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Genetic diversity of a Daugava basin brown trout (*Salmo trutta*) brood stock

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Abstract – Genetics play an increasingly important role in the conservation of threatened fish populations. We have examined twelve microsatellite markers to determine the genetic diversity of a brood stock of brown trout from the Latvian Daugava river basin, used in a local supportive breeding program and compared diversity values to other Baltic populations. Allelic data was further inspected for indications of increased inbreeding. Additionally, we have analyzed the mitochondrial control region to classify the population within a broader phylogenetic framework. We found that the genetic diversity was comparatively low, but there was no strong evidence of high inbreeding. A newly detected mitochondrial haplotype indicates unnoticed genetic diversity of "Atlantic lineage" brown trout in the Daugava basin region. Our study provides first genetic details on resident brown trout from the Baltic Daugava river basin to improve the regional conservation management of this valuable genetic resource and contributes phylogeographically useful information.

Keywords: *ex situ* conservation / individual inbreeding coefficient F / D-loop / effective population size N_e / salmonids

Résumé – Diversité génétique d'un stock de géniteurs de truite brune (*Salmo trutta*) du bassin de la Daugava. La génétique joue un rôle de plus en plus important dans la conservation des populations de poissons menacées. Nous avons examiné douze marqueurs microsatellites pour déterminer la diversité génétique d'un stock de géniteurs de truite du bassin letton de la Daugava, utilisé dans le cadre d'un programme de reproduction locale et comparé les valeurs de diversité à celles d'autres populations baltes. Les données sur les allèles ont été analysées plus à fond afin de déceler des signes d'augmentation de la consanguinité. De plus, nous avons analysé la région de contrôle mitochondrial pour classer la population dans un cadre phylogénétique plus large. Nous avons constaté que la diversité génétique était comparativement faible, mais qu'il n' y avait pas de preuves solides de consanguinité élevée. Un haplotype mitochondrial nouvellement détecté indique une diversité génétique inaperçue de la truite de "lignée atlantique" dans la région du bassin de la Daugava. Notre étude fournit les premiers détails génétiques sur la truite résidente du bassin de la Daugava baltique afin d'améliorer la gestion régionale de la conservation de cette ressource génétique précieuse et fournit des informations utiles sur le plan phylogéographique.

Mots-clés : conservation *ex situ* / coefficient de consanguinité individuelle F / boucle D / taille effective de la population N_e / salmonidés

1 Introduction

Numerous fish populations are threatened by different environmental factors and human activities (*e.g.* Freyhof and Brooks, 2011), and diverse measures are taken to conserve such populations. Populations involved in conservation programs should be genetically characterized and compared to other populations of the species to set up appropriate conservation strategies (*e.g.* Attard *et al.*, 2016; Luck *et al.*, 2003; Palsbøll *et al.*, 2007). Such information may help to prioritize conservation strategies (Araguas *et al.*, 2007; Fraser and Bernatchez, 2001; Luck *et al.*, 2003; Nunney and Campbell, 1993).

Supportive breeding is a common *ex situ* conservation strategy, often used after or along with *in situ* strategies like

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habitat restoration (*e.g.* Anderson *et al.*, 2013; Black *et al.*, 2016; Hundt *et al.*, 2015; Saura and Faria, 2011), which aims at directly increasing population size. In the ideal case, specimen for supportive breeding originate from the threatened population itself to take into account possible local adaptions and best conserve the global genetic diversity of species (*e.g.* George *et al.*, 2009). However, the number of spawners in breeding programs is often restricted. Thus, typically the effective population size N_e of brood stocks is low, which may lead to adverse genetic effects, such as inbreeding depressions (*e.g.* Fraser, 2008; Naish *et al.*, 2013). Genetic analyses of brood stocks (spawners or descendants) may indicate such issues early (*e.g.* Naish *et al.*, 2013).

Brown trout (*Salmo trutta*) is a species with a high level of genetic diversity and complicated spatial patterns of genetic variability indicate a complex evolutionary history (*e.g.* Bernatchez, 2001; Cortey *et al.*, 2009; Laikre, 1999; Lerceteau-Kohler *et al.*, 2013; McKeown *et al.*, 2010). Based on analyses of mitochondrial DNA (mtDNA) at least six major genetic lineages have been detected in different regions of the Eurasian native range of the species (Bernatchez, 2001; Cortey *et al.*, 2009; Suarez *et al.*, 2001; Susnik *et al.*, 2005). The "(Northern-) Atlantic lineage" is the most widespread lineage and natively distributed from western to northern Europe. Beside this large scale variability, brown trout may show considerable regional or even local differentiation (*e.g.* Lehtonen *et al.*, 2009; Palmé *et al.*, 2013), which may indicate local adaption (Meier *et al.*, 2011), and plays an increasingly important role in conservation (*e.g.* Fruciano *et al.*, 2014; Vilas *et al.*, 2010). Additionally, natural patterns of diversity have been altered by various human activities (Kohout *et al.*, 2012 and citations therein) more recently, which adds further complexity. Nevertheless, in several regions and certain populations human influence on the genetic diversity of brown trout may be still absent or negligible (Lerceteau-Kohler *et al.*, 2013; Van Houdt *et al.*, 2005). Although genetic diversity of brown trout has been studied frequently, genetic data on this species are still lacking for important regions within its natural distribution range, like the Baltic Daugava river basin.

