672. https://doi.org/10.1111/eva.12373

Laggis, A., Baxevanis, A. D., Charalampidou, A., Maniatsi, S., Triantafyllidis, A., & Abatzopoulos, T. J. (2017). Microevolution of the noble crayfish (*Astacus astacus*) in the Southern Balkan Peninsula. *BMC Evolutionary Biology*, 17, 122. https://doi.org/10.1186/s12862-017-0971-6

Laikre, L. (2010). Genetic diversity is overlooked in international conservation policy implementation. *Conservation Genetics*, 11, 349–354. https://doi.org/10.1007/s10592-009-0037-4

Laikre, L., & Ryman, N. (1996). Effects on intraspecific biodiversity from harvesting and enhancing natural populations. *Ambio*, 25, 504–509.

Makkonen, J., Kokko, H., & Jussila, J. (2015). Mitochondrial cytochrome oxidase I gene analysis indicates a restricted genetic background in Finnish noble crayfish (*Astacus astacus*) stocks. *Knowledge and Management of Aquatic Ecosystems*, 416, 21. https://doi.org/10.1051/kmae/2015017

Martin-Torrijos, L., Kokko, H., Makkonen, J., Jussila, J., & Dieguez-Uribeondo, J. (2019). Mapping 15 years of crayfish plague in the Iberian Peninsula: The impact of two invasive species on the endangered native crayfish. *PLoS ONE*, 14, 1–14. https://doi.org/10.1371/journal.pone.0219223

Moritz, C. (1994). Defining evolutionarily-significant-units for conservation. *Trends in Ecology & Evolution*, 9, 373–375. https://doi.org/10.1016/0169-5347(94)90057-4

Nesbø, C. L., Fossheim, T., Vøllestad, L. A., & Jakobsen, K. S. (1999). Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. *Molecular Ecology*, 8, 1387–1404. https://doi.org/10.1046/j.1365-294x.1999.00699.x

Nilsson, J., Gross, R., Asplund, T., Dove, O., Jansson, H., Kelloniemi, J., ... Lumme, J. (2001). Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonization of the Baltic Sea area. *Molecular Ecology*, 10, 89–102. https://doi.org/10.1046/j.1365-294X.2001.01168.x

Perdikaris, C., Kozak, P., Kouba, A., Konstantinidis, E., & Paschos, I. (2012). Socio-economic drivers and non-indigenous freshwater crayfish species in Europe. *Knowledge and Management of Aquatic Ecosystems*, 404, 01. https://doi.org/10.1051/kmae/2011077

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959. https://doi.org/10.1093/genetics/155.2.945

Richman, N. I., Boehm, M., Adams, S. B., Alvarez, F., Bergey, E. A., Bunn, J. J. S., ... Collen, B. (2015). Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 370, 20140060. https://doi.org/10.1098/rstb.2014.0060

Rogell, B., Dannewitz, J., Palm, S., Petersson, E., Dahl, J., Prestegaard, T., ... Laurila, A. (2012). Strong divergence in trait means but not in plasticity across hatchery and wild populations of sea-run brown trout *Salmo trutta*. *Molecular Ecology*, 21, 2963–2976. https://doi.org/10.1111/j.1365-294X.2012.05590.x

Rosenberg, N. A. (2004). DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, 4, 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Ruokonen, T. J., Sjovik, R., Erkamo, E., Tulonen, J., Ercoli, F., Kokko, H., & Jussila, J. (2018). Introduced alien signal crayfish (*Pacifastacus leniusculus*) in Finland — Uncontrollable expansion despite numerous crayfisheries strategies. *Knowledge and Management of Aquatic Ecosystems*, 419, 27. https://doi.org/10.1051/kmae/2018016

Ryman, N., Jorde, P. E., & Laikre, L. (1995). Supportive breeding and variance effective population size. *Conservation Biology*, 9, 1619–1628. https://doi.org/10.1046/j.1523-1739.1995.09061619.x

Ryman, N., & Laikre, L. (1991). Effects of supportive breeding on the genetically effective population-size. *Conservation Biology*, 5, 325–329. https://doi.org/10.1111/j.1523-1739.1991.tb00144.x

