Lajbner (2011) Human-aided dispersal has altered but not erased the phylogeography of the tench

Evolutionary applications

Multiple – gene sequencing approach and barrier detection statistics to test whether the range wide genetic variation of the tench shows a significant phylogeographic structure that can be explained by natural processes during the last glacial and inter glacial cycle.

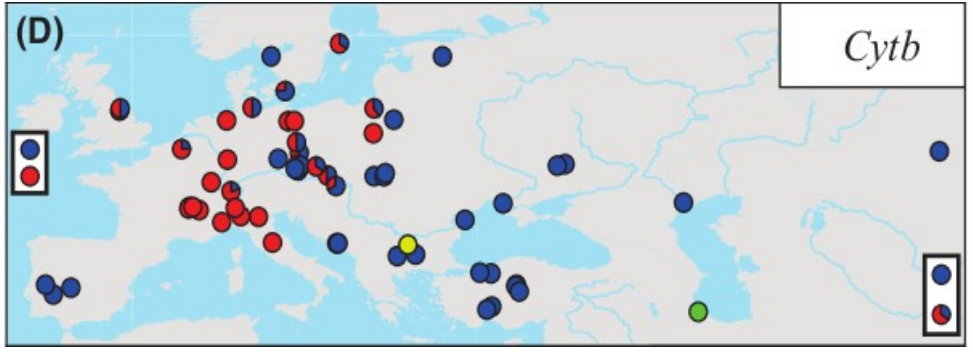
Sample: 225 individuals from 76 populations

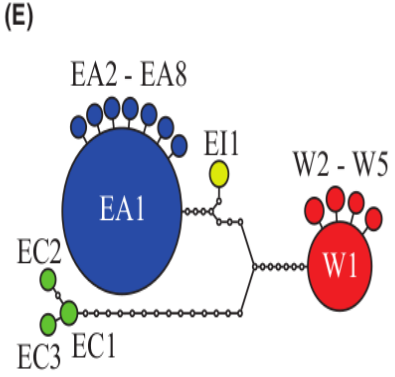
Methods: Introns of **three nuclear genes** and **a complete sequence of one mitochondrial gene** were analyzed by PCR. For each locus the haplotype and nucleotide diversities and their variance was estimated.(Act, ATPase, RpS7, **Cytb**)

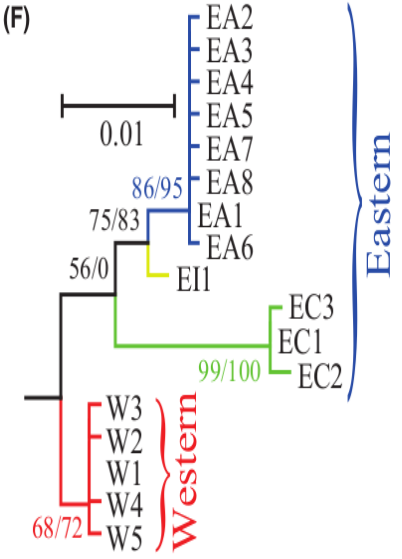
Figure 1 Putative native (olive) and part of non-native (violet) distribution range of the tench. Large areas where the origin is considered ambiguous are highlighted by orange. Locations of major freshwater glacial refugia in Europe, Western/Atlantic (R1), Danubian (R2), and Ponto-Caspian (R3) are indicated. Sampling countries are labeled (codes: B, Belgium; BG, Bulgaria; BIH, Bosnia and Herzegovina; CH, Switzerland; CZ, Czech Republic; D, Germany; EST, Estonia; GB, Great Britain; H, Hungary; I, Italy; P, Portugal; RO, Romania; S, Sweden; SK, Slovakia). References to the map: Urchinov 1995; Brylińska et al. 1999; Mitrofanov and Petr 1999; Sawaitova and Petr 1999, Economidis et al. 2000; Wang et al. 2004; Innal and Erk'akan 2006; Hesthagen and Sandlund 2007; Popov 2009; Mamilov et al. 2010.