Against this background, we have analyzed genetically a brood stock of brown trout to enhance a local conservation initiative in Latvia. This brood stock is used to restock and support a threatened population of resident brown trout in Virgulica creek in the Daugava river basin. Virgulica creek brown trout are mainly threatened by the loss of suitable spawning grounds caused by extensively increased dam buildings of beavers (*Castor fiber*). This had led to a considerable decline in population size, and several stretches of the creek have been totally without trout. To protect the fish population of Virgulica creek, spawning grounds have been restored and offspring of a locally derived brood stock of brown trout was used to repopulate the creek afterwards. These efforts were undertaken by local, private initiatives, which may be considered a common situation for conservation efforts of single, specific fish stocks. Typically, comprehensive genetic analyses, including populations outside the focal area, are far beyond the capabilities of such initiatives. However, local breeding programs may benefit from genetic analyses of the population under consideration itself and further comparisons with data from other regions – if available (cp. George *et al.*, 2009).

To improve the Virgulica creek trout restoration efforts, we have used nuclear and mitochondrial markers and compared the genetic diversity of the brood stock to other Baltic populations. Further, we have examined the allelic data for indications of inbreeding. Finally, our study provides first genetic details on resident brown trout from the Baltic Daugava river basin and contributes new phylogeographically relevant information on brown trout from an understudied region. Overall, this study may improve the regional management of the valuable genetic diversity of brown trout in the Daugava river basin.

2 Materials and methods

2.1 Location and brood stock

Virgulica creek is a small (length ca. 20 km) tributary of River Pededze, in the Daugava river basin in Latvia (57.44°N, 27.33°E) and a typical salmonid creek of the region (Fig. 1). The dominant land use around Virgulica creek is forestry, but also some agriculture. In the 1960/70s the creek was partly straightened for agricultural land reclamation.

In recent years the main threat to brown trout in Virgulica creek was loss of spawning grounds and habitat fragmentation caused by beaver dams. Licensed recreational angling takes place at the creek, but fishing pressure is generally low. However, illegal poaching has been observed.

Virgulica creek has a historic watermill dam 150 m before joining River Pededze, which presumably prevents fish upstream migrations. No stocking with foreign brown trout happened within the last 20 years and there is no indication for earlier introductions. Thus, we consider the Virgulica creek brown trout as autochthonous.

The Virgulica creek brood stock was derived from 50 wild brown trout, caught in the most downstream, largely unmodified stretch of the creek in 2009. Eggs of several randomly selected females were fertilized with the milt of the respective number of likewise randomly selected males. Thereby, all available specimen (50), regardless of phenotypic properties (*e.g.* size, early/late maturity), were used to conserve genetic diversity and avoid artificial selection.

2.2 Sampling and genetic analysis

In November 2011 adipose fin tissue of 25 specimens of the first generation offspring of the breeding program was clipped and tissue samples were preserved in 96% ethanol and stored at −20 °C in the lab. DNA was extracted using a modified (Wetjen *et al.*, 2017) salt protocol (Aljanabi and Martinez, 1997).

Nuclear DNA of all 25 specimens was examined at twelve microsatellite loci in two multiplex-PCRs (Type-it Microsatellite PCR Kit, QIAGEN) and one single PCR (Tab. 1). The 5 µl reaction volumes contained different volumes of the primers (Tab. 1), 1x Type-it Multiplex PCR Master Mix, 0.5x Q-Solution (QIAGEN) and 10 ng DNA. Cycling parameters were: initial denaturation (95 °C, 5 min), 30 cycles at 94 °C (30 s), 57 °C (90 s), 72 °C (60 s), and final extension at 60 °C (30 min). The loci were analyzed on an automated sequencer (CEQ 8000, Beckman Coulter) using the GenomeLab DNA Size Standard Kit (400 and 600 respectively, Beckman Coulter).