Saitou, N., & Nei, M. (1987). The Neighbor-Joining method — A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Schrimpf, A., Piscione, M., Cammaerts, R., Collas, M., Herman, D., Jung, A., ... Theissinger, K. (2017). Genetic characterization of Western European noble crayfish populations (*Astacus astacus*) for advanced conservation management strategies. *Conservation Genetics*, 18, 1299–1315. https://doi.org/10.1007/s10592-017-0981-3

Schrimpf, A., Theissinger, K., Dahlem, J., Maguire, I., Parvulescu, L., Schulz, H. K., & Schulz, R. (2014). Phylogeography of noble crayfish (*Astacus astacus*) reveals multiple refugia. *Freshwater Biology*, 59, 761–776. https://doi.org/10.1111/fwb.12302

Skurdal, J., Taugbøl, T., Burba, A., Edsman, L., Söderbäck, B., Styrishave, B., ... Westman, K. (1999). Crayfish introductions in the Nordic and Baltic countries. In F. Gherardi & D. M. Holdich (Eds.), *Crayfish in Europe as alien species. How to make the best of a bad situation* (pp. 193–219). Rotterdam: AA Balkema.

SLU Artdatabanken. (2020). Rödlistade arter i Sverige 2020 (The 2020 Swedish red list; in Swedish with English summary). Retrieved from https://www.artdatabanken.se/globalassets/ew/subw/artd/2.-var-verksamhet/publikationer/31.-rodlista-2020/rodlista-2020

Strand, D. A., Johnsen, S. I., Rusch, J. C., Agersnap, S., Larsen, W. B., Knudsen, S. W., ... Vralstad, T. (2019). Monitoring a Norwegian freshwater crayfish tragedy: eDNA snapshots of invasion, infection and extinction. *Journal of Applied Ecology*, 56, 1661–1673. https://doi.org/10.1111/1365-2664.13404

Strand, D. A., Jussila, J., Johnsen, S. I., Viljamaa-Dirks, S., Edsman, L., Wiik-Nielsen, J., ... Vrålstad, T. (2014). Detection of crayfish plague spores in large freshwater systems. *Journal of Applied Ecology*, 51, 544–553. https://doi.org/10.1111/1365-2664.12218

Swahn, J. O. (2004). The cultural history of crayfish. *Bulletin Francais de la Peche et de la Pisciculture*, 372-73, 243–251.

Takezaki, N., & Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, 144, 389–399. https://doi.org/10.1093/genetics/144.1.389

Tallmon, D. A., Luikart, G., & Waples, R. S. (2004). The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution*, 19, 489–496. https://doi.org/10.1016/j.tree.2004.07.003

Taugbøl, T. (2004). Exploitation is a prerequisite for conservation of *Astacus astacus*. *Bulletin Francais de la Peche et de la Pisciculture*, 372-73, 275–279. https://doi.org/10.1051/kmae:2004002

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. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Vrålstad, T., Johnsen, S. I., Fristad, R. F., Edsman, L., & Strand, D. (2011). Potent infection reservoir of crayfish plague now permanently established in Norway. *Diseases of Aquatic Organisms*, 97, 75–83. https://doi.org/10.3354/dao02386

Walsh, P. S., Metzger, D. A., & Higuchi, R. (1991). Chelex-100 as a medium for simple extraction of DNA for pcr-based typing from forensic material. *BioTechniques*, 10, 506–513.

Wang, J. L., & Ryman, N. (2001). Genetic effects of multiple generations of supportive breeding. *Conservation Biology*, 15, 1619–1631. https://doi.org/10.1046/j.1523-1739.2001.00173.x

Weeks, A. R., Stoklosa, J., & Hoffmann, A. A. (2016). Conservation of genetic uniqueness of populations may increase extinction likelihood of endangered species: The case of Australian mammals. *Frontiers in Zoology*, 13, 31. https://doi.org/10.1186/s12983-016-0163-z

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population-structure. *Evolution*, 38, 1358–1370. https://doi.org/10.2307/2408641

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.