

**Phylogeographic and phylogenetic perspectives on
Hucho hucho, *Hucho taimen* *Hucho bleekeri* and
Parahucho perryi – the world's largest salmonid fishes**

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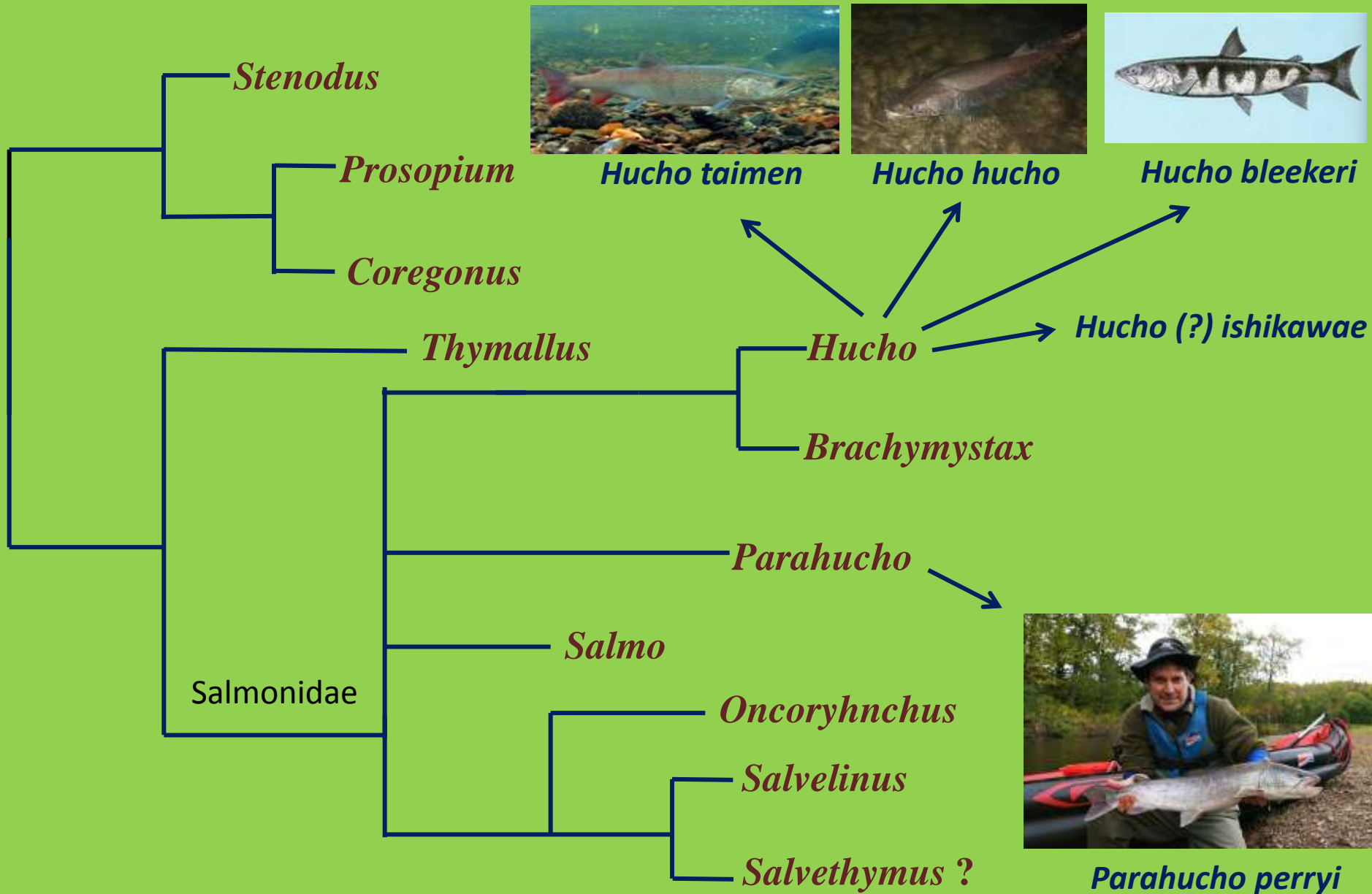