The phylogenetic and network analyses split the range-wide data set for the mitochondrial Cytb into **two distinct phylogroups, (Clades W and E)** separated with 1.6% fo genetic distance, translating to a divergence time of about 64x10.3 to 1600x10,3 years ago.

The Western phylogroup was found in Europe between the British Isle and Poland, whereas the **Eastern phylogroup was present from Europe throughout Asia to China** with a broad zone of overlap with Western phylogroup in Europe







Clade W (red), Clade EA (blue), EC (green) and

Clade EI (yellow). Clade E was partitioned into three sub-clades.

EC haplotypes in Caspian Sea in Iran and the EI haplotype in the Iskar River in Bulgaria

Rooted phylogenies were reconstructed by the maximum-likelihood criterion using PhyML.

Lajbner (2010) Lack of reproductive isolation between the Western and Eastern phylogroups of the tench

Reviews in Fish Biology and Fisheries

Hypothesis: **The two phylogroups (Western and Eastern) may represent distinct species**.

Analysis variation at introns of **nuclear genes**, **microsatellites**, **allozymes**, and **mitochondrial DNA** in population from two post glacial lakes within the contact zone in Germany.

Sample: 49 tench collected from a wild population from Lake Felchowsee and supplemented by an additionbal 19 individuals from Lake Dollnsee (Germany).

Actin gene (336b), **RPS7 gene (900 bp), mtDNA 1225bp** (entire gene for Cytb).

6 microsatellite loci for fish from both lakes were taken from studies of Kohlmann and Kersten (1998,2006,2007).

Methods: PCR-GENEPOP to test Hardy-Weinberg equilibrium.

Results:The result of the study support the hypothesis of free interbreeding between the two phylogroups of tench.

Samples from both lakes did **show significant disequilibria but they were limited to individual loci** and were not concordant between populations.

Therefore, although the phylogroups may be considered as separate phylogenetic species the present data suggest that **they are a single species under the biological species concept**.

Lajbner (2011) PCR_RFLP assays to distinguish the Western ans Eastern phylogroups in wild and cultured tench Tinca tinca Molecular Ecology Resources.

New method to distinguish between Western and Eastern phylogroups of the tench. The method relies on **PCR-RFLP** assay of two independent nuclear-encoded exon-primed intron-crossing markers (EPIC) markers and on of the **mtDNA markers**

Sample: Act gene, S7 ribosomal protein (RpS7) and **mtDNA marker of Cytb gene**. **225 tench** *(from a wide range of geographical regions)*

Results: A Western phylogroup (clade W) found in Europe from the British Isle to Poland and and an Eastern phylogroup (clade E) distributed from Europe through out Asia to China.

- Within the Eastern phylogroup, population with mtDNA (but not nuclear markers) distinct from the **major Eastern clade (EA)** were found in in a southern tributary of thte Danube River in **Bulgaria (Clade EI)** and in the southern part of the **Caspian Sea basin in Iran (EC)**.
- **Separation into the phylogroups most reasonably reflects long-term evolutionary isolation of populations in different parts of the native range.**

Presti (2014) Sequence variability at the mitochondrial ND1, ND6, cyt b and D-loop segments in tench (Tinca tinca L.)

Applied Ichthyology

Sample: 29 individuals from 17 populations (Spain,Germany,Czech Republic ,Italy, Hungary, Romania, Turkey, China).

Genetic markers: **ND1** *1019bp*, **ND6** *576 bp*, ***Cytb*** *1146*, **D-loop** *996-1001bp*), overall *3743bp* representing 22.5% of the total mtDNA

Methods:PCR-RFLP, MEGA4 sequence alignment and reconstruction of Neighbor-Joining trees

Results: The combination of the haplotypes at the single segments determined 20 composites haplotypes: 7 **(H1a-H1g) as H1** and **6 (H2a-H2f) as H2**.

