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1 **Mixed Stock Assessment of Lake-run Caspian Sea Trout *Salmo caspius* in the Lar National Park,**
2 **Iran**

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11

12 **Running title:** Mixed Stock assessment of *Salmo caspius*

13

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22

23 **Abstract**

24 A total of 120 Caspian Sea trout specimens from five streams and the Lar Lake of the Lar National Park
25 plus 27 Caspian Sea trout specimens (out-group) from the Babolrud River drainage in the Caspian Sea
26 Basin (Iran) were analyzed using 12 microsatellite loci to assess population genetic structure and to
27 estimate the contribution of each population to the lake-run stock. In addition to the Babolrud River
28 population that was significantly differentiated ($P<0.001$) from the populations in the Lar National Park,
29 the Dalichay population was significantly differentiated from other populations ($P<0.001$). The Dalichay
30 and Absefid populations showed no contribution to the lake-run trout stock, while the others that did not
31 show population genetic structure showed a 100% contribution to the lake-run trout stock. The different
32 contributions to the lake-run trout stock estimated here may be related to the habitat qualities that make
33 the habitats suitable for natural Caspian Sea trout production or to different migratory behaviors between
34 resident and lake-run trout. The results show that more stringent protection of the stream habitats for the
35 highly contributing populations should be considered in order to sustain recreational fishing in the lake.

36 **Keywords:** Recreational fishery management, Southern Caspian Sea basin, *Salmo caspius*, Mixed-stock
37 analysis, Conservation

38 **1. Introduction**

39 *Salmo trutta*, commonly known as brown trout, have a wide native distribution ranging from Iceland in
40 the most westerly reaches to the tributaries of the rivers draining to the Aral Sea in the east, and from the
41 northernmost rivers of Finland to the Atlas Mountains of North-West Africa at the southern edges of their
42 distribution (Bernatchez, 2001). In Iran, there are three basins hosting native trout populations, namely the
43 Caspian Sea, the Uromia Salt Lake, and the Namak Lake basins (Hashemzadeh Segherloo et al., 2012).

44 Caspian Sea trout, *Salmo caspius* Kessler, 1877 is restricted to the southern Caspian Sea basin (Kottelat
45 and Freyhof, 2007). Its populations are locally vulnerable, and they have been exposed to restocking
46 activities since 1983 (Abdoli, 2000; Kiabi et al., 1999; Niksirat and Abdoli, 2009). The main threats to the

47 sustainability of the species in Iran are dam construction, pollution, rainbow trout *Oncorhynchus mykiss*
48 (Walbaum 1792) aquaculture, habitat degradation, and overfishing (Hashemzadeh Segherloo et al., 2012).

49 In the Southern Caspian Sea basin, the Lar National Park system -including the Lar Lake, Dalichay,
50 Absefid, Elarm, Kamardasht, and other tributaries of the Lar River drainage- provides a unique habitat for
51 the Caspian Sea trout (Esteve et al., 2017). The Lar National Park is located in the north of Iran between
52 Tehran and Mazandaran Provinces. Since the water resources of the park are exploited to supply drinking
53 water for Tehran city, and due to the protected nature of the park, no industrial or agro-aquaculture
54 activities are permitted in the park area. For these reasons, habitat quality is generally higher than in other
55 parts of the southern Caspian Sea basin, which is beneficial for wildlife and especially the Caspian Sea
56 trout populations. The Caspian Sea trout populations of the park have not been subjected to restocking
57 activities. Both resident and lake-run forms of Caspian Sea trout are observed in the Lar National Park
58 region. The lake-run trout migrate to the lake as juveniles and return to the natal streams as adults for
59 spawning (Esteve et al. 2017). Lake-run individuals are phenotypically distinguishable from resident
60 individuals. Lake-run individuals usually are larger in size with fewer red spots, darker coloration, and
61 more frequent star-like black spots. Until recently, every year during the summer, a number of permits
62 (~20,000 permits with a maximum of five fish allowed by each permit) for angling in the Lar Lake were
63 issued by the Iranian Environment Organization. As the lake-run Trout landings showed a decreasing
64 trend, fishing in the National Park has been stopped by the Department of Environment since a few years
65 ago. Other anthropogenic activities that go on every summer include the settlement of temporary residents
66 and tourist camping in the park. They usually settle near the streams and their activities may negatively
67 affect the quality of the stream habitats that are the main spawning grounds of the migratory and resident
68 fish.