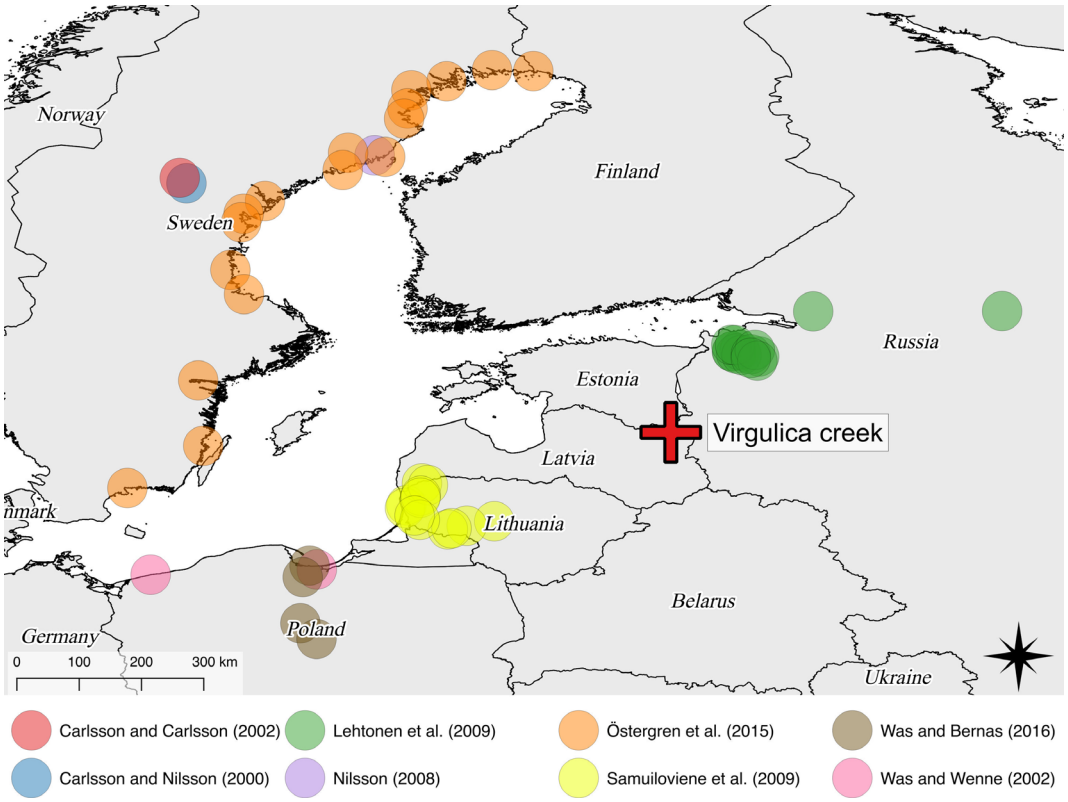

Fig. 1. Location of the Virgulica creek (red cross) in Latvia and approximate locations of non-hatchery samples from Carlsson and Carlsson (2002), Carlsson and Nilsson (2000), Lehtonen *et al.* (2009), Nilsson *et al.* (2008), Östergren *et al.* (2015), Samuiloviene *et al.* (2009), Was and Bernas (2016), and Was and Wenne (2002) (Tab. 2). Locations for Östergren *et al.* (2015) are indicated at the respective river outlets.

Table 1. Primer concentrations, batches, size ranges and references of the microsatellite loci.

Locus	Concentration [μM]	PCR	Size range [bp] (Ref.)	Reference
MST 15	0.05	multiplex A	214–224	Presa and Guyomard (1996)
Sco 216	1.0	single	329–347	Dehaan and Ardren (2005)
Ssa 85	0.0125	multiplex A	110–138	OReilly *et al.* (1996)
SsaA 86	0.025	multiplex A	169–241	King *et al.* (2005)
SSOSL 85	0.15	multiplex A	194	Slettan *et al.* (1995)
MST 60	0.05	multiplex B	97–111	Presa and Guyomard (1996)
MST 73	0.075	multiplex B	140–158	Presa and Guyomard (1996)
OMM 1310	0.5	multiplex B	175–289	Palti *et al.* (2002)
Sco 204	0.035	multiplex B	107–173	Dehaan and Ardren (2005)
Ssa 410 UOS	0.5	multiplex B	198–324	Cairney *et al.* (2000)
OMM 1323	0.05	multiplex A	101–206	Palti *et al.* (2002)
Ssa 417 OUS	0.15	multiplex B	265–424	Cairney *et al.* (2000)

Further, the control region (CR) of the mtDNA of 11 specimens was amplified with primers Str-L19 (5'-CCAC-TAGCTCCCAAAGCTA-3') and Str-H17 (5'-ACTTTC-TAGGGTCCATC-3') (Bernatchez *et al.*, 1992), as detailed in Wetjen *et al.* (2017). Bidirectional sequencing was done by SeqIT GmbH & Co KG.

2.3 Data analysis

Prior to further analyses we checked completeness of allelic data and determined polymorphism of loci to reject uncomplete and monomorphic loci. MICRO-CHECKER Version 2.2.3 (Van Oosterhout *et al.*, 2004) was used to test for null alleles. We determined the size range of alleles [base pairs (bp)], the number of alleles and genotypes, the allele frequencies (Supplemental Information Tab. S1), the allelic richness (AR) and observed and expected heterozygosity (H_{obs} and H_{exp}) for each locus and calculated the difference $H_{exp}-H_{obs}$ and the fixation index F_{IS} $[(H_{exp}-H_{obs})/H_{exp}]$. Further, we tested conformity to the Hardy–Weinberg equilibrium (HWE) per locus. At population level we calculated the expected heterozygosity H_s and the means of

Table 2. Number of population samples, mean number of specimens per sample, total number of specimens, number of analyzed microsatellites (N_{MS}), river and sea basin of sample origin, and years of sampling from eight studies from which genetic diversity data were acquired for comparison with our results on the Virgulica creek brood stock sample. Comparisons of diversity values per sample are shown in Figure 2.

