

Genetic diversity of noble crayfish in Finland based on ITS1 microsatellite-like repeat variation: implications to the conservation and management

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Abstract – During the last two centuries, native noble crayfish (*Astacus astacus*) has been introduced and re-introduced into many waters in Finland, to widen the area of distribution, and to re-establish the collapsed populations. Recent studies have revealed narrowed genetic diversity in *A. astacus* populations, especially in North Europe, due to crayfish plagues and due to the past translocation policies with numerous introductions also with small number of individuals. However, preliminary data based on the microsatellite like-repeat variation in ITS1 (Internal Transcribed Spacer 1) have indicated also genetic heterogeneity among Swedish and Finnish populations. In this study, the genetic diversity of 38 Finnish *A. astacus* populations was analysed based on variation in ITS1 region. Stocking histories of populations were obtained from official records and from local fishermen. Eighth out of 38 populations showed divergence in paired comparison with other analysed populations (Population Divergence Test, $p < 0.05$) indicating either possibility of autochthonous origin or donor. Potential autochthonous populations, as well as refugee areas within original distribution range and within designated protection area, should be further distinguished and monitored to maintain the remaining genetic diversity of the populations.

Keywords: Indigenous species / introduction / natural distribution area / biodiversity / autochthonous population / protection area

1 Introduction

Noble crayfish (*Astacus astacus*) has a threatened conservation status throughout Europe (Jussila and Edsman, 2020). In Finland the conservation status was updated from vulnerable (Edsman *et al.*, 2010) to endangered (IUCN Red List status; EN A2a,b,c,e) (Hyvärinen *et al.*, 2019), but the species is still exploited. The original distribution area of noble crayfish, with the northern distribution limit being roughly at 62°N latitude after the last glaciations (Nylander, 1859; Helle, 1904; Järvi, 1910), has spread northward (Ruokonen *et al.*, 2023). This spread has been aided by anthropogenic establishments as several translocations have been made into many new water bodies up to the latitude 68°N (Westman, 1973; Souty-Grosset *et al.*, 2006; Pursiainen and Rajala, 2009). The translocations have been typical in all Nordic countries, where

some exploitable stocks still exist, and the more recent stocking programs have been initiated to stop the declining of populations (Jussila and Mannonen, 2004; Paaver and Hurt, 2009; Dannewitz *et al.*, 2021). As a result, a total of 2.4 million noble crayfish have been introduced or reintroduced into more than 1 000 water bodies between 1989–2021 in Finland (Ruokonen *et al.*, 2023). In addition, numerous not recorded stockings have also occurred.

A National Crayfish Strategy, implemented by the Finnish Fisheries Authority first in 1989 (Kirjavainen, 1989) and renewed in 2000 (Mannonen A, Halonen T, TE-keskusten työryhmä, 2000), in 2013 (Ministry of Agriculture and Forestry, 2014), in 2019 (Erkamo *et al.*, 2019) and in 2023 (Ruokonen *et al.*, 2023), has guided the management and conservation of noble crayfish, and defined and re-defined the geographical limits for previously licensed introductions of alien signal crayfish (*Pacifastacus leniusculus*). Stockings and farming of signal crayfish were banned in 2014 in European Union legislation (EU Regulation on Invasive Alien Species 1143/2014), entered into force in 2015

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together with the national regulation and implement to the national crayfish strategy in 2019 in Finland (Erkamo *et al.*, 2019). However, these national strategies are without any legislative force, and the nature of these strategies has received criticism from stakeholders (Ruokonen *et al.*, 2018; Jussila and Edsman, 2020; Jussila *et al.*, 2021).

National Crayfish Strategy 2023–2032 (Ruokonen *et al.*, 2023) defines the protection areas to preserve the noble crayfish populations (*i.e.*, water basins without known signal crayfish), and the management area that includes the water basins currently with signal crayfish. Current distribution of alien signal crayfish is remarkably overlapping with the original distribution range of noble crayfish (Ruokonen *et al.*, 2023). Numerous illegal stockings of signal crayfish, along with crayfish plague (*Aphanomyces astaci*) outbreaks, have caused ongoing threat for the noble crayfish populations (Westman, 1991; Jussila and Mannonen, 2004; Souty-Grosset *et al.*, 2006; Pursiainen, 2012; Ruokonen *et al.*, 2018; Jussila and Edsman, 2020; Jussila *et al.*, 2021; Ruokonen *et al.*, 2023). In addition, changes in the fragile aquatic habitat can cause increased mortality, emigration, reduction in growth rate and production, and problems in reproduction for noble crayfish (Mannonen and Westman, 1998).

Previous studies concerning the noble crayfish genetic diversity in Finland are based on the microsatellite-like repeat located in the internal transcribed spacer 1-region (ITS1) indicating some genetic heterogeneity and characteristic fragmentation (Alaranta *et al.*, 2006, see also Edsman *et al.*, 2002; Alaranta *et al.*, 2011a), and on the novel microsatellites showing some genetic heterogeneity (Gross *et al.*, 2013). However, these studies are focused more to the development of the genetic analyses and included only limited number of Finnish populations. In addition to these data, the mitochondrial cytochrome oxidase I (*COI*) gene was used to assess genetic diversity of a total of 55 Finnish populations; however, no genetic variation was observed (Makkonen *et al.*, 2015), and the single haplotype detected from all analysed individuals was the most common found in whole Europe (Schrimpf *et al.*, 2011; Schrimpf *et al.*, 2014). The colonization history and human translocations show significant role in noble crayfish populations in Fennoscandia. It has been suggested that the postglacial recolonization of Fennoscandia involved two independent colonization events following separate routes from a common refugium in south-eastern Europe (Dannewitz *et al.*, 2021; see also Schrimpf *et al.*, 2014; Gross *et al.*, 2021). In Europe, populations have experienced significant declines, caused by anthropogenic pressures on the habitats, together with climate change and the spread of invasive species (Lovrenčić *et al.*, 2022). However, except study based on the *COI* haplotype variation (Makkonen *et al.*, 2015), the previous studies have not comprehensively covered the distribution range of noble crayfish in Finland, and therefore implications to the conservation and management have mostly been lacking.