Only D-loop has good resolution

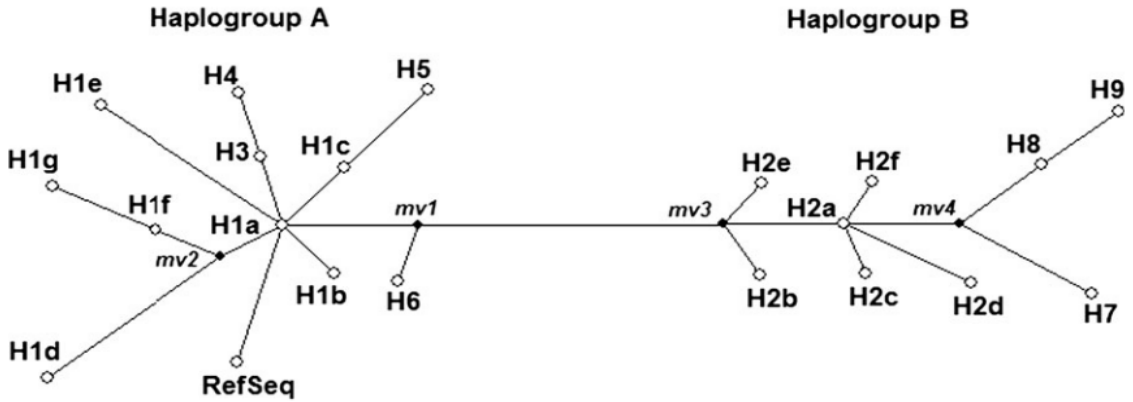


Fig. 2. Median-Joining network of the tench mtDNA composite haplotypes. Median vectors (*mv*) represent hypothesized sequences required to connect existing sequences.

- **The phylogenetic trees constructed to verify the discriminatory power of the four segments showed that each of them was informative enough to clearly identify (bootstrap value of 100%) the two highly divergent haplogroups.**

Table 5
Occurrence of composite mtDNA haplotypes in examined tench populations

Haplotype	Population
H1a	DÖL, KÖW, VAL
H1b	FEL
H1c	ROM
H1d	VEM
H1e	PIA
H1f	ALC, BOL
H1g	TRA
H2a	FEL, HUN, VOD
H2b	BAD
H2c	CHI
H2d	TUR
H2e	ROM
H2f	PIA, VEM
H3	ALC, BOL, TRA
H4	TRA
H5	VAL
H6	FEL
H7	TUR
H8	MAL
H9	FEL, GOL