Samples	Specimens		N_{MS}	River basin(s)	Sea basin(s)	Year(s) of sampling	Reference
	Mean (SEM)	$\sum$					
14	47.21 (3.74)	661	5	Ammerån	Bothnian Bay	1996, 1997	Carlsson and Nilsson (2000)
1	48	48	14	Ammerån	Bothnian Bay	1997	Carlsson and Carlsson (2002)
16	35.62 (4.25)	570	9	Luga	Gulf of Finland	2000–2004	Lehtonen *et al.* (2009)
1	49	49	8	Sävarån	Bothnian Bay	2005–2006	Nilsson *et al.* (2008)
17	47.06 (6.97)	800	10	Torneälven, Kalixälven, Luleälven, Piteälven, Byskeälven, Skellefteälven, Sävarån, Ume-Vindelälven, Oreälven, Ångermanälven, Indalsälven, Ljungan, Ljusnan, Dalälven, Bråvikena, Emån, Mörrumsån	Bothnian Bay, Bothnian Sea, Southern Baltic Sea	1995, 1999, 2001–2007, 2009	Östergren *et al.* (2015)
13	21.77 (2.19)	283	8	Akmena-Dane, Bartuva, Dubysa, Jura, Minija	Southern Baltic Sea	2003–2005	Samuiloviene *et al.* (2009)
6	39.83 (0.17)	239	5	Vistula, Rega, hatchery	Southern Baltic Sea	1996, 1997	Was and Wenne (2002)
9	101.00 (8.72)	909	7, 12	Vistula	Southern Baltic Sea	1971, 2003, 2011	Was and Bernas (2016)

the above genetic diversity values per locus and specimen respectively with standard errors (SEM). For comparison of our results on $n = 25$ specimens from Virgulica creek we acquired genetic diversity values for 77 Baltic population samples from Carlsson and Carlsson (2002) Carlsson and Nilsson (2000) Lehtonen *et al.* (2009) Nilsson *et al.* (2008) Östergren *et al.* (2015) Samuiloviene *et al.* (2009) Was and Bernas (2016), and Was and Wenne (2002) (Fig. 1, Tab. 2). We obtained the mean number of alleles ($n = 34$), the mean AR ($n = 55$), H_{obs} ($n = 35$) and H_{exp} ($n = 77$) per sample.

For each specimen we derived a likelihood function of the individual inbreeding coefficient F and estimated a mean F by randomly sampling 1000 F-values from the distribution of the probability density from this function. Further, we estimated the pairwise relatedness over all loci M_{xy} (Blouin *et al.*, 1996) between all specimens. We used 'adegenet' v. 1.3-9.2 (Jombart, 2008), 'hierfstat' v. 0.04-10 (Goudet, 2013) and 'Demerelate' v. 0.9-3 (Kraemer and Gerlach, 2017) in R v. 3.0.2 (R Core Team, 2013).

MtDNA CR sequences were aligned and assigned to previously published haplotypes (Bernatchez, 2001; Bernatchez *et al.*, 1992; Cortey and Garcia-Marin, 2002; Duftner *et al.*, 2003; Kohout *et al.*, 2012; Weiss *et al.*, 2001) and major mtDNA lineages (Bernatchez, 2001; Bernatchez *et al.*, 1992) using the Geneious 6.0 software (Biomatters). Haplotype diversity h was estimated as $h = n/(n-1)(1-\sum x_i^2)$, with sample size n and frequency of haplotype$_i$ x_i (Nei and Tajima, 1981). For comparison we obtained or calculated haplotype diversities from Cortey and Garcia-Marin (2002; $n = 10$), Duftner *et al.* (2003; $n = 5$), and Kohout *et al.* (2012; $n = 29$) for 44 populations with at least 10 specimens genotyped.

3 Results

The locus Ssa 417 UOS could not be amplified in 17 samples (68%), while for all other loci percentage of missing data was within an acceptable range (≤12%). Loci Ssa 417 UOS and OMM 1323 were 100% monomorphic, and thus rejected, so that further analyses included allelic data from 25 individuals at 10 polymorphic loci. No evidence for null alleles was found.

For the Virgulica stock the mean number of alleles was 3.40 (SEM 0.43) and the mean AR was 3.80 (SEM 0.04). The mean H_{obs} was 0.52 (SEM 0.02) and the mean H_{exp} was 0.53 (SEM 0.05). Table 3 shows detailed characteristics per locus. All four diversity values were in the lower quartile of the respective values obtained from other studies (Fig. 2). The mean number of alleles in the reference samples ranged from 3.2 to 8.0 (median 4.72, mean 4.75, SEM 0.23) and the mean AR from 3.26 to 8.57 (median 4.61, mean 5.28, SEM 0.20). The range of H_{obs} was 0.39 to 0.80 (median 0.64, mean 0.62, SEM 0.02) and 0.47 to 0.75 (median 0.66, mean 0.65, SEM 0.01) for H_{exp}.

The mean difference of $H_{exp}-H_{obs}$ was 0.01 (SEM 0.43), mean F_{IS} was 0.04 (SEM 0.56) and significant deviation from HWE was observed at locus Ssa410UOS (Tab. 3). The genetic diversity within the population H_s was 0.53.