Dagi River, Sahkalin Island, Russia

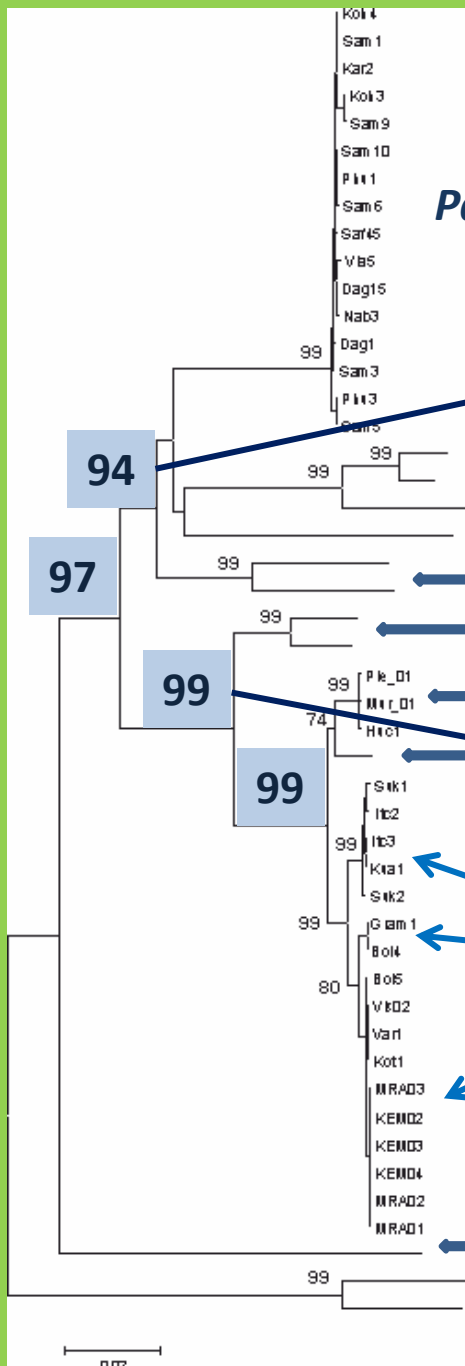
Global Distribution of the Species



Salmonoid Phylogeny (schematic)



Salmonidae Phylogeny (mtDNA, ca. 1700 bases) (ATP6 + partial Control region + partial NADH-1)



Parahucho perryi

all exhibit anadromy

Salvelinus – alpinus + fontinalis

Onchorhynchus mykiss

Salmo

Brachymystax

Hucho hucho

Hucho bleekeri

Hucho and Brachymystax do not exhibit anadromy

Hucho taimen

Strong support for Salmonidae, sister-group status for Hucho and Brachymystax and monophyly for Hucho

Coregonus

Thymallus

0.02

Distribution Area of Danube salmon *Hucho hucho* (Danube endemic)



Map of the Danubian basin, showing the original distribution range of *Hucho hucho* (in grey) as well as the sampling locations (numbered 1-11, and corresponding to the names listed in Table 1 (Weiss et al 2010)

The original distribution was limited to montane tributaries and thus much of this territory, and especially the lowlands of Hungary, Romania and Bulgaria are not *Hucho* habitat.

H. hucho has lost approximately two thirds of its range overall (Holčík et al. 1988) and 90% of its range within Austria (Schmutz et al 2003). In Austria there are reproducing populations in at least the Mur, Gail und Pielach rivers. *Hucho* exists in Germany (Bavaria), Slovenia, Bosnia-Herzegovina, Ukraine, Czech Republic, Croatia, Serbia, Montenegro, Slovakia, Hungary and Poland.

In many rivers, the question arises whether or not populations are self-sustaining, that is would they exist without stocking measures. In the most southern regions (e.g. Montenegro) there is no stocking of *Hucho*. Nonetheless, illegal fishing is a major problem there, and population densities are extremely low.

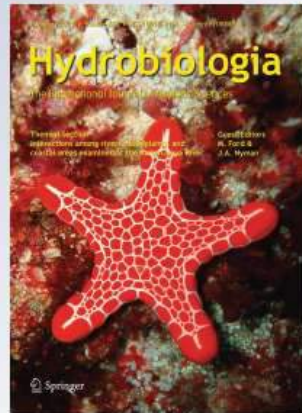
Very low levels of genetic diversity across the entire distribution area – four closely related mtDNA haplotypes across over 1800 base pairs

*Regional structure despite limited mtDNA sequence diversity found in the endangered Huchen, *Hucho hucho* (Linnaeus, 1758)*

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PRIMARY RESEARCH PAPER

Regional structure despite limited mtDNA sequence diversity found in the endangered Huchen, *Hucho hucho* (Linnaeus, 1758)