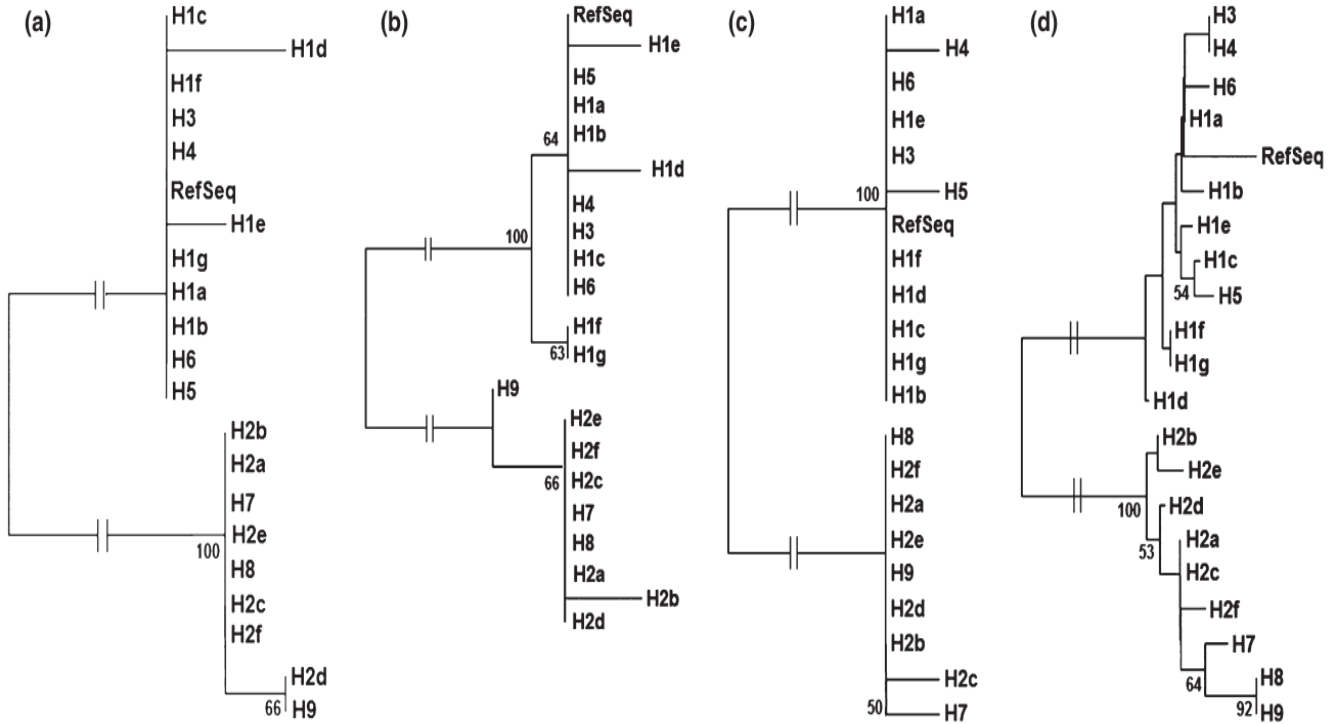


Fig. 1. NJ tree constructed with data of the single segments: a) ND1, b) ND6, c) cyt *b*, d) D-loop.

Presti (2012) Genetic variability in tench (Tinca tinca L.) as revealed by PCR-RFLP analysis of mitochondrial DNA

Italian Journal of animal Sciences

Sample: **126 individuals** from **14 wild and cultured populations** located in different European and Asian countries (Italy, Spain, Germany, China, Turkey, Czech Republic).(Data from 5 Italian population from Presti 2010) for the same mtDNA also included

Four mitochondrial DNA segments, **ND1, ND6, Cytb, D-loop**.

Methods: PCR-RFLP , Neighbour-Joiining tree (PHYLIP), ARLEQUIN to evaluate variability within population by haplotype and nucleotide diversity

Results: All segments were polymorphic and originated **9 haplotypes** which clustered into **2 haplogroups A, and B (Western – Eastern).**

9 out of 19 populations showed polymorphism

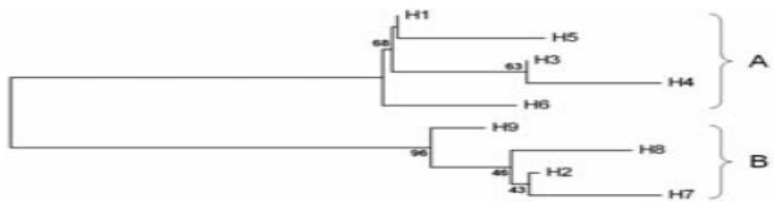


Figure 1. Neighbour-Joining tree of the composite haplotypes. Only bootstrap values higher than 40% are shown.

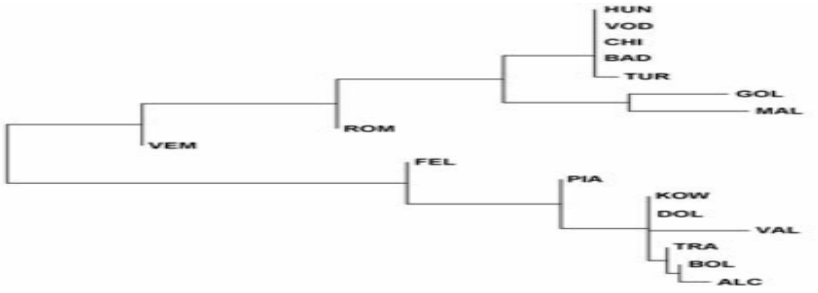


Figure 2. Neighbour-Joining tree of 19 tench populations.