Estimates of mean F ranged from 0.147 (sample ID: F1459) to 0.53 (F1464). The mean F of 20 specimens were below 0.33, slightly exceeded 0.4 for two specimens (0.41; F1456, F1461) and were higher than 0.5 for another three (0.53; F1464, F1467, F1473) (Supplemental Information Tab. S2). Figure 3 shows a graphical representation of the likelihood functions of F. At population level, the mean of individual F–values was 0.26 (SEM 0.03). Pairwise relatedness M_{xy} ranged from 0.25 (F1461–F1468) to 0.83 (F1464–F1470) with a mean of 0.53 (SEM 0.0065) (Fig. 4).

Based on a comparison of 247 bp of haplotype At-s1 (310 bp; GenBank accession number M97969; Bernatchez, 2001; Bernatchez *et al.*, 1992) and based on 401 bp to haplotype At1 (464 bp; AF321990; Weiss *et al.*, 2001) all mtDNA CR sequences were identical. A comparison of the full 946 bp segments assigned eight specimens to haplotype H2 (1012 bp; AF273087) and two specimens to haplotype H3 (1012 bp; AF274574) in Cortey and Garcia-Marin (2002). The haplotypes H2 and H3 are identical to the haplotypes At1b and

Table 3. Genetic diversity values of the $n = 25$ specimens determined per locus. Bold numbers indicate significant ($p < 0.0001$) deviations from Hardy–Weinberg equilibrium.

Locus	Alleles	Size range [bp]	Genotypes	AR	H_{obs}	H_{exp}	$H_{exp}-H_{obs}$	F_{IS}	HWE		
									χ^2	*d.f.*	*p*
MST 15	3	221–227	5	3.00	0.40	0.52	0.12	0.24	5.8164	3	0.1209
Sco 216	3	151–179	5	3.00	0.50	0.57	0.07	0.13	2.5972	3	0.4580
Ssa 85	3	111–115	6	3.00	0.64	0.62	−0.02	−0.02	1.8441	3	0.6054
SsAa 86	2	174–182	3	2.00	0.32	0.36	0.04	0.12	0.3770	1	0.5392
SSOSL 85	3	178–188	4	2.99	0.32	0.42	0.10	0.24	3.3179	3	0.3452
MST 60	2	93–97	3	4.00	0.64	0.49	−0.15	−0.30	2.2306	1	0.1353
MST 73	2	141–143	3	2.00	0.48	0.48	0.00	0.00	0.0000	1	1.0000
OMM 1310	4	182–192	6	3.92	0.58	0.61	0.03	0.05	2.1724	6	0.9032
Sco 204	6	98–172	9	8.49	0.64	0.62	−0.02	−0.03	4.9649	15	0.9924
Ssa 410 UOS	6	202–254	7	5.64	0.64	0.59	−0.05	−0.08	**54.5449**	**15**	**<0.0001**
OMM 1323	1	153	1								
Ssa 417 OUS	1	369	1								
Mean (SEM)	3.40 (0.43)		5.10 (0.03)	3.80 (0.04)	0.52 (0.02)	0.53 (0.05)	0.01 (0.43)	0.04 (0.56)			

AR allelic richness, H_{obs} observed heterozygosity, H_{exp} expected heterozygosity, F_{IS} fixation index, HWE deviation from Hardy–Weinberg equilibrium

At1d in Duftner *et al.* (2003). A third haplotype, represented by one specimen, was not found in any previous study. It differs from haplotypes At1b and At1d by one mutation at nucleotide position 527 (Tab. 4). This sequence was named At1q, following the attempt of Duftner *et al.* (2003) to standardize haplotype nomenclature, and deposited in GenBank (KT360957).

Additionally, we compared haplotypes At1b, At1d, and At1q to a 285 bp segment at the 3' end of the CR associated to haplotype At1 (328 bp; M97968; Bernatchez *et al.*, 1992). Haplotype At1d differs at three positions from At1, while haplotypes At1b and At1q, which are identical in this segment, differ at four positions. Differences include two insertions/deletions in either of these cases (Tab. 4).

The estimated haplotype diversity h was 0.47, and thus within a medium range: It is between the lower quartile and the median of haplotype diversities found in populations from earlier studies ($n = 44$, range 0.00 to 0.90, mean 0.53, SEM 0.04; Fig. 5).

4 Discussion

The comparison of genetic diversity values of Baltic brown trout populations based on neutral nuclear markers overall revealed that the diversity of the Virgulica creek brood stock is rather low. Low genetic diversity is often regarded as a warning signal that a population might be or become threatened by increased inbreeding or deleterious genetic drift (*e.g.* Naish *et al.*, 2013 and citations therein). Thus, maintenance or establishment of high levels of genetic diversity is a common aim of conservation efforts (Saura and Faria, 2011). Nevertheless, low genetic diversity may occur in small, wild salmonid populations without preventing survival and adaption (Pujolar *et al.*, 2016) and citations therein), so that the comparatively low genetic diversity of the Virgulica brood stock itself is not necessarily a major concern.