69 The rivers in Lar National Park are different in their size and physico-chemical properties (Sedighkia et
70 al., 2018) and their populations may provide different contributions to the lake-run trout stock and related
71 recreational fishing, but this has never been assessed before. Genetic Mixed Stock Assessment (MSA) can

72 be helpful in identifying the most important habitats contributing to the lake trout fishery stock and can
73 provide the basis for conservation of the trout and related fisheries management (Carlsson and Nilsson,
74 2000; Carlsson et al., 1999; Mäkinen et al., 2015). We therefore used MSA to assess the contribution of
75 each stream population to the lake-run trout using microsatellite DNA loci and field data records, with a
76 view to identify and prioritize the populations and their habitats for conservation.

77 **2. Materials and Methods**

78 *Salmo caspius* (N=120) were collected in 2009 by electrofishing in five rivers and in Lar Lake (Table 1;
79 Fig. 1). The numbers collected were restricted by the availability of fish (Table 1). Electrofishing was
80 performed in a length of the rivers over 100 meters to avoid the problem of family sampling (Hansen et
81 al., 1997). The pectoral fin of the fish was clipped and fixed in 96% ethanol. During sampling, hydro-
82 chemical parameters were recorded using a HACH portable water metre (Table 1). In addition, 27
83 individuals from Babolrud River drainage in the southern Caspian Sea basin were also included as an out-
84 group.

85 Total genomic DNA was extracted from the fin clips using a salt extraction method (Aljanabi and
86 Martinez, 1997). Samples were genotyped for a total of 12 dinucleotide microsatellite loci (Hashemzadeh
87 Segherloo et al., 2012). The samples were genotyped using single PCR reactions optimised for each
88 primer set. Four different fluorescent dyes were used, and unlabelled primers had a GTTT tail added to
89 their 5' end to promote the adenylation of the nascent DNA strand to facilitate accurate genotyping
90 (Brownstein et al., 1996). The primers for the loci Str15INRA, Str543INRA, Str85INRA, and Strutta58
91 were re-designed to overcome allele size overlaps (Swatdipong, 2009). The single PCR reactions (10 µl)
92 consisted 70-80 ng of template DNA, 0.5 pM of both primers, 1X PCR buffer, 1.5 mM MgCl₂, 0.25 mM
93 dNTPs and 0.1 U BioTaq DNA polymerase (Bioline GmbH). Amplifications were carried out in a
94 PTC100 thermal cycler (MJ Research), a 2720 Thermal Cycler (Thermo Fisher Scientific) or a Piko
95 thermal cycler (Thermo Fisher Scientific). PCR amplifications of all microsatellite loci, except

96 Str15INRA, Str543INRA, Str85INRA and Strutta58, were run under the following thermal conditions: 3
97 min at 94°C; followed by 30 cycles of 30 sec at 94°C, 30 sec at an annealing temperature specific to each
98 primer, and 30 sec at 72°C; and, finally, 5 min at 72°C. For the other loci, the conditions were 3 min at
99 94°C; followed by 30 cycles of 30 sec at 94°C, 30 sec at the annealing temperature, and 1 min at 72°C;
100 and, finally, 10 min at 72°C (for more details see Hashemzadeh Segherloo et al. (2012)).