The aims of this study are 1) to assess the genetic diversity of noble crayfish in Finland based on ITS1 microsatellite-like repeat variation, and 2) to assess the possible existence of autochthonous populations with implications for the future conservation and management of the species in Finland.

2 Materials and methods

2.1 Samples

Noble crayfish samples were collected by trapping, in collaboration with local fishermen, during the crayfish trapping seasons (from late July to late October) in 2004. In addition, previously collected samples from Lake Iso-Lauas population (sampling year 1995) and Lake Mäntyjärvi population (1997) were also included to this study. The stocking histories of the populations were obtained from the local fishermen, government's fisheries authority and from the literature. A total of 1140 individual samples (10–69 crayfish from each site), were collected and analysed from a total of 38 water systems (Fig. 1, Tab. 1). Nine populations were locating within original distribution area below latitude 62°N (Järvi, 1910) while 29 populations were located in more northern areas (Tab. 1). A total of eight populations were from a drainage basin within a current protection range (Ruokonen *et al.*, 2023) and one out of those was locating within original distribution area (Fig. 1, Tab. 1).

2.2 DNA extraction

Individual DNA was extracted from the walking leg muscle removed by using sterile wooden toothpick by using protocol described in Alaranta *et al.* (2006). DNA concentration was measured using spectrophotometer (NanoDrop® ND 1000) and DNA extractions were stored at – 70 °C.

2.3 PCR, ITS1 fragment analysis and Population Divergence Test (PDT)

PCR was performed using the primers Asa1F (5'-tca ctc cgt cag cag tga gtc gct- 3'; Cy-5 labelled) and Asa1R (5'- gag tca aga cgt gca gcc tag gcc c-3') (Edsman *et al.*, 2002), ranging fragment sizes from 162 bp to 216 bp. Laboratory protocol and PCR reaction are described in Alaranta *et al.* (2006). In ITS1 fragment length analyses the PCR products were loaded to the Hydrolink Long Read 6% gel and the fragment lengths were separated by using ALFexpress automated sequences. CY-5 labelled external size markers (50–250 bp in 50 bp intervals) and two internal size markers (50 bp and 250 bp) were used. Fragment sizes were determined by using ALFwin Fragment Analyser (Pharmacia Biotech).

The population Divergence Test (PDT) measures differences between populations based on the frequencies of the fragments, and it produces probabilities (*p*-values from 0 to 1) expressing the difference between two populations in paired comparison (*p*-values < 0.05), identical populations having *p*-value 1. PDT is described more detailed by Edsman *et al.*, (2002). PDT was performed in the Laboratory of Molecular Systematics (Swedish Museum of Natural History, Sweden).

3 Results

3.1 Genetic analyses

The amplified ITS1 fragment lengths varied from 168 bp to 216 bp. The following local fragments were observed from

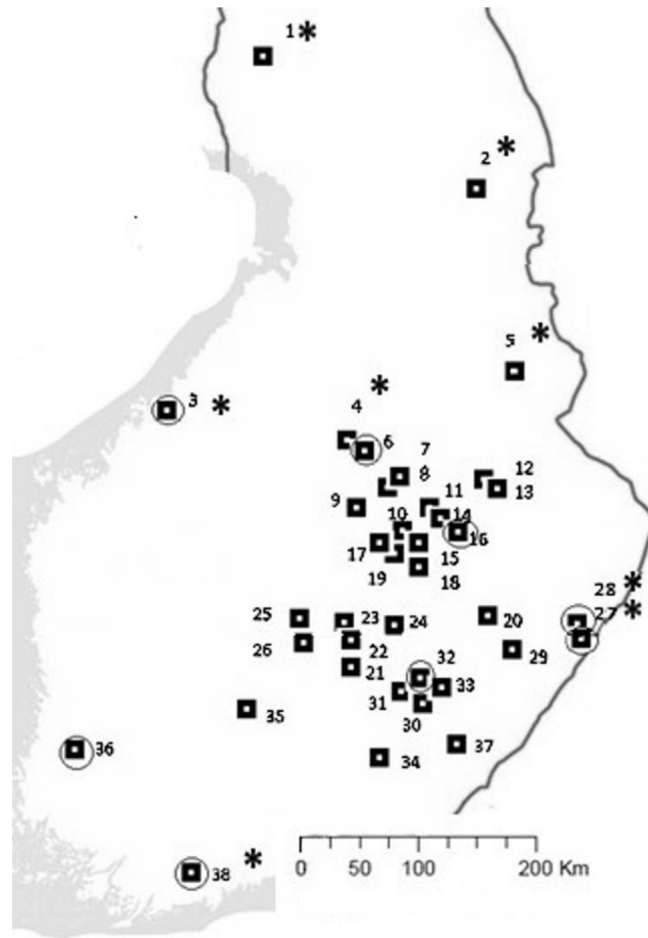


Fig. 1. Geographical distribution of 38 sampled noble crayfish populations. Eight populations with p -values < 0.05 (PDT) in paired comparison (see Tab. 3) are marked with circle, and eight populations locating in the protection area (Ruokonen *et al.*, 2023) are marked with asterisk (*). Map © Maanmittauslaitos.

only one population: fragment of 214 bp (frequency of 3%, Lake Viitajärvi), fragment of 172 bp (frequency of 7%, Lake Mäntyjärvi), fragment of 180 bp (frequency of 4%; Lake Saimaa) and fragment of 216 bp (frequency of 19, Lake Linkullasjön) (Tab. 2).