Direct comparisons of genetic diversity between microsatellite based studies might be affected *e.g.* by the selection of different markers (Ryman *et al.*, 2006). However, this effect should be reduced at population level by averaging over a number of loci with different levels of polymorphism. The number of loci in the studies used here for comparison ranged from 5 to 14 (mean 8.1, SEM 0.25; Tab. 2). However, the necessary number of loci is disputable (Selkoe and Toonen, 2006). Further, rare alleles might be missed because of low sampling sizes, which also makes comparisons between studies difficult. Our sample of the Virgulica creek brood stock is at the lower range of sampling sizes per population used for comparison (Tab. 2). This might partly explain the comparatively low diversity found here. However, measured by the low number of 50 specimens in the parental generation, we believe that our sample is representative for the brood stock. The range of genetic diversity values compared here, might in part reflect the ecological range of Baltic brown trout (*e.g.* effective population sizes, life history traits, isolation, or population history, like bottlenecks), so that overall, despite methodological difficulties, we believe that this approach is helpful in providing a context for the further assessment of the Virgulica creek population.

The mean difference of $H_{exp}-H_{obs}$, the mean F_{IS}, and deviations from HWE, provide better evidence for inbreeding than above genetic diversity values. Both, mean $H_{exp}-H_{obs}$ and F_{IS}, were nearly zero. Significant deviation from HWE was found only at a single locus. Thus, all three values did not indicate significant inbreeding at the population level. In contrast we have found certain indications for inbreeding at the individual level, *i.e.* mean $F > 0.4$, in 5 specimens (20%). We consider this indication as weak ($0.4 < F \leq 0.5$) for two (8%) specimens and as reasonable ($F > 0.5$) for three (12%) specimens. Higher values of F in first generation offspring may be explained by kinship within the sample or the parental generation. The mean, range and frequency distribution of M_{xy} in the brood stock sample match the expectations for full