101 For all samples, the amplicons were diluted and denatured, then subjected to electrophoresis on an ABI
102 Prism 3130X1 genetic analyser (Thermo Fisher Scientific), along with a GeneScan 600 LIZ size standard
103 (Thermo Fisher Scientific). The sizes of the DNA fragments were called using GeneMapper 4.0 (Thermo
104 Fisher Scientific), and all genotypes were manually inspected.

105 Exact probability tests for deviations from Hardy-Weinberg equilibrium (HWE) across populations
106 (within loci) and loci (within populations) and exact tests for deviations from genotypic linkage
107 equilibrium (LE) across populations were performed using GenePop 4.0.10 (Rousset, 2008).

108 Inter-population genetic divergence estimates (F_{ST} and Jost's estimate of differentiation (D_{est})) were
109 calculated using GenAlex 6.5 (Peakall and Smouse, 2012). P -values for genetic differentiation were
110 calculated using 999 permutations. Analysis of molecular variances (AMOVA) was performed using
111 Arlequin 3.5 (Excoffier and Lischer, 2010). To clarify clustering pattern existing among the specimens,
112 STRUCTURE V. 2.3.4 (Pritchard et al., 2000) was ran using admixture model for $K=1-10$ clusters. The
113 model run was repeated five times for each K value. The pre-burn-in and MC runs were 10,000 and
114 100,000, respectively. The results of the structure analysis were analysed using online Structure Harvester
115 available at <http://taylor0.biology.ucla.edu> (Earl and vonHoldt, 2012). The most appropriate K was
116 selected based on the mean ln probability of data, genetic differentiation values, the results from
117 individual assignment tests, and the geographic features of the studied area.

118 To assess the possible contribution of each stream population to the lake-run trout, we used stream
119 specimens as the baseline and the lake-run specimens as mixture and assigned the mixture to stream

120 samples using the maximum likelihood method implemented in the program Oncor (Kalinowski et al.,
121 2007) and a Bayesian method (Rannala and Mountain, 1997) implemented in GeneClass 2.0 (Piry et al.,
122 2004). For MSA using the maximum likelihood method of Oncor, 1,000 bootstrap replicates were used.
123 To compute the probabilities of membership in the Bayesian method the simulation algorithm of Paetkau
124 et al. (2004) with 1,000 simulated individuals and a type I error (alpha) of 0.01 was used. In cases where
125 particular populations were not differentiated from one another, we also pooled them and performed MSA
126 considering them as a single population.

127 **3. Results**

128 Among the studied localities in the Lar National Park, Dalichay, and Absefid streams were similar in
129 electro-conductivity (EC) and salinity measurements, compared to other streams. Other localities were
130 similar to one another with respect to the measured parameters (Table 1).

131 All loci were found to be in H-W equilibrium ($P \geq 0.05$). The F_{ST} and D_{EST} values showed no significant
132 levels of differentiation among the Caspian trout populations from different streams in the Lar National
133 Park with the exception of the Dalichay Stream population that was significantly differentiated from other
134 populations ($P < 0.001$). The population from the Babolrud River in a southern Caspian Sea river drainage
135 was also significantly differentiated from samples belonging to the Lar National Park ($P < 0.001$; Table 2).
136 Based on analysis of molecular variances (AMOVA), the genetic variation estimates among groups,
137 populations, individuals within populations, and within individuals, were 61.12% ($P < 0.001$), 3.66%
138 ($P < 0.001$), 1.07% ($P > 0.05$), and 34.15% ($P < 0.001$), respectively (Table 3). Based on the criteria we
139 considered to select the most appropriate number of clusters (K) among the studied populations, $K = 4$
140 was selected as an appropriate measure of the clustering pattern. At $K > 1$ in all cases the Babolrud
141 population had its own cluster and at $K > 3$ a second cluster appeared in three individuals in the Babolrud
142 sample set (Fig., 2 top panel). Population structure analysis for $K > 2$ revealed that trout population
143 inhabiting the Dalichay Stream belonged to a different cluster (Figs. 2 a and b). In the mixed-stock

144 analysis via both the Maximum Likelihood and the Bayesian approaches, all lake-run individuals were
145 assigned to the pooled sample of undifferentiated populations (Kamardasht, Elarm, and Khoshkehrood
146 streams), but not to the Babolrud River or the Dalichay and Absefid streams (Table 4).