Numbers of different fragments in a fragment profile varied from five (Lake Ahvenlampi) to 13 (Lake Saimaa) within populations and relative frequency of samples with unique fragment profiles within population from 17% (Lake Ahvenlampi) to 88% (Lake Saimaa) (Tab. 2).

According to PDT, the following eight populations differed (p -values < 0.05) from the other assessed populations in paired comparison: River Perhonjoki, Lake Ahvenlampi and Lake Suuri-Heinäjärvi, all locating within the protection area (see Ruokonen *et al.*, 2023) but outside of the original range, Lake Koivujärvi and River Kasijoki locating in the management area outside of the original range, Lake Suuri-Vahvanen and Lake Köyliönjärvi locating within the original distribution area, and finally Lake Linkullasjön locating within the original distribution area and in the current protection area (Fig. 1, Tabs. 2 and 3). The remaining 30 populations showed no statistically significant differences (p -values > 0.05) in paired comparison with one (*e.g.*, River Pajakkajoki together with

seven populations) or up to 12 populations (Pond Valkeinen) (Tabs. 2 and 3).

4 Discussion

The determination of genetic structure, *e.g.*, ITS-fingerprints, of natural and managed populations forms a basis for conservation genetics that generally aims to conserve and restore the biodiversity (Coates *et al.*, 2018). Genetically different populations are suggested as candidates for special management efforts to prevent the loss of unique genetic variants (Dowling and Childs, 1992). Therefore, management strategies are, or should be, focused to preserve genetic diversity within populations while separate stocks are recommended to be managed as distinct conservation units (Souty-Grosset *et al.*, 1997). Considering the conservation and management of noble crayfish, the local populations may be better adapted to their local environment and introduction of genetically different individuals could adversely alter the gene pool (Souty-Grosset *et al.*, 1997).

The populations with naturally high levels of genetic variation are thought to be capable to adapt more successfully to environmental changes while lack of genetic variation

Table 1. Noble crayfish populations, location, size of the water system (ha), drainage basins code with an asterisk (*) if within protection area (Ruokonen *et al.*, 2023); number of samples, historical information, and findings from previous genetic analyses.

Noble crayfish populations	Location (lat; long)	Size (ha)	Drainage basin code	Number of samples	Historical information	Findings from previous analyses
Established populations, located above 62 °N						
1. River Sirkkakoski	66.683164; 24.406271	–	67*	30	–	No difference with Swedish population (Alaranta <i>et al.</i> , 2006)
2. Lake Jokijärvi	65.910623; 28.732909	638	61*	69	–	–
3. River Perhonjoki	63.838474; 23.248587	>1	49*	30	Established (Ignatius, 1885 according to Westman, 1991), crayfish plague 1962, 1977, 1992 (Mannonen <i>et al.</i> , 2006)	No difference with Perhonjoki, Koivujärvi and Pitkäjoki (Gross <i>et al.</i> , 2013)
4. Lake Pyhäjärvi	63.684715; 26.001706	12 179	54*	30	Translocations within lake according to local fishermen, crayfish plague 1956 and 1998 (Mannonen <i>et al.</i> , 2006)	–
5. River Pajakkajoki	64.123641; 29.481611	–	59*	30	Used as stocking resource, crayfish plague after sampling (2009) (Makkonen <i>et al.</i> , 2015)	–
6. Lake Koivujärvi	63.485313; 26.270324	2 607	14	30	Used as stocking resource according to local fishermen	No difference with Perhonjoki, Koivujärvi and Pitkäjoki (Gross <i>et al.</i> , 2013)
7. Lake Viitajärvi	63.032237; 26.800604	87	14	30	–	–
8. Lake Saarinen	63.053015; 26.806283	150	14	30	–	–
9. Lake Horonjärvi	62.866147; 26.286328	369	14	40	–	–
10. Lake Rytky	62.856242; 27.421818	156	04	30	–	–
11. River Siilinjoki	63.077664; 27.676949	–	04	30	–	–
12. Lake Mäntyjärvi	63.074632; 28.707261	52	04	30	Crayfish plague after sampling (2009) (Makkonen <i>et al.</i> , 2015)	–
13. River Pisankoski	63.186702; 28.414235	–	04	22	Observed mortality 1972 (Mannonen <i>et al.</i> , 2006)	–
14. Pond Poljanlampi	62.970829; 27.871599	5	04	22	–	–
15. Pond Valkainen	62.841045; 27.588294	10	04	30	–	–
16. River Kasjoki	62.838145; 28.133751	–	04	35	–	–
17. Lake Iso-Lauas	62.777193; 27.381992	611	14	30	Established (Oksman and Lindqvist, 1977), used heavily as stocking resource according to local fishermen (Korhonen, 2010), crayfish plague 1996, 2000 (Mannonen <i>et al.</i> , 2006)	–
18. Lake Valkainen	62.841358; 27.586620	18	04	30	–	–
19. Lake Iivesjärvi	62.250763; 24.789097	26	04	30	–	–
20. Lake Pyhtäänjärvi	62.313978; 26.024866	397	14	30	–	–
21. Lake Ala-Siili	62.303050; 27.027540	210	14	30	Crayfish plague 1950 (Mannonen <i>et al.</i> , 2006)	–
22. Lake Suuri-Pölläkkä	62.278385; 28.915644	119	04	30	–	–
23. Lake Ylä-Kintaus	62.327056; 25.365686	572	14	30	–	–

Table 1. (continued).