- 75% of the pairwise comparisons were significant indicating a high between population variability.
- **GOL, MAL (Czech Republic)** and **VAL (Italy**) differed statistically from all the other populations, while most of the non-significant comparisons involved the **Eastern populations (CHI, TUR, HUN, ROM, VEM and VOD)** and the one from Spain (BAD).
- No differences were observed between the **German populations (FEL, KÖW, DÖL**) or between those of **Central- Southern Italy (TRA, BOL, ALC**).

Table 5. Nucleotide divergence within population (diagonal) and between populations (above the diagonal); significance (*) of the exact test of Raymond and Rousset (1995) for population differentiation (below the diagonal).

	BAD	CHI	TUR	FEL	KOW	DOE	HUN	ROM	GOL	MAL	VEM	VOD	PIA	VAL	BOL	TRA	ALC
BAD	*0.00*	0.00	0.11	3.43	6.00	6.00	0.00	0.13	2.00	1.00	0.40	0.00	4.42	6.46	6.05	6.03	6.30
CHI	.	*0.00*	0.11	3.43	6.00	6.00	0.00	0.13	2.00	1.00	0.40	0.00	4.42	6.46	6.05	6.03	6.30
TUR	.	.	*0.51*	3.45	6.11	6.11	0.11	0.24	2.11	1.11	0.51	0.11	4.53	6.57	6.16	6.14	6.41
FEL	*	*	*	*2.89*	0.18	0.18	3.43	1.71	3.43	4.18	1.05	3.43	0.10	0.64	0.23	0.21	0.48
KÖW	*	*	*	.	*0.00*	0.00	6.00	3.73	6.00	7.00	2.80	6.00	0.11	0.46	0.05	0.03	0.30
DÖL	*	*	*	.	.	*0.00*	6.00	3.73	6.00	7.00	2.80	6.00	0.11	0.46	0.05	0.03	0.30
HUN	.	.	.	*	*	*	*0.00*	0.13	2.00	1.00	0.40	0.00	4.42	6.46	6.05	6.03	6.30
ROM	.	.	.	*	*	*	.	*2.13*	1.73	1.13	-0.19	0.13	2.49	4.19	3.78	3.76	4.03
GOL	*	*	*	*	*	*	*	*	*0.00*	1.00	1.80	2.00	4.70	6.46	6.05	6.03	6.30
MAL	*	*	*	*	*	*	*	*	*	*0.00*	1.40	1.00	5.42	7.46	7.05	7.03	7.30
VEM	.	.	*	*	*	*	.	.	*	*	*2.80*	0.40	1.73	3.26	2.85	2.83	3.10
VOD	.	.	.	*	*	*	.	.	*	*	.	*0.00*	4.42	6.46	6.05	6.03	6.30
PIA	*	*	*	*	.	.	*	*	*	*	*	*	*1.47*	0.57	0.15	0.13	0.41
VAL	*	*	*	*	*	*	*	*	*	*	*	*	*	*0.46*	0.51	0.49	0.76
BOL	*	*	*	*	.	.	*	*	*	*	*	*	*	*	*0.38*	-0.03	0.06
TRA	*	*	*	.	.	.	*	*	*	*	*	*	*	*	.	*0.61*	0.06
ALC	*	*	*	.	*	*	*	*	*	*	*	*	*	*	.	.	*0.60*

Presti (2009) PCR-RFLP analysis of mitochondrial DNA in tench Tinca tinca

Journal of Fish Biology

Sample: 105 samples from different areas of Italy. **ND1** (1019bp), **ND6** (576bp), (146bp) **Cytb**, (998bp) for **D-loop**. Overall **25% of *T.tinca* mitochondrial genome**

Methods:PCR-RFLP

Results: The polymorphism at the four segments originated a total of **5 composite haplotypes (H1-H5)** with H1 corresponding to the reference sequence.