147 **4. Discussion**

148 Our results revealed significant genetic differentiation between the Dalichay population and all other
149 populations analysed in the Lar National Park and also between the Babolrud population relative to the
150 populations in the national park. In the case of Babolrud population the existing differences is expected,
151 since this population is geographically isolated from populations in the Lar River drainage. The
152 population structure observed in the Lar National Park might be related to the artificial barrier blocking
153 the trout from migrating to Dalichay and vice versa. Field observations revealed that there was an
154 artificial barrier (wire mesh and a very steep stream reach) in the river constructed around 30-50 years
155 (10-17 trout generations) before sampling date (June-2009) that may block the gene flow between
156 Dalichay Stream and other rivers and the lake. As no lake-run fish were assigned to the Delichay Stream,
157 it may indicate that there is no, or very rare, downstream migration from Dalichay Stream. This is further
158 supported by the highly significant genetic differentiation measures and the population structure observed.
159 In contrast, using morphological analysis of otolith and scale, Rashidabadi et al. (2019) could not clearly
160 delimit Dalichay population from other populations. Our observation parallels the results presented in
161 Pettersson et al. (2001). In their study on the Brown trout *Salmo trutta* populations in a stream on the
162 Swedish west coast, they observed that no/insignificant migration occurred from upstream of a waterfall
163 and downstream. Because the barrier does not block migration from Dalichay to the lake, no contribution
164 of this population to the lake-run stock may also be related to limited movements of resident trout (Olsson
165 and Greenberg, 2004; Pettersson et al., 2001; Vøllestad et al., 2012). Among other streams, the Absefid
166 Stream population, although not highly diverged, also had no apparent contribution to the lake-run trout
167 stock. This may be a result of the small sample size collected from the Absefid Stream, which may have
168 an adverse effect on MSA analysis (Anderson et al., 2008). Further, downstream of the sampled locality

169 in the Absefid stream, sulphorus springs discharge into the stream and the high turbidity of the water may
170 result in this section of the stream being less suitable, or less attractive, for trout (Sedighkia et al., 2018).
171 The Absefid population was not significantly differentiated from other populations; however this may be
172 due to low sample size, as the estimated F_{ST} values were higher. Analysis of a higher number of
173 individuals would be beneficial to resolve this issue. The Elarm Stream, which was one of the three
174 streams (pooled sample of undifferentiated populations) that were shown to have the highest contribution
175 to the lake-run stock also had been reported to be a highly suitable habitat regarding ecological conditions
176 for the Caspian Sea trout (Sedighkia et al., 2018), which is concordant with our results. Based on our
177 results the western streams, including the Kamardasht and Elarm Streams, are the habitats with significant
178 contributions to the lake-run Caspian Sea trout fishing activities. Further, due to its divergence and
179 artificial isolation, the eastern Dalichay Stream population should be considered for separate
180 management. Hence, we propose to prioritize the western habitats for more intense protection against
181 anthropogenic activities that may lead to spawning habitat deterioration. In the case of the Dalichay
182 population we propose removing the barrier to restore the natural connection between this habitat and the
183 lake for possible resumption of the natural gene flow and contribution of this stream habitat to the lake-
184 run trout stock. As the Dalichay Stream is one of the largest streams in the Lar National Park, restoration
185 of its connectivity to the lake may be an effective management measure in sustainability of fishing in the
186 national Park via its possible contribution. More detailed and robust conclusions about population
187 structure and mixed-stock assessment in the Lar National Park would be possible by analysis of more
188 specimens from all localities in the National Park.