Noble crayfish populations	Location (lat; long)	Size (ha)	Drainage basin code	Number of samples	Historical information	Findings from previous analyses
24. Lake Ala-Kintaus	62.292516; 25.356388	722	14	30	Farmed population, crayfish plague 1964-1965, 1999 (Mannonen <i>et al.</i> , 2006)	–
25. Lake Riihijärvi	62.02185; 26.056294	89	14	25	Farmed population, used as stocking resource, crayfish plague 1964 (Mannonen <i>et al.</i> , 2006)	–
26. River Pitkäjoki	62.119420; 26.047111	–	14	26	–	No difference with Perhonjoki, Koivujärvi and Pitkäjoki (Gross <i>et al.</i> , 2013)
27. Lake Ahvenlampi	62.068805; 30.364909	29	02*	30	–	Origin or donor autochthonous (Alaranta <i>et al.</i> , 2006)
28. Lake Suuri-Heinäjärvi	62.091913; 30.329275	351	02*	30	Established/re-established according to local fishermen, observed mortality 1995 (Mannonen <i>et al.</i> , 2006)	–
29. Lake Ylä-Luotojärvi	62.025555; 29.244862	586	04	30	Used as a donor according to local fisherman	–
Populations within natural distribution area below 62°N latitude						
30. Lake Kitere	61.527360; 27.273874	44	04	30	Established	–
31. Lake Säimijärvi	61.556562; 27.007384	69	14	30	–	–
32. Lake Suuri-Vahvanen	61.683725; 27.539792	132	04	30	Established, stockings	–
33. Lake Ylä-Säynätjärvi	61.663362; 27.405896	52	04	30	Established, stockings	–
34. Lake Hosusjärvi	61.254510; 26.802335	31	14	10	–	–
35. Pond Mikkolanjärvi	61.614456; 24.594347	23	35	27	–	–
36. Lake Köyliönjärvi	61.106264; 22.347164	1423	34	30	Farmed population	Origin or donor probably autochthonous (Alaranta <i>et al.</i> , 2006)
37. Lake Saimaa	61.212337; 27.651544	137703	04	30	Stockings	No difference with two Swedish population and one Estonian population (Alaranta <i>et al.</i> , 2006)
38. Lake Linkullasjön	60.097355; 23.879507	60	82*	30	–	Origin or donor autochthonous (Alaranta <i>et al.</i> , 2006), more similar with Swedish than Finnish populations (Gross <i>et al.</i> , 2013)

Table 2. Population Divergence Test (PDT) results based on ITS1 microsatellite-like repeat variation; populations with no statistical difference (PDT $p > 0.05$) in paired comparison (see p -values in Tab. 3.), and relative frequency (%) of different ITS1 fragment profiles within population, total number of fragments within population and local fragments (bp) if detected with the frequencies.

Population	Populations with no statistically significant difference (PDT, $p > 0.05$) in paired comparison	Relative frequency of samples with different fragment profiles within population (%)	Total number of fragments detected in population	Local fragments (bp) and frequencies (%)
Established populations, located above 62°N				
1. River Sirkkakoski	2, 20, 26	40	10	–
2. Lake Jokijärvi	1, 14	22	9	–
3. River Perhonjoki	–	43	9	–
4. Lake Pyhäjärvi	9, 15, 18, 24, 34	40	8	–
5. River Pajakkajoki	12	57	8	–
6. Lake Koivujärvi	–	30	8	–
7. Lake Viitajärvi	8, 9, 15, 17, 24, 25, 26, 31	40	8	214 bp, 3%
8. Lake Saarinen	7, 15, 24, 25, 26	40	6	–
9. Lake Horonjärvi	4, 7, 15, 20, 24	48	8	–
10. Lake Rytty	17	60	10	–
11. River Siilinjoki	13, 17, 37	33	8	–
12. Lake Mäntyjärvi	5, 14, 20	60	10	172 bp, 7%
13. River Pisankoski	11, 17	50	7	–
14. Pond Pohjanlampi	2, 12, 15, 17, 24, 26, 30	73	7	–
15. Pond Valkeinen	4, 7, 8, 9, 14, 17, 20, 23, 24, 25, 26, 37	40	7	–
16. River Kasijoki	–	31	7	–
17. Lake Iso-Lauas	7, 10, 11, 13, 14, 15, 21, 23, 25, 26, 31	63	8	–
18. Lake Valkeinen	4, 23, 34	40	7	–
19. Lake Ilvesjärvi	22	46	9	–
20. Lake Pyhtäänjärvi	1, 9, 12, 15, 24	40	7	–
21. Lake Ala-Siili	17, 33	50	6	–
22. Lake Suuri-Pölläkkä	19	30	7	–
23. Lake Ylä-Kintaus	15, 17, 18	60	10	–
24. Lake Ala-Kintaus	4, 7, 8, 9, 14, 15, 20, 25, 26	57	7	–
25. Lake Riihijärvi	7, 8, 15, 17, 24, 35	68	8	–
26. River Pitkäjoki	1, 7, 8, 14, 15, 17, 20, 24	58	7	–
27. Lake Ahvenlampi	–	17	5	–
28. Lake Suuri-Heinäjärvi	–	27	6	–
29. Lake Ylä-Luotojärvi	34	30	7	–
Populations within natural distribution area below 62°N latitude				
30. Lake Kitere	14	50	9	–
31. Lake Säiniönjärvi	7, 17,	43	6	–
32. Lake Suuri-Vahvanen	–	40	6	–
33. Lake Ylä-Säynätjärvi	21	33	6	–
34. Lake Hosusjärvi	4, 18, 29	60	6	–
35. Pond Mikkolanjärvi	25	56	8	–
36. Lake Köyliönjärvi	–	63	10	–
37. Lake Saimaa	11, 15	88	13	180 bp, 4%
38. Lake Linkulasjön*	–	53	6	216 bp, 19%