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Abstract We evaluate the hypothesis of no geographic structure in the Huchen (*Hucho hucho*), a large, predatory salmonid endemic to the Danube basin. Forty-seven individuals sampled from throughout the Huchen's native range were genetically characterized. Extremely limited sequence diversity across 1,800 bases of mtDNA (the complete control region and partial NDH1-L subunit) evidenced by four closely related mtDNA haplotypes was found. Nonetheless, the geographic distribution of mtDNA repeats (5–10, 82-bp long copies per individual) as well as allelic diversity across two microsatellite loci indicated large-scale geographic structure between the north-western (Austria and Slovenia) distribution area and eastern (Slovakia and Ukraine) or southern (Bosnia-Herzegovina and Montenegro) sample sites. An extremely slow rate of substitution for the

H. hucho mtDNA is considered along with the alternative hypotheses to explain the limited mtDNA diversity. Considering the regional genetic structure implied by our data, we advocate restrictions on the transport of brood fish or yearlings across the range of the species distribution and sale of Huchen across international boundaries. Future genetic analysis to support local conservation and monitoring efforts must focus on developing a high-resolution screen that may be applied to identify hatchery versus naturally reproduced individuals in the wild.

Keywords Danube salmon · Danubian basin · Phylogeography · Microsatellites · mtDNA

Introduction

Huchen *Hucho hucho* (Linnaeus, 1758) also known as Danube salmon is one of the most endangered members of the family Salmonidae (Holcik et al., 1993). They are endemic to the Danube basin and historically were common over large areas of the drainage (Holcik 1995). *H. hucho*, along with its sister species taimen *Hucho taimen* (Pallas, 1773), are obligate freshwater residents and nonetheless the largest of all salmonid fishes. While the taimen may exceed 100 kg (Holcik et al., 1993), the largest reported Huchen was 60 kg, caught in the Danube River in 1873, whereas specimens up to 30 kg were still being reported in the mid-twentieth century

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For the four sequence haplotypes, no phylogeographic structure across the range could be found. However, by sequencing across an 82 bp repeat in the mtDNA control region, a statistically significant structure was revealed based on the number of repeats.

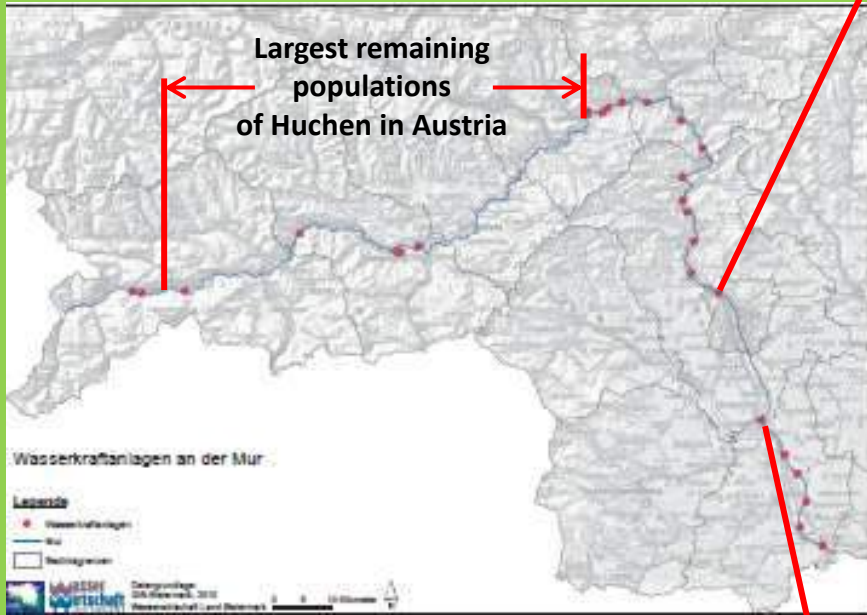
Table 2 Frequency of the 82-bp repeats in the 3'-end of the mtDNA control region across 11 sample sites of *H. hucho*

Population/region	Number of 82-bp long repeats in the control region						Total
	5	6	7	8	9	10	
Austria							
Drau River	–	2	1	3	–	–	6
Gail River	–	2	2	1	–	–	5
Mur River	1	–	1	1	–	1	4
Slovenia							
Krka River	–	–	–	5	–	–	5
Sava River	2	–	–	–	–	–	2
Sora River	–	–	2	–	–	–	2
Bosnia-Herzogevenia							
Drina River	–	–	–	6	1	–	7
Vrbas River	–	–	–	–	2	–	2
Montenegro							
Lake Plav	–	–	–	2	–	–	2
Slovakia							
Vah River	–	–	–	3	1	–	4
Ukraine							
Tisa River	–	–	–	3	–	–	3
Total	3	4	6	24	4	1	41



Caught in Plav Lake, Montenegro in 2004, reportedly 135 cm in total length and 37 kg in weight.