The identified markers provide a powerful tool for further studies on this species

TABLE III. Pair-wise test for homogeneity of haplotype frequencies between *Tinca tinca* populations

	PI	VA	TR	BO	AL
PI	—				
VA	***	—			
TR	ns	*	—		
BO	**	***	ns	—	
AL	**	*	ns	ns	—

PI, Pianalto; VA, Valagola Lake; TR, Trasimeno Lake; BO, Bolsena Lake; AL, Alcantara River.
***, $P < 0{\cdot}001$; **, $P < 0{\cdot}01$; *, $P < 0{\cdot}05$; ns, not significant.

Lujic (2017) Phylogeographic identification of Tench from the Northern Balkans and Adjacent Regions and its Implications for Conservation

Zoological studies

Sample: 70 Tench individuals from (Serbia,FYROM Macedonia, Hungary, Croatia)

2 nuclear markers (***Act, RpS7***) and one mitochondrial marker (***Cytb***)

Methods: PCR-RFLP

Results:

1) All markers enabled the identification of two major clades (**Western and Eastern**).

2) Nuclear markers enabled the identification of hybrids between the two clades.

3) Based on **Cytb**, tench is separated in areas north of the Danube River (dominant Western origin) and areas south of the Danube River (dominant Easter origin).

4) **Cytb** also identified the rare western **haplotype W2** in southern balkans which clearly indicated human-aided dispersal of tench.

Kohlmann (2009) Genetic variability and differentiation of wild and cultured tench populations inferred from microsatellite loci

Fish Biology Fisheries

Sample: **792 tench individuals from 21 wild and cultured populations**.(Germany, Czech republic, Spanish, Chinese, Romanian, Turkish, Polish).

Two out of three wild populations and 4 out of 18 strains were already used in (Kohlmann 2007).

Methods: 9 tench specific loci were amplified by PCR.

CEQ 8000 to record microsatellites genotypes. **GENEPOP** to calculate allele numbers, expected heterozygosities and to test for deviations from Hardy-Weinberg equilibrium. **FSTAT** to calculate genetic differentiation between populations.

Results: **66 alleles recorded across loci with 20 of them being private alleles**.

Genetic differentiation between population was moderate to high.

In contrast the **highest variability** were found in wild tench from L**ake Felchowsee** and the cultured strain from **Konigswartha Lake** (Germany).

The smallest genetic distances were found between the geographically most distant populations.

The **Spanish cultured tench strain was homozygous at all loci for all 50 individuals**. Low variability also detected in Turkish wild population and in Chinese population

A Neighbor-Joining tree showed only **two major clades** consisting of 4 and 17 populations respectively. Within the smaller clade the Turkish wild and Spanish, Chinese cultured tench populations formed a sub-cluster with 100% bootstrap support.