makes populations more vulnerable (Grandjean and Souty-Grosset, 2000). In addition, introductions by using small number of individuals can cause a strong bottleneck effect, and small populations are most likely to be affected by the loss of genetic variation due to the excessive harvest because of their small effective population size (Ryman *et al.*, 1995). Knowledge of genetic and geographical origin is a key for effective conservation and sustainable exploitation. The lack of knowledge on the genetic structure may lead to the genetic

contamination or homogenization of local populations (Largiadèr *et al.*, 2000; Gross *et al.*, 2017) and for the noble crayfish this has been already seen in other Baltic Sea area countries (Gross *et al.*, 2013; Dannewitz *et al.*, 2021; Gross *et al.*, 2021).

In our study, a total of 30 out of 38 analysed populations showed no genetic difference with one or up to 12 populations in paired comparison. These results support the previous findings, based on *COI*-gene haplotype variation (Makkonen *et al.*, 2015)

and microsatellites (Gross *et al.*, 2013; Dannewitz *et al.*, 2021), indicating, in general, that the genetic diversity of noble crayfish in its northern distribution range, including Finland, is remarkably narrow due to the homogenizing effect caused by past crayfish introduction policies.

Interestingly, we were able to detect a total of eight noble crayfish populations that had statistically significant difference with all other analysed populations indicating as least some level of remaining heterogeneity. This finding might be, depending on the location of the population, also an indication of an autochthonous origin or autochthonous donor. Nevertheless, due to the introduction policy in the past the determination of autochthonous origin is not always unambiguous. It should be noted that stocking histories in Finland are impossible to assess comprehensively, as noble crayfish are inhabiting thousands of waters, and millions of individuals have been introduced. Our results here, however, could be used as a base for future analyses.

Criteria for determination of an autochthonous origin suggested here, based on the background information and results of this study, are 1) population is locating within original distribution range *e.g.*, below the 62°N latitude (Järvi, 1910), 2) statistically significant genetic difference with other analysed populations and 3) history of the populations observed from fisheries authority and local fishermen reveals no anthropogenic disturbance, *e.g.*, introductions. The present data supports our preliminary findings (Alaranta *et al.*, 2006) indicating Lake Linkulasjön population being possible solely autochthonous or it was established from autochthonous donor population since its location within original distribution area. In addition, Lake Köyliönjärvi and Lake Suuri-Vahvanen populations are locating within original distribution area but with some known anthropogenic disturbance. In addition, River Perhonjoki, Lake Ahvenlampi, Lake Suuri-Heinäjärvi, Lake Koivujärvi, River Kasijoki populations are located outside of the original noble crayfish distribution range and therefore they are most likely established.

We were also able to detect variation within populations, as for example majority of Lake Saimaa individuals (88%) showed individual fragmentation and the population showed the highest number of detected fragments. This is possibly due to the effect of one or multiple stockings of the presence of subpopulations due to the size of the water area (Lake Saimaa, 4 400 km², one sampling site). In addition, Lake Ahvenlampi individuals were most homogenous: 83% of samples showed the identical fragmentation and the lowest number of detected fragments within population indicating a bottleneck effect as a possible consequence of establishing population with a low number of individuals from a single donor population. Individuals displayed distinctly different fragment patterns also within Swedish populations in previous analyses (Edsman *et al.*, 2002). Harris and Crandall (2000) found considerable intragenomic variation in the genera *Procambarus* and *Orconectes* (Faxonius), even to the extent that variation within individuals exceeded that between different species.

In this study local *e.g.*, private fragments, as described in Alaranta *et al.* (2006), were found from five populations with frequencies varying from 3% to 7%, except in Lake Linkulasjön, where local fragment frequency was 19%. Fragment of 214 bp (Lake Viitajärvi) was neither observed in other populations in Sweden nor in Estonia (Edsman *et al.*, 2002;

Alaranta *et al.*, 2006). The fragment of 180 bp present in Lake Saimaa has earlier been found in some Estonian samples (Alaranta *et al.*, 2006) and in one population from Montenegro (Edsman *et al.*, 2002), while 172 bp present in Lake Mäntyjärvi has been found in one Swedish and Estonian population, and 216 bp (Lake Linkulasjön) in one Estonian population (Alaranta *et al.*, 2006). Shared fragments are one indication of shared origin and sign of human-made translocation. The presence of local fragments is interesting, however, as seen here the existence is highly dependent of the number of populations compared.