Chain of low-head (7 to 15 MW) hydropower plants on the Mur River in Austria

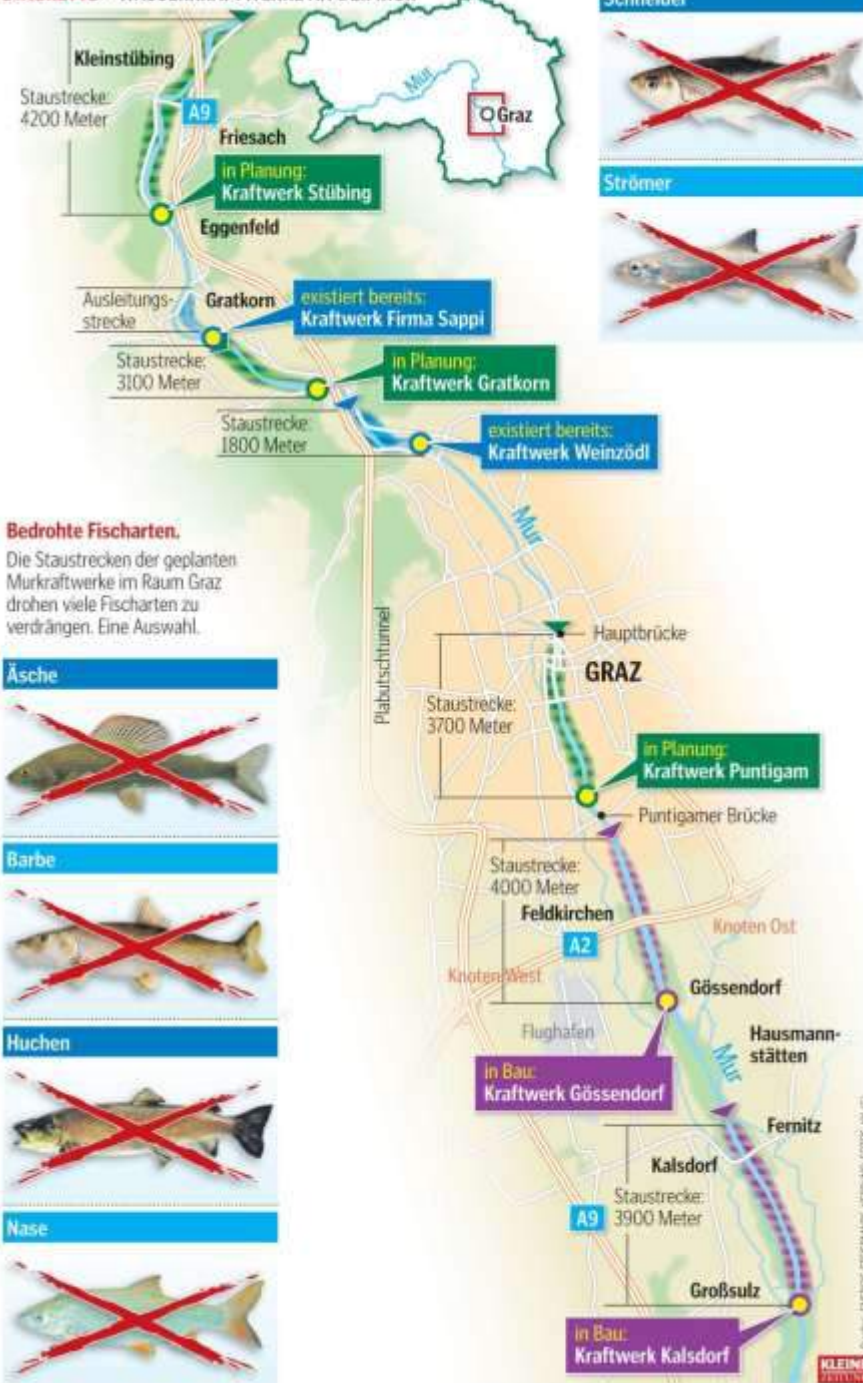


Estimated 1500 adults left in the Mur River, by far the largest population in Austria.

31 dams existing, four more in advanced stages of planning, including the first new dam in the upper Mur, inside a „Natura 2000“ area.

No sign of governmental interest in stopping ANY dam construction due to species protection interests.

STAUKETTE – WASSERKRAFTWERKE AN DER MUR



Media engagement against government and power company propaganda



Abbildung 7: Huchen-Laichplatz, fotografiert von der Hauptbrücke im Grazer Stadtgebiet am 13.5.2011 welche von Weiss an 12.05.2011 im Rahmen der Lehrveranstaltung „Allgemeine Ökologie“ den Studenten vorgeführt wurde. Einige Tage zuvor wurden hier Huchen beim Ablaichen beobachtet.