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290 **FIGURE CAPTIONS**

291 **FIG. 1.** Map showing the location of the Lar River in the Caspian Sea basin (Top panel) and sampling
292 localities in the Lar National Park (Bottom panel): Red circles denote sampling localities. Black circle in
293 the top panel denote the locality of specimens from Babolrud (out-group).

294 **FIG. 2.** Population structure at $K=4$ clusters in the Lar National Park. a) Structure graph with the
295 Babolrud population (out-group), b) pie graphs showing average percentage of each structure cluster in
296 each locality in the Lar National Park. Green colour denotes Dalichay cluster and red colour denotes the
297 common cluster observed in other streams and in the lake-run fish. Structure clusters revealed in Babolrud
298 River are not mapped as pie graphs. In all pie graphs the percentages of clusters from the Lar National
299 Park sum up to 99% and the remaining 1% was attributed to other sources that we have not presented
300 them beside the pie graphs.

301 **TABLES**302 **Table 1.** Sampling data and environmental parameters

Locality	Locality		N	River characters			Conductivity ($\mu\text{s}/\text{cm}$) ($m\pm\text{SD}$)	TDS (mg/litre) ($m\pm\text{SD}$)	Salinity (ppt)($m\pm\text{SD}$)	T (C) ($m\pm\text{SD}$)	DO (mg/lit)
	Lat	Long		Elevation (m)	Width (m)	Depth (cm)					
Absefid	35° 57' 21.9" N	51° 55' 12.7" E	5	2684	4.35	23.60	509±83	254±41	0.023±0.005	9.1±4.00	7.20
Elarm	35° 55' 22.8" N	51° 52' 10.2" E	28	2537	4.70	26.20	281±8	140±4	0.01±0.000	9.9±2.06	9.80
Kamardasht	35° 54' 23.5" N	51° 49' 32.3" E	19	2564	12.95	30.78	253±21	125±12	0.01±0.000	13.5±4.80	8.70
Dalichay	35° 55' 19.8" N	51° 59' 37.7" E	16	2560	8.64	22.70	493±87	246±43	0.024±0.005	8.3±4.50	7.26
Khoshkehrud	35° 53' 48.2" E	51° 49' 15.5" E	12	2947	-	-	-	-	-	-	-
Lar Lake	-	-	40	-	-	-	276±35	138±17	0.012	14.4±4.70	7.08
Babolrud	36°13'01.3"N	36°13'01.3"N	27	-	-	-	-	-	-	-	-

303 N, Sample size; TDS, Total dissolved solids; T, Temperature; DO, Dissolved oxygen.

304 **Table 2.** Pairwise F_{ST} (lower diagonal) and D_{EST} (upper diagonal) values for the analyzed populations.

Population	Babolrud	Absefid	Elarm	Kamardasht	Dalichay	Khoshkehrud
Babolrud	-	0.897	0.912	0.935	0.879	0.912
Absefid	0.597	-	0.000	0.008	0.104	0.000
Elarm	0.566	0.026	-	0.003	0.137	0.000
Kamardasht	0.578	0.035	0.014	-	0.186	0.000
Dalichay	0.602	0.111	0.110	0.144	-	0.124
Khoshkehrud	0.564	0.036	0.009	0.018	0.107	-

305 Bold F_{ST} and D_{EST} values are significant at $P < 0.001$.

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315 **Table 3.** Analysis of molecular variances (AMOVA) in studied populations

Source of variation	Sum of squares	Variance components	Percentage of variation	<i>F</i> -statistics
Among groups	211.249	2.649	61.12	$F_{CT} = 0.61^*$
Among populations within groups	24.082	0.158	3.66	$F_{SC} = 0.09^*$
Among individuals within populations	145.560	0.046	1.07	$F_{IS} = 0.03$
Within individuals	145.500	1.480	34.15	$F_{IT} = 0.66^*$
Total	526.391	3.017	100.00	

316 .* Significant at $P < 0.001$.