Historical information proved to be extremely valuable when assessing the genetic data, however, it is usually lacking from the official records. According to our data, Lake Linkulasjön shows divergence in paired comparison, presents a local fragment and approximately 50% of the samples share the same fragmentation. Lake Linkulasjön is a privately owned small lake (60 ha) in South Finland (noble crayfish natural range) with good availability of historical information provided by the owner of the property. Based on the location and known history of Lake Linkulasjön as well as on the base of our previous (Alaranta *et al.*, 2006) and current results, we suggest that population in Lake Linkulasjön might be of an autochthonous origin. However, individuals from Lake Linkulasjön share a local fragment with Estonia samples (Alaranta *et al.*, 2006), and according to microsatellite analysis made by Gross *et al.* (2013) Lake Linkulasjön population tended to be genetically similar to Swedish populations rather than other analysed Finnish populations, indicating Swedish origin. Transfers of crayfish are known to have occurred across the Baltic Sea in both directions (Alm, 1929; Edsman, 2004). As a second example, established Lake Iso-Lauas population (Oksman and Lindqvist, 1977) shows no difference with 10 populations in paired comparison with geographical distant populations (distances varying from 20 km to 200 km). Results from Lake Iso-Lauas population indicate anthropogenetic effects due to the absence of local fragments and prominent level of individual fragmentation (63% of samples). According to local fishermen, prior crayfish plague outbreaks in 1996 and 2000 (Mannonen *et al.*, 2006), Lake Iso-Lauas had produced a massive number of crayfish not reaching the consumable size of >10 cm (total length). Best catchments, with no consumable value, were up to 1 000 crayfish/day/fishermen. Therefore tens of thousands of small sized crayfish were sold for stocking; however, this data (Lake Iso-Lauas population acting as a donor) is totally lacking from the official stocking records (Korhonen, 2010).

Most common method of managing crayfish population has been, and still is, stockings: introduction to new areas, reintroductions to the areas where they have become extinct and restocking to boost existing populations. As perspective in the species level conservation, the creation of large-scale conservation areas has been discussed in Scandinavian countries (Mannonen and Westman, 1998; Mannonen *et al.*, 2000; Edsman and Schröder, 2009) and launched also in Finland (Ministry of Agriculture and Forestry, 2014; Erkamo *et al.*, 2019; Ruokonen *et al.*, 2023). However, the current protection area in Finland is mainly outside of the original distribution range (Ruokonen *et al.*, 2023), and the conservation efforts are focused now more on species, not on genetically distinct populations. In Europe, among the

crucial for interpreting genetic data accurately. Secondly, due to the lack of information of the occurrence of the species, especially in the small size water bodies and subpopulations in large water bodies, monitoring programs within original range, designated protection area, and natural parks is highly recommended. The new genetic tools, *e.g.*, eDNA applications (see Beng and Corlett, 2020; King *et al.*, 2022), have shown potential in monitoring of aquatic systems in providing precise information about distribution and population size (Bohmann *et al.*, 2014; Takahara *et al.*, 2013). These tools have been already tested in Finnish conditions with promising results (Mäkinen *et al.*, 2021). In addition, combinations of methods *e.g.*, fine scale phylogenetics, population genetics with novel microsatellites and species distribution modelling (Lovrenčić *et al.*, 2022) might be possibly used to assist the conservation actions needed. As a perspective of species conservation, preserving genetic variation and biodiversity is crucial for the evolutionary potential and sustainable exploitation of noble crayfish. Despite advancements in genetic analysis and genotyping methods, caution is needed due to potential risks in data interpretation (Selkoe and Toonen, 2006). Utilizing modern crayfish-specific population models is also recommended (Koivu-Jolma *et al.*, 2023). To secure the evolutionary potential of noble crayfish stocks in Fennoscandia, it is essential to preserve genetic variation and biodiversity, in collaboration and involvement with all the national stakeholders. This would provide a solid base for effective conservation and potentially sustainable exploitation for the future decades.

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References

- Alaranta A, Henttonen P, Jussila J, Kokko H, Prestegard T, Edsman L, Halmekytö M. 2006. Genetic differences between noble crayfish (*Astacus astacus*) stocks in Finland, Sweden and Estonia based on ITS region. *B Fr Peche Piscic* 380–381: 965–976.
- Alaranta A, Laakkonen M, Partanen T, Sarajärvi K, Hupli H, Ryyppö P, Korhonen P. 2010. The conservation and management plan of noble crayfish (*Astacus astacus*) in state –owned areas in Finland. *European Crayfish: food, flagships and ecosystem services*. 26–29 October 2010, Poitiers, France –Conference Proceedings. Abstract, p. 30.
- Alaranta A, Jussila J, Kokko H. 2011a. Inheritance of ITS1 region microsatellite-like repeats in the noble crayfish, *Astacus astacus* (Decapoda, Astacidea). *Crustaceana* 84: 1325–1336.
- Alaranta A, Laakkonen M, Partanen T, Sarajärvi K, Hupli H, Ryyppö P, Korhonen P. 2011b. Metsähallituksen raputalouden toimintaohjelma 2011–2016. *Metsähallituksen luonnonsuojelujulkaisuja*. Sarja C 113. In Finnish.
- Alm G. 1929. Der Krebs und die Krebspest in Schweden. *Zeitschrift für Fisherei* 27: 123–138. In German.
- Beng KC, Corlett RT. 2020. Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodivers Conserv* 29: 2089–2121.
- Bohmann K, Evans A, Gilbert MTP, Carvalho GR, Creer S, Knapp M, Yu DW, de Bruyn M. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol Evol* 29: 358–367.
- Coates DJ, Byrne M, Moritz C. 2018. Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. *Front Ecol Evol* 6: 165.
- Dannewitz J, Palm S, Edsman L. 2021. 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. *Aquat Conserv: Mar Freshw Ecosyst* 31: 1927–2303.
- Dowling TE, Childs MR. 1992. Impact of hybridization on a threatened trout of the southwestern United States. *Conserv Biol* 6: 355–364.
- Edsman L. 2004. The Swedish story about import of live crayfish. *Bull Fr Pêche Piscic* 372–373: 281–288.
- Edsman L, Farris JS, Källersjö M, Prestegard T. 2002. Genetic differentiation between noble crayfish, *Astacus astacus* (L.), populations detected by microsatellite length variation in the rDNA ITS1 region. *B Fr Peche Piscic* 367: 691–706.
- Edsman L, Füreder L, Gherardi F, Souty-Grosset C. 2010. *Astacus astacus*. *The IUCN Red List of Threatened Species 2010*: e.T2191 A9338388. <https://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T2191 A9338388>. en. Accessed on 28 October 2023.
- Erkamo E, Tulonen J, Kirjavainen J. 2019. Kansallinen rapustrategia 2019–2022. *Maa- ja metsätalousministeriön julkaisuja* 4. In Finnish.
- Edsman L, Schröder S. 2009. Action plan for the noble crayfish (*Astacus astacus*), 2008–2013. *Swedish Environmental Protection Agency*, report 5955.
- Grandjean F, Souty-Grosset C. 2000. Mitochondrial DNA variation and population genetic structure of the white clawed crayfish, *Austropotamobius pallipes pallipes*. *Conserv Genet* 1: 309–319.
- Gross R, Kõiv K, Pukk L, Kaldre K. 2017. Development and characterization of novel tetranucleotide microsatellite markers in the noble crayfish (*Astacus astacus*) suitable for highly multiplexing and for detecting hybrids between the noble crayfish and narrow-clawed crayfish (*A. leptodactylus*). *Aquaculture* 472: 50–56.
- Gross R, Lovrenčić L, Jelić M, Grandjean F, Ćuretanić S, Simić V, Burimski O, Bonassin L, Groza MI, Maguire I. 2021. Genetic diversity and structure of the noble crayfish populations in the Balkan Peninsula revealed by mitochondrial and microsatellite DNA markers. *PeerJ* 9: e11838.
- Gross R, Palm S, Kõiv K, Prestegard T, Jussila J, Paaver T, Geist J, Kokko H, Karjalainen A, Edsman L. 2013. Microsatellite markers reveal clear geographic structuring among endangered noble crayfish (*Astacus astacus*) populations in Northern and Central Europe. *Conserv Genet* 14: 809–821.