Abbildung 2: Interview mit dem Kamerateam im Auftrag von ServusTV in der Mur nahe der Mündung des Grazbachs am 2.4.2011, etwa 10 m von der Uferlinie in 20-50 cm tiefen Wasser.

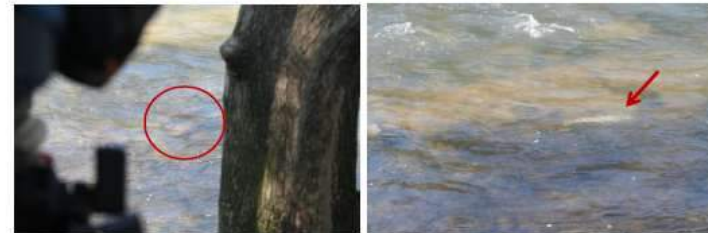
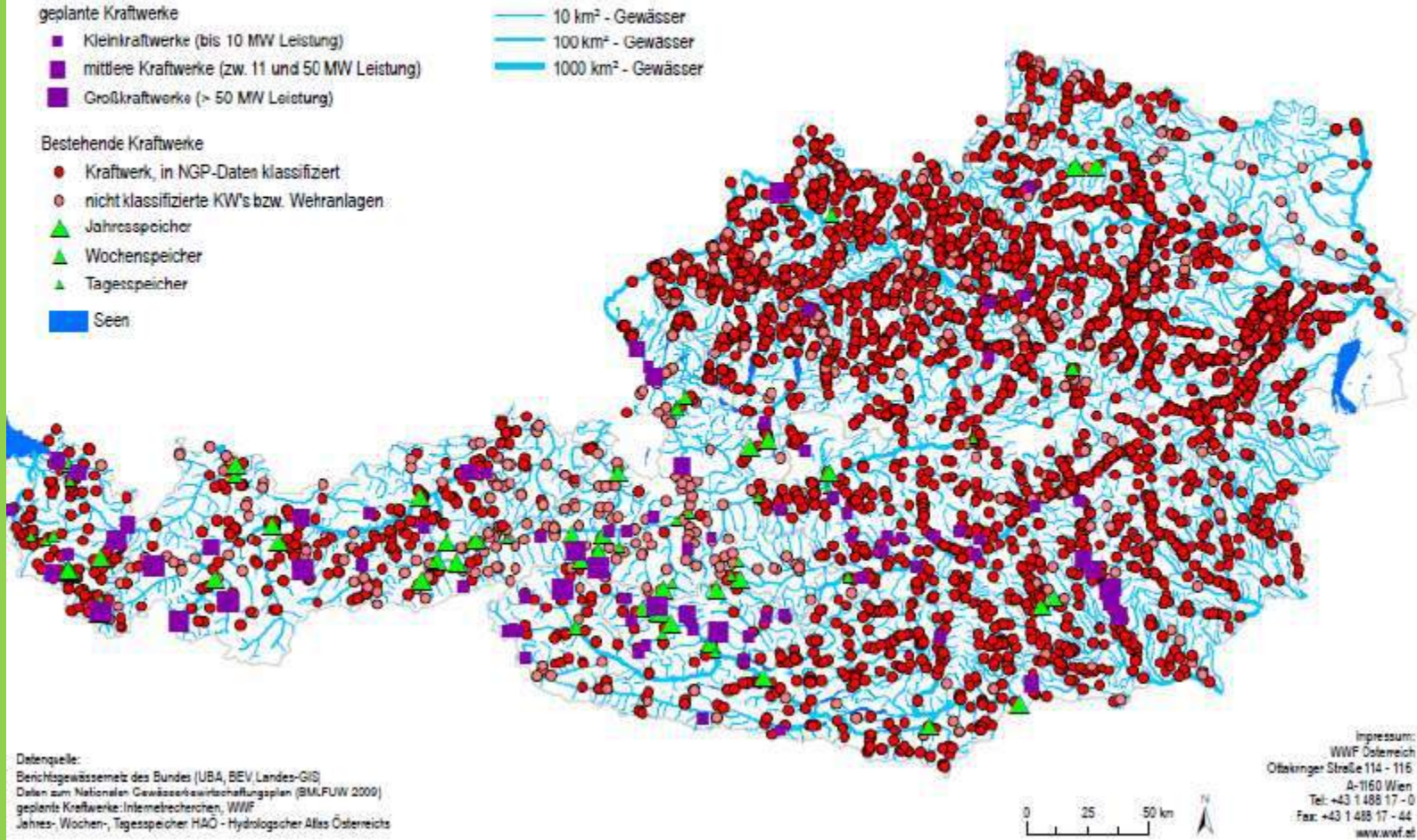


Abbildung 3: Fernsehkamera und ablaichende Huchen (roter Kreis) in der Mur, etwa sechs Meter von der Uferlinie entfernt, ca. 75 m flussabwärts der Grazbach-Mündung (02.04.2011).