317 **Table 4.** Assignment analysis of lake-run fish to pooled sample of populations with non-significant differentiation, indicating individual fish
 318 origin

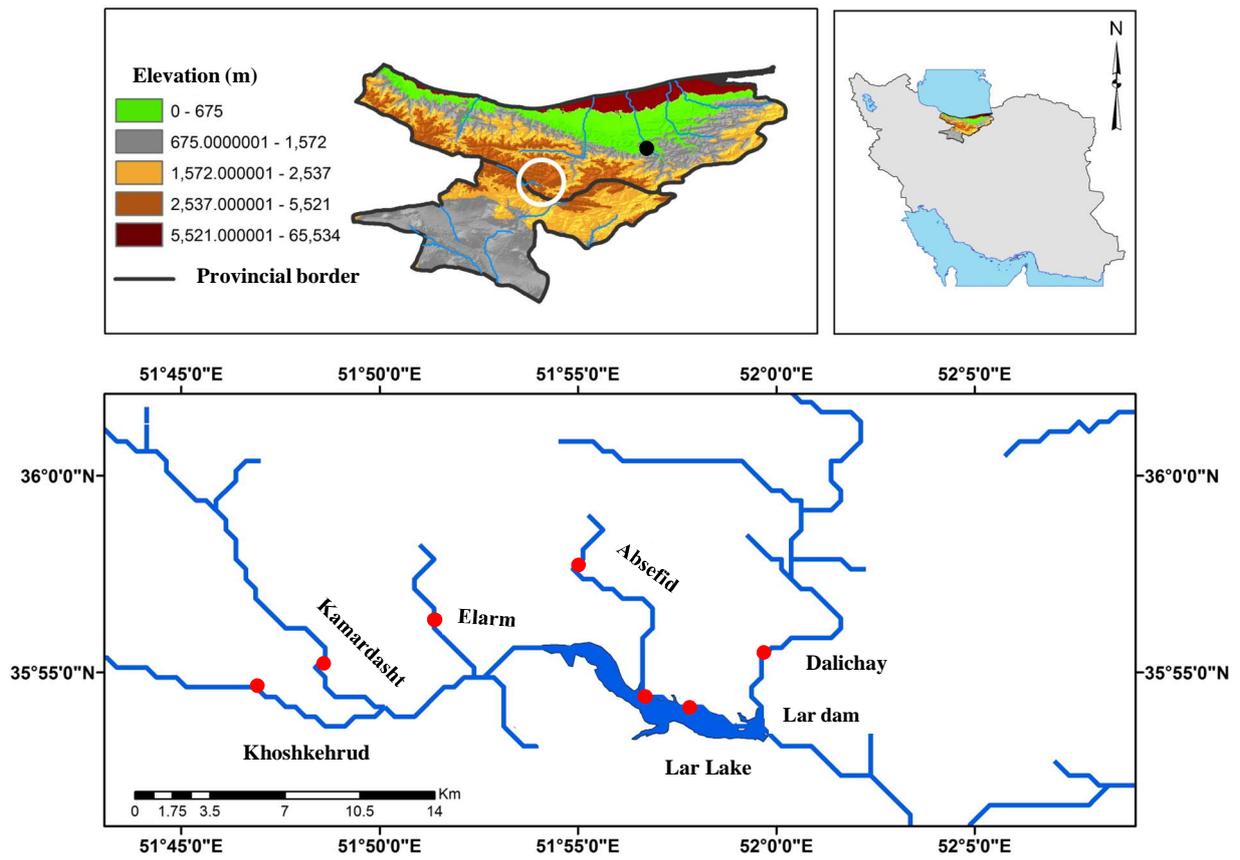
Stream	Maximum Likelihood		Bayesian
	Proportion of lake-run fish assigned to each pop.	95% CI	Proportion of lake-run fish assigned to each pop.
Babolrud	0.00	0.000-0.000	0.00
Pooled sample	1.00	0.537-1.000	1.00
Absefid	0.00	0.000-0.463	0.00
Dalichay	0.00	0.000-0.000	0.00