- Harris DJ, Crandall KA. 2000. Intragenomic variation within ITS1 and ITS2 of freshwater crayfishes (Decapoda: Cambaridae): implications for phylogenetic and microsatellite studies. *Mol Biol Evol* 17: 284–291.
- Helle L. 1904. Vähän jokiäyriäisen (*Astacus fluviatilis*) esiintymisestä Suomessa. *Luonnon Ystävä* 8: 168–170. In Finnish.
- Holdich DM, Sibley P, Peay S. 2004. The white-clawed crayfish – a decade on. *Br Wildl* 15: 153–164.
- Horton MP. 2009. Establishing the island of Ireland’s first ark site for the white-clawed crayfish *Austropotamobius pallipes* in the Ballinderry river system, Co. Tyrone. In Brickland J, Holdich DM, Imhoff EM, eds. Crayfish Conservation in the British Isles, Proceedings of a Conference Held in Leeds pp. 71–84.
- Hyvärinen E, Juslén A, Kempainen E, Uddström A, Liukko U-M (eds.). 2019. The 2019 Red list of finnish species. Helsinki, Finland: Ympäristöministeriö & Suomen ympäristökeskus.
- Ignatius KEF. 1885. Suomen maantiede kansalaisille. *Ensimmäinen osa. Toinen vihko. Helsinki.* p. 251, 423. In Finnish.
- Jussila J, Edsman L. 2020. Relaxed attitude towards spreading of alien crayfish species affects protection of native crayfish species: case studies and lessons learnt from a Fennoscandian viewpoint. *Freshw Crayfish* 25: 39–46.
- Jussila J, Edsman L, Maguire I, Diéguez-Urbeondo J, Theissing K. 2021. Money kills native ecosystems: European crayfish as an example. *Front Ecol Evol* 9: 648495.
- Jussila J, Mannonen A. 2004. Crayfisheries in Finland, a short overview. *B Fr Peche Piscic* 372–373: 263–273.
- Järvi TH. 1910. Ravusta ja rapukulkutaudeista Suomessa. *Luonnon ystävä* 14: 42–53. In Finnish.
- King AC, Krieg R, Weston A, Zenker AK. 2022. Using eDNA to simultaneously detect the distribution of native and invasive crayfish within an entire country. *J Environ Manage* 302: 113929.
- Kirjavainen J. 1989. Täplärapu 2000. Ehdotus Suomen täplärapustrategiaksi. Keski-Suomen kalastuspiiri. *Tiedotus nro* 6: 5–23. In Finnish.
- Koivu-Jolma M, Kortet R, Vainikka A, Kaitala V. 2023. Crayfish population size under different routes of pathogen transmission. *Ecol Evol* 13: e9647.
- Korhonen L. 2010. Jokiravun (*Astacus astacus*) levinneisyydestä ja istutuskäytännöistä Suomessa. Thesis, *Turku University of Applied Sciences. Kala- ja ympäristötalous.* 46 p. In Finnish.
- Largiadèr CR, Heger F, Lörtscher M, Scholl A. 2000. Assessment of natural and artificial propagation of the white-clawed crayfish (*Austropotamobius pallipes* species complex) in the Alpine region with nuclear and mitochondrial markers. *Mol Ecol* 9: 25–37.
- Lovrenčić L, Temunović M, Gross R, Grgurev M, Maguire I. 2022. Integrating population genetics and species distribution modelling to guide conservation of the noble crayfish, *Astacus astacus*, in Croatia. *Sci Rep* 12: 2040.
- 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. *Knowl Manag Aquat Ecosyst* 416: 21.
- Mannonen A, Halonen T, Nylund V, Westman K, Westman P. 2006. Raputaurekisteri. Raputauren esiintyminen Suomessa 1893–2000. *Maa- ja metsätalousministeriö.* 44 p. In Finnish.
- Mannonen A, Halonen T, TE-keskusten työryhmä. 2000. Kalataloushallinnon rapustrategia. Maa- ja metsätalousministeriö, Hämeenlinna. 44 p. In Finnish.
- Mannonen A, Westman K. 1998. Crayfish situation in Finland. In Taugbøl T, ed. Nordic-Baltic workshop on freshwater crayfish research and management, May 22–26, 1998, Sagadi Training Centre, Estonia. *üstlandforskning, ùF-Rapport nr. 26/1998,* 29–38.
- Ministry of Agriculture and Forestry. 2014. Kansallinen rapustrategia 2013–2022. Maa- ja metsätalousministeriö 6/2014. In Finnish.
- Mäkinen M, Jussila J, Sjövik R, Ventelä E, Kokko H, Pesonen A, Ruokonen TJ. 2021. Monitoring of noble crayfish and signal crayfish using eDNA. *Proceedings of the department of biological and environmental science, University of Jyväskylä,* 27 p.