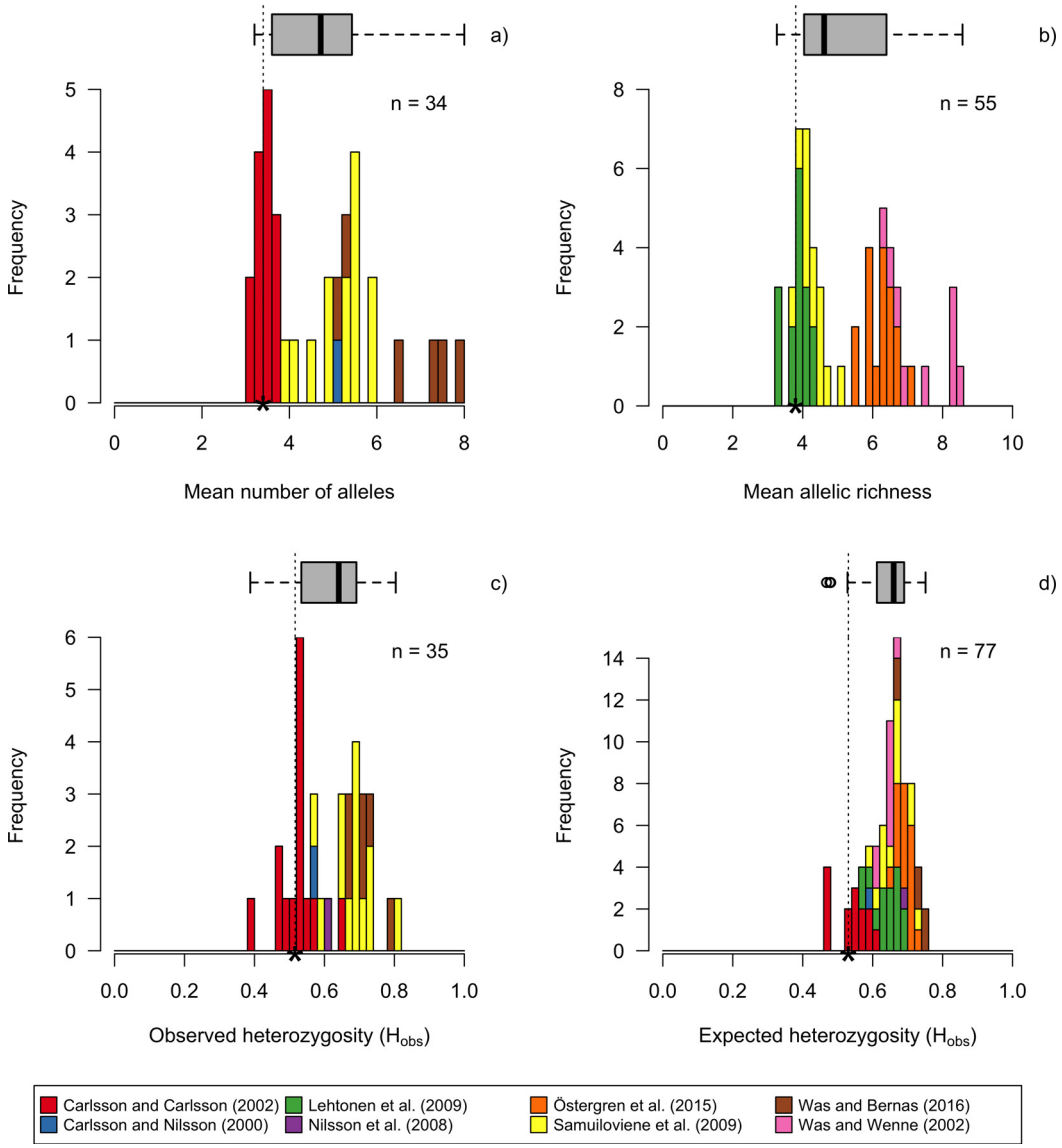

Fig. 2. Frequency (bars) and distribution (boxplots) of the genetic diversity values a) mean number of alleles, b) mean allelic richness, c) observed heterozygosity, and d) expected heterozygosity of 77 samples of Baltic brown trout from Carlsson and Carlsson (2002), Carlsson and Nilsson (2000), Lehtonen *et al.* (2009), Nilsson *et al.* (2008), Östergren *et al.* (2015), Samuiloviene *et al.* (2009), Was and Bernas (2016), and Was and Wenne (2002) (Tab. 2). Asterisks and dashed lines mark the respective values of the Virgulica creek brood stock of which $n = 25$ specimens were examined in the present study (a: 3.40, b: 3.80, c: 0.52, d: 0.53).

sibs (or parent–offspring pairs). Our results come very close to the findings of Blouin *et al.* (1996) for a breed of full siblings from wild parents in mice (*Mus musculus*). Thus, our sample apparently includes mostly closely related specimens. The spawners of the brood stock were mainly sampled from just several stretches of a rather small section of Virgulica creek. Thus, already this sample may have contained (half) siblings. By mixing sperm and eggs of several spawners the risk of producing offspring exclusively from one pair of siblings was minimized. However, mating of (half) siblings may also occur in the wild, especially in headwaters where effective population sizes are typically low (Hansen and Jensen, 2005). Also, at the population level, mean *F* appears uncritically low. Thus, we believe that the proportion of specimens with indications of reasonable inbreeding of less than 15% is acceptable in a local breeding program (cp. Ruzzante *et al.*, 2001). However, in a future perspective the Virgulica creek breeding program could benefit from conducting sib-avoidance matings to further delay inbreeding. Overall, our analyses of microsatellite data confirm that low genetic diversity itself is a rather insufficient indicator of inbreeding. Thus, aiming at increasing genetic diversity in conservation programs may not be simply justifiable by avoidance of inbreeding depressions.

Our analyses of the mtDNA CR have demonstrated that the brown trout population of Virgulica creek shows – as expected for the Daugava river basin – exclusively (northern) "Atlantic" haplotypes. Haplotypes At1b and At1d are the most common haplotypes in central and northern Europe. This is well reflected in our results: 91% of the specimens

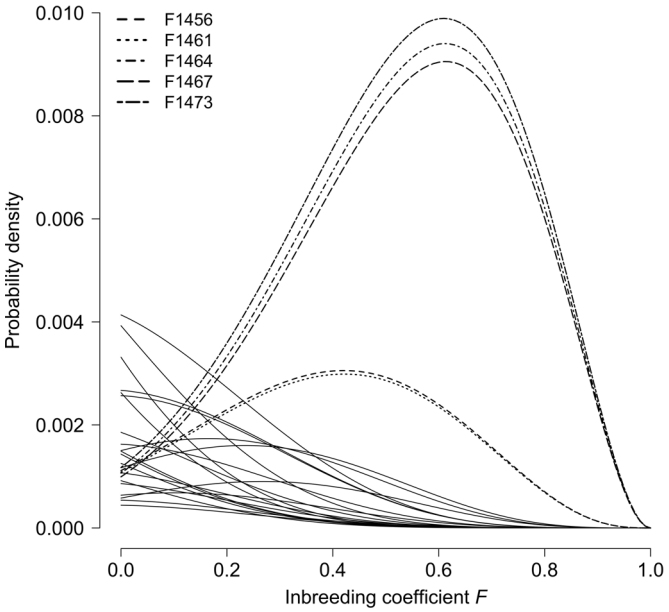

Fig. 3. Likelihood functions of the inbreeding coefficient F of the $n = 25$ specimens. Dashed lines indicate specimen with mean F-values >0.4 and solid lines specimen with mean F-values <0.4. Note that the maxima of the probability density for the specimens F1456 and F1461 are in the same order of magnitude (~0.4), while they are higher for specimens F1464, F1467, and F1473 (~0.6) compared to mean F (Supplemental Information Tab. S2).