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321 FIGURS

322 FIG. 1



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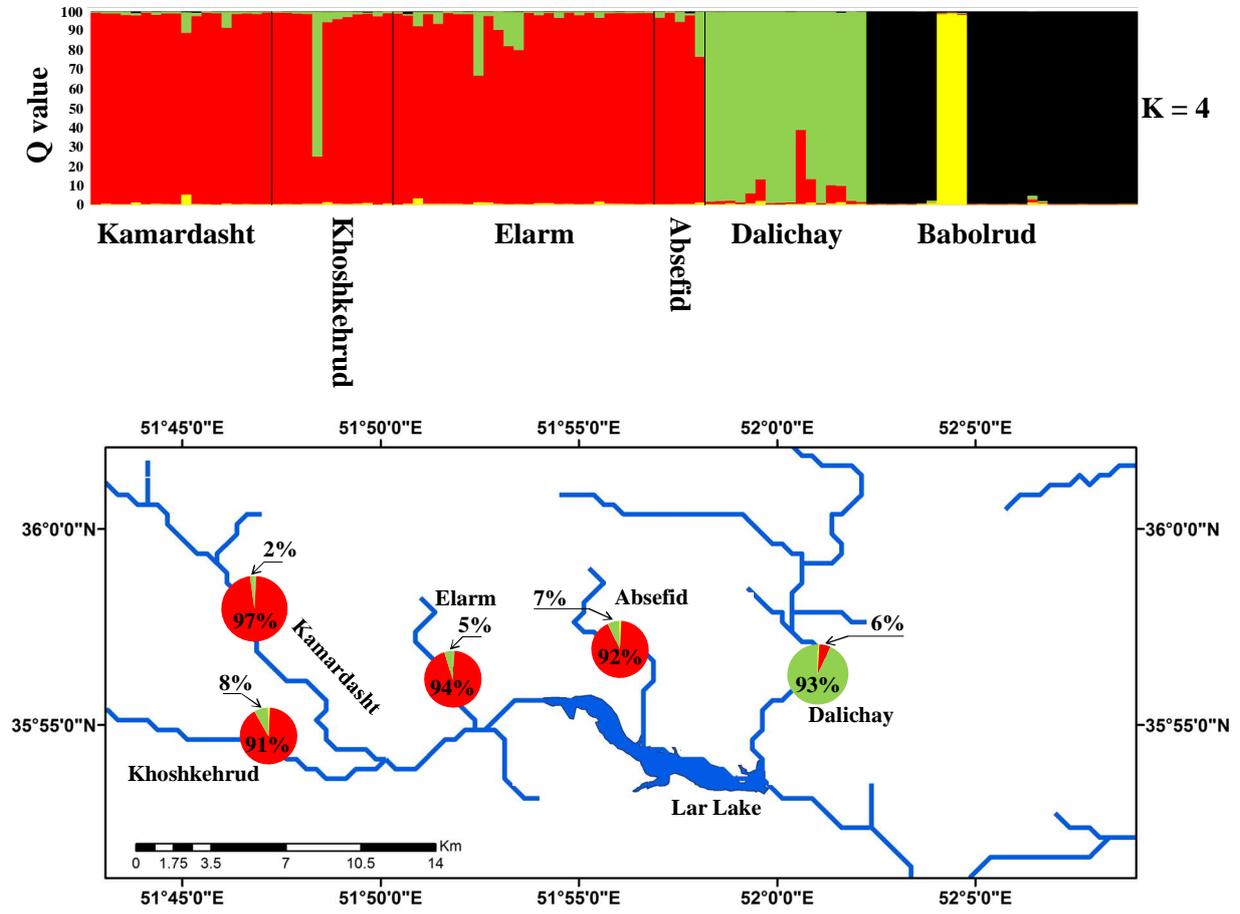
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330 FIG. 2



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