- Nylander W. 1859. Strödda anteckningar (On kräftans utbredning in Finland). *Not Sallsk Fauna Fl Fenn Forh* 4: 248–248. In Swedish.
- Oksman H, Lindqvist OV. 1977. *Kuopion läänin raputaloustutkimus 1976–1977.* In Finnish.
- Paaver T, Hurt M. 2009. Status and management of noble crayfish *Astacus astacus* in Estonia. *Knowl Managt Aquatic Ecosyst* 294–395: 18.
- Peay S. 2009. Selection criteria for “ark-sites” for white-clawed crayfish. In Brickland J, Holdich DM Imhoff EM, eds. Crayfish Conservation in the British Isles, Proceedings of a Conference Held in Leeds, pp. 63–69.
- Pursiainen M. 2012. Jokiravun ja täplärapun levinneisyys (‘Distribution of the noble and signal crayfish’) In Pursiainen M, Mattila J, eds. Crayfish distribution and production in Finland in 2010. *Riista- ja kalatalous – Tutkimuksia ja selvityksiä/2010:* 7–9.
- Pursiainen M, Rajala J. (eds.). 2009. Crayfish Review 2008. *Riista ja kalatalous –Selvityksiä 5/2009,* 52 p. In Finnish, English abstract.
- Reynolds J, Souty-Grosset C. 2012. Management of freshwater biodiversity: crayfish as bioindicators. *Cambridge University Press,* 374 p.
- Ruokonen T, Erkamo E, Jussila J, Kirjavainen J. 2023. Kansallinen rapustrategia 2023–2032. *Maa- ja metsätalousministeriön julkaisuja 2023:20.* In Finnish.
- Ruokonen TJ, Sjövik 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. *Knowl Manag Aquat Ecosyst* 419: 27.
- Ryman N, Utter F, Laikre L. 1995. Protection of intraspecific biodiversity of exploited fish. *Rev Fish Biol Fish* 5: 417–446.
- Schrimpf A, Schulz HK, Theissing K, Pärvolescu L, Schulz, R. 2011. The first large-scale genetic analysis of the vulnerable noble crayfish *Astacus astacus* reveals low haplotype diversity in central European populations. *Knowl Manag Aquatic Ecosyst* 401: 1–35.
- Schrimpf A, Theissing K, Dahlem J, Maguire I, Pärvolescu L, Schultz H, Schulz R. 2014. Phylogeography of noble crayfish (*Astacus astacus*) reveals multiple refugia. *Freshw Biol* 59: 761–776.
- Selkoe KA, Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol Lett* 29: 615–629.
- Sibley P, Clarkson M, Frayling M, Stenson C. 2007. Translocating the white-clawed crayfish *Austropotamobius pallipes*. In: Hickley P, Axford S. (eds.), Fisheries and Conservation Successes and Failures, Institute of Fisheries Management Conference Proceedings, 42–51.
- Souty-Grosset C, Grandjean F, Raimond R, Frelon M, Debenest C, Bramard M. 1997. Conservation genetics of the white-clawed crayfish *Austropotamobius pallipes*: the usefulness of the mitochondrial DNA marker. *B Fr Peche Piscic* 347: 677–692.
- Souty-Grosset C, Holdich DM, Noël PY, Reynolds JD, Haffner P. 2006. Atlas of Crayfish in Europe. *Patrimoines naturels,* 187 pp. Muséum national d’Histoire naturelle, Paris.

- Souty-Grosset C, Reynolds JD. 2009. Current ideas of methodological approaches in European crayfish conservation and restocking procedures. *Knowl Manag Aquatic Ecosyst* 394–395: 01.
- Takahara T, Minamoto T, Doi H. 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS One* 8: 1–5.
- Vrijenhoek RC, Douglas ME, Meffe GK. 1985. Conservation genetics of endangered fish populations in Arizona. *Science* 229: 400–402.
- Weiss S, Persat H, Epe R, Schlötterer C, Uiblein F. 2002. Complex patterns of colonization and refugia revealed for European grayling *Thymallus thymallus*, based on complete sequencing of the mitochondrial DNA control region. *Mol Ecol* 11: 1139–1407.
- Westman K. 1973. The population of crayfish *Astacus astacus* L. in Finland and the introduction of the American crayfish *Pacifastacus leniusculus* Dana. *Freshw Crayfish* 1: 41–55.
- Westman K. 1991. The crayfish fishery in Finland its past, present and future. *Finn Fish Res* 12: 187–216.

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