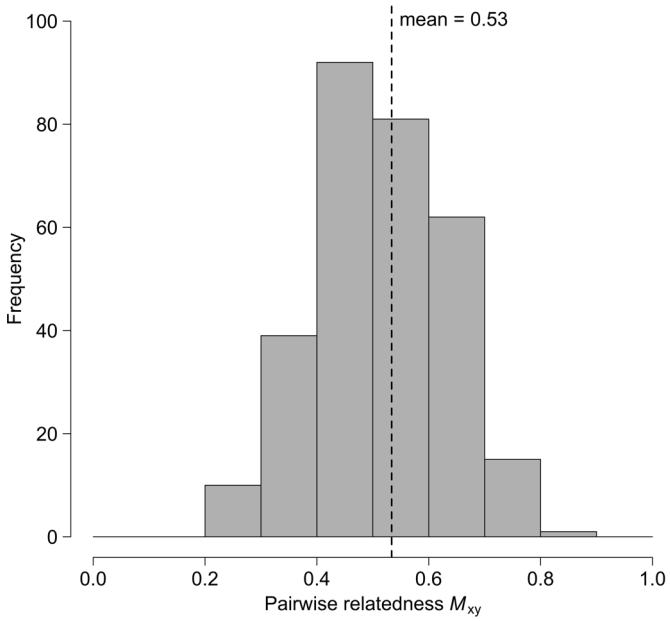

Fig. 4. Frequency of pairwise relatedness M_{xy} between all analyzed specimens from Virgulica creek brood stock. Dashed line indicates the mean (0.53, SEM 0.0065) of all M_{xy}.

Table 4. Variable base positions among the three haplotypes of the $n = 11$ specimens based upon 946 bp of the mtDNA CR and additionally a 285 bp segment of the 3' end of haplotype At1 (328 bp; acc. no. M97968; Bernatchez *et al.*, 1992). Nucleotide positions are numbered according to the reference sequence 'haplotype 2' (AF273087; Cortey and Garcia-Marin, 2002). Identity with the reference sequence is indicated with . and indels are marked with −. Number (N) and relative frequency (Freq.) of each haplotype is given.

Haplotype name†	Variable sites					N	Freq.
	527	714	731‡	750§	935		
AT-s1|At1|H2|At1b	C	C	C	A	A	8	0.73
AT-s1|At1|H3|At1d	.	.	.	.	C	2	0.18
AT-s1|At1|–|At1q	T	.	.	.	.	1	0.09
At1 − 3' end		A	–	–	C		

†The haplotype names are composed of the haplotype names of the reference sequences and separated by |, as follows: Bernatchez *et al.* (1992) and Bernatchez (2001) | Weiss *et al.* (2001) | Cortey and Garcia-Marin (2002) | Duftner *et al.* (2003) and this study.
‡Position 731 is followed by another three cytosine nucleotides in haplotypes At1b, At1d and At1q.
§Position 750 is followed by two more adenine nucleotides in haplotypes At1b, At1d and At1q.

were assigned to either of them. One out of just eleven analyzed specimens, however, revealed a previously undescribed haplotype. This suggests that the Daugava river basin may hold undetected genetic diversity of brown trout. This assumption is supported by the variation at the 3' end of the CR compared to haplotype At1 (*sensu* Bernatchez *et al.*, 1992). Such previously undetected diversity in understudied regions is potentially phylogeographically relevant and lastly important to establish appropriate conservation strategies at a larger scale (*e.g.* Cortey and Garcia-Marin, 2002; Schenekar *et al.*, 2014). Our study provides a first basis towards future conservation strategies for brown trout in the Daugava river basin.

From an applied perspective, our study shows the importance of comparing genetic diversity data between studies to better evaluate values measured in a single population of interest. This comparison revealed, that the genetic diversity of the Virgulica creek brood stock is relatively low. Analyses of inbreeding showed, that − despite overall low diversity − the supportive breeding procedures applied in the Virgulica creek program appear appropriate to conserve the valuable genetic diversity of brown trout at a local scale.

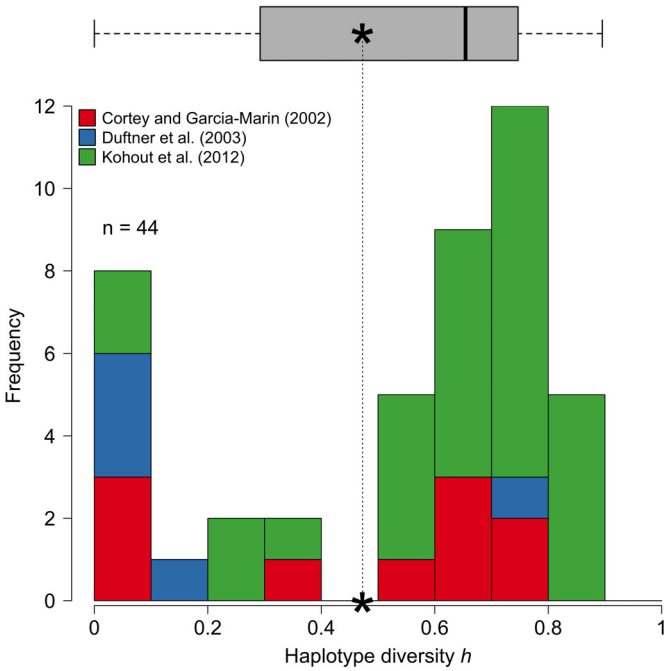

Fig. 5. Frequency (bars) and distribution (boxplot) of haplotype diversity *h* of 44 samples of brown trout from Cortey and Garcia-Marin (2002; $n = 10$), Duftner *et al.* (2003; $n = 5$) and Kohout *et al.* (2012; $n = 29$). Asterisks and dashed line mark the haplotype diversity of the Virgulica creek brood stock (0.47).

Supplementary Material

Supplementary tables.
The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2017048/olm.

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