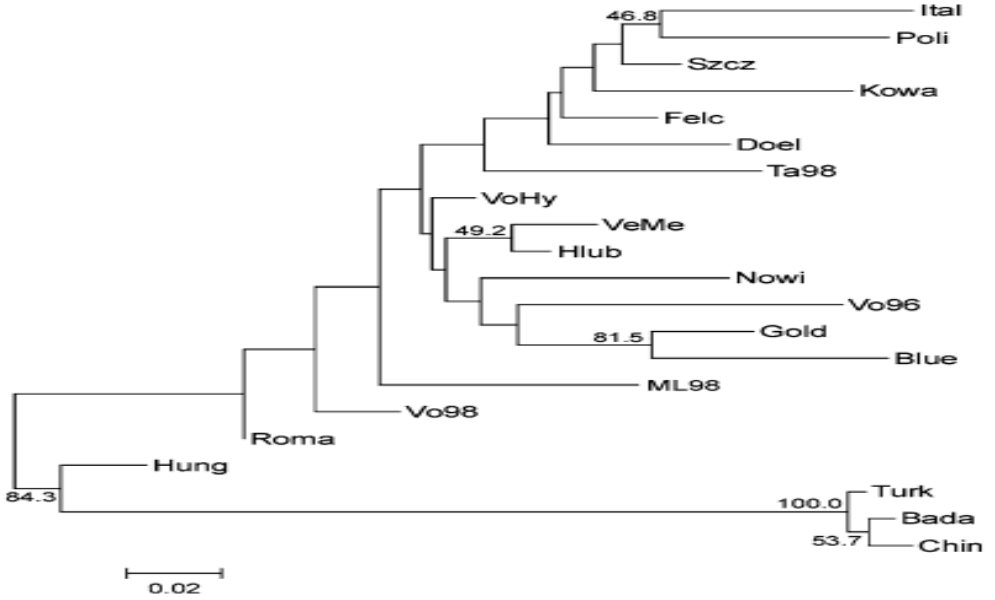


Fig. 1 Neighbor-Joining tree of 21 tench populations based on D_A genetic distances (Nei et al. 1983) and 1,000 bootstrap replicates (only bootstrap support higher than 40% is displayed on tree nodes)

Kohlmann (2005) Microsatellite loci in tench: isolation and variability in a test population

Aquaculture International

Sample: **50 tench individuals** originating from a wild population of Lake Dollnsee Germany.

Methods: Microsatellite loci amplified by PCR based method of generating microsatellite enriched DNA fragment libraries. GENEPOP software to calculate allele genotype frequencies and test deviations from Hardy-Weinberg equilibrium.

Results: Identified 9 loci (MTT-1 to MTT-9). (**MTT-3, MTT-4, MTT-7**) did not show any polymorphims. (**MTT2-2 alleles and MTT-9 9 alleles**)

Conclusion: Tench microsatellite loci **are more suitable markers** for genetic research than enzyme loci.

Table 2 Variability of nine microsatellite loci in tench originating from a wild population.

Locus	Alleles		All examined fish (n = 50)			Wild caught fish only (n = 19)		
	Total number	Size range	H_O	H_E	P_{HW}	H_O	H_E	P_{HW}
MTT-1	5	167–177	0.700	0.683	0.000	0.632	0.740	0.043
MTT-2	2	236–240	0.540	0.416	0.040	0.579	0.462	0.345
MTT-3	1	160	–	–	–	–	–	–
MTT-4	1	211	–	–	–	–	–	–
MTT-5	5	207–217	0.500	0.651	0.053	0.474	0.661	0.089
MTT-6	6	160–174	0.659	0.548	0.199	0.722	0.624	0.385
MTT-7	1	213	–	–	–	–	–	–
MTT-8	3	230–236	0.167	0.173	0.106	0.118	0.169	0.091
MTT-9	9	130–178	0.959	0.844	0.020	0.889	0.859	0.542

H_O, observed heterozygosity; H_E, expected heterozygosity; P_{HW}, exact p-value of Hardy–Weinberg probability test.

Kohlmann (2007) Comparison of microsatellite variability in wild and cultured tench (Tinca tinca)

Aquaculture

Sample: Tench were collected from two wild populations Lake Dollnsee and Felchwsee (Germany) and four cultured strains, (40 to 50 individuals).

Methods: PCR, genotypes recorded and used as input data for the GENEPOP software FSTAT software to calculate genetic variability within group of populations as well as differentiation between populations.

Results:

7 out of the 9 microsatellite were polymorphic with at total number of **49 alleles being recorded**

The number of distinct alleles at polymorphic loci displayed large variation from **2 to locus MTT2** to **9 to locus MTT9**

In contrast to the variability within populations genetic diffierentiation was significant between all of them.

Summary

Arthor	Lujic (2017)	Presti 2014	Presti 2012	Presti 2009	Lajbner (2011)	Lajbner (2011)a	Lajbner (2010)	Kohlmann (2009)
Genes	*Cytb*	*Cytb D-loop*	*Cytb D-loop*	*Cytb D-loop*	*Cytb (full)*	*Cytb (full)*	*Cytb (full)*	*?*
Region	-	*Spain Czech R Romania*	*Spain Czech R*	-	*Spain Czech R Romania England*	*Spain Czech R Romania England*	-	-
Trees		*		*	*			*