Abbildung 4: Das gleiche Huchen-Pärchen. Das vermutliche Weibchen (roter Pfeil) ist im Zuge des Schlagens der Laichgrube seitlich gedreht (02.04.2011).



Documented hydropower plants on Austrian rivers with a catchment area > 10 sq. km



The environmental ministry as well as numerous lobbying groups are calling for expanding hydropower to its fullest potential in Austrian rivers, with no regard to protected areas, outside of a few high elevation national parks

Siberian Taimen Phylogeography - 2005



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FIG. 1. Sample locations for this study. See Table 1 for population codes and names.

Comparative mtDNA sequence (control region, ATPase 6 and NADH-1) divergence in *Hucho taimen* (Pallas) across four Siberian river basins

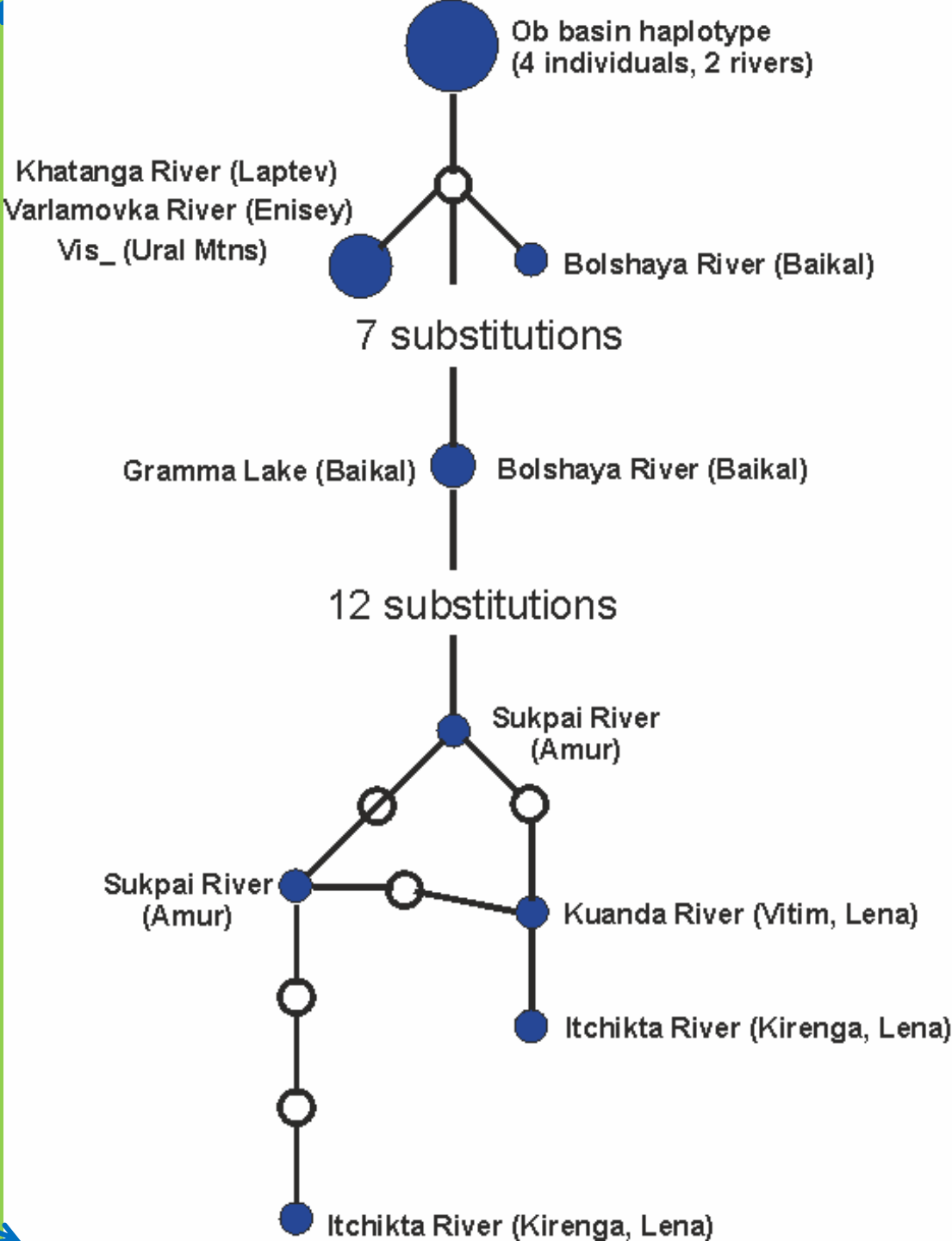
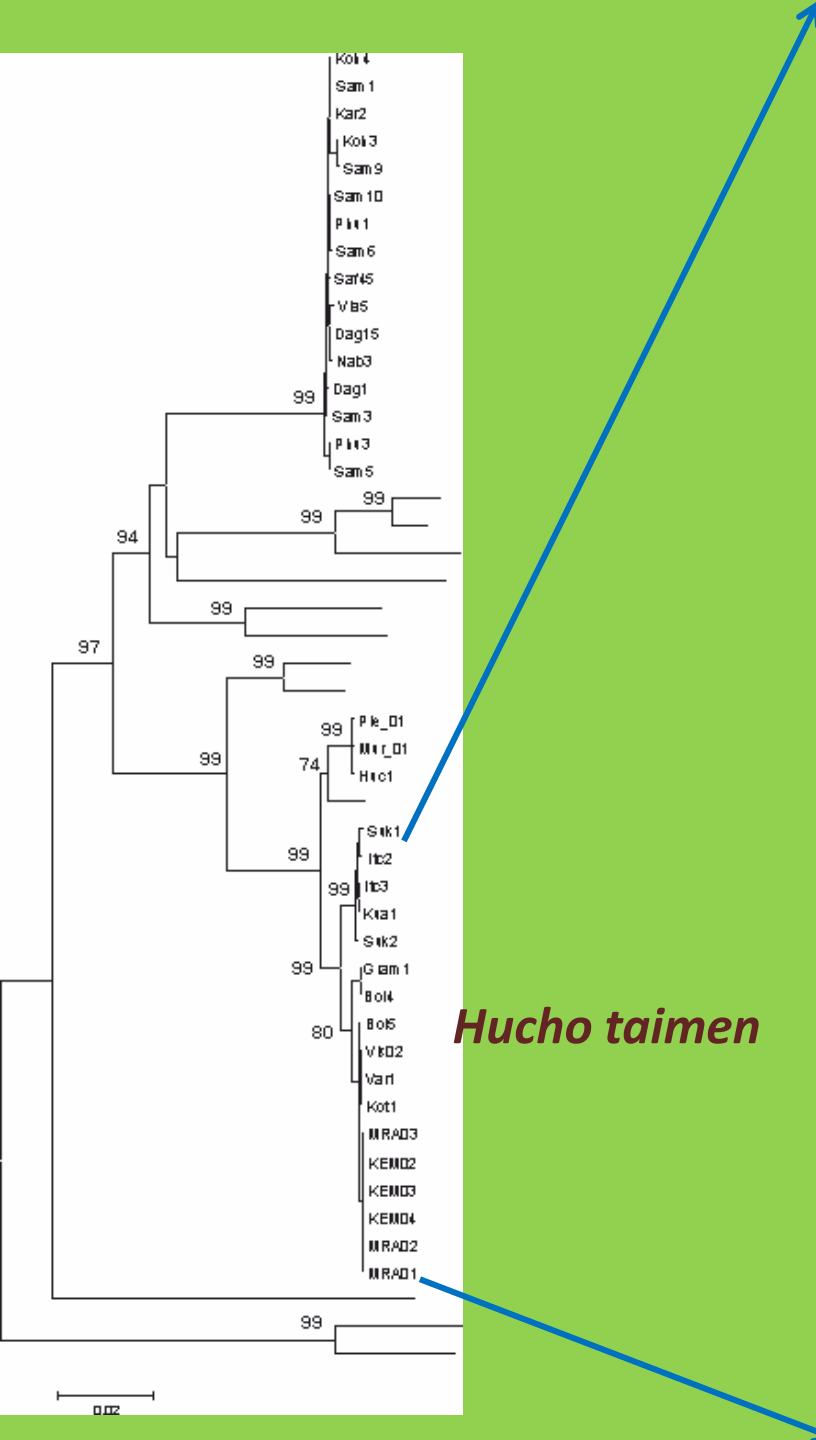
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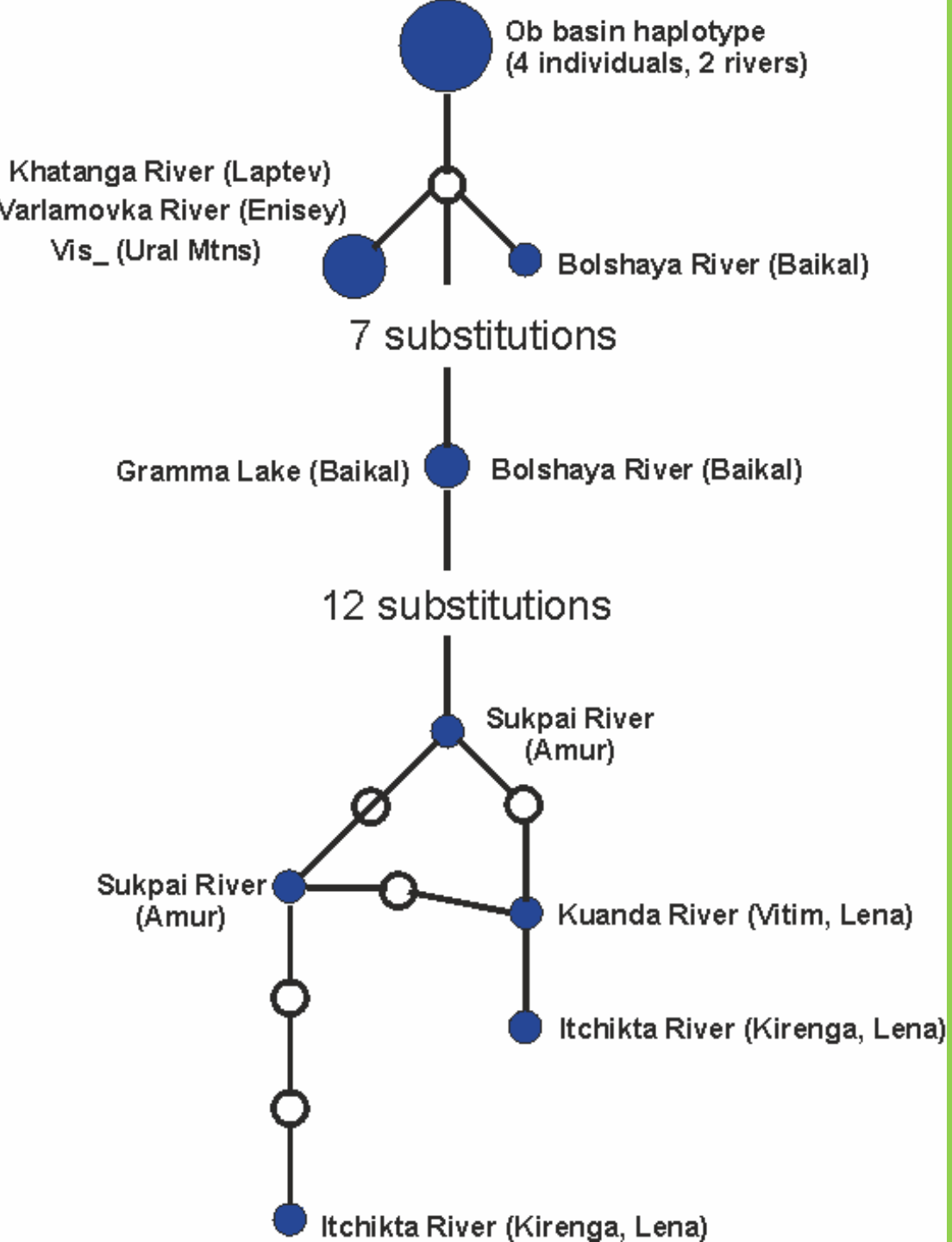
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Salmon from eight populations spanning five drainage basins (Amur, Lena, Enkel and Khatanga) were analysed for nucleotide sequence variation across three mitochondrial genes (ATP6, NADH1 and control region). Samples of *H. hucho*, *Salvelinus leuiscus* (deep-seated and mountainous forms) and *Parachanna percyi* were also included for comparison. Nucleotide variation across a total of 1028 base pairs in *H. taimen* revealed shared haplotypes between the Amur and Lena basins, further supporting a previous hypothesis of late to post-Pleistocene hydrological exchange between these now disparate basins. In contrast to an earlier study using the control region alone, clear phylogeographic structure was seen at a large geographic scale, reflected by two phylogenetic clades, one corresponding to the Amur and Lena basins, and the other to the Enkel and Khatanga basins. Comparative rates of divergence revealed intermediate rates and low heterogeneous substitution rates for the two coding genes, respectively at interspecific levels compared to the mtDNA control region. © 2005 The Authors. Journal compilation © 2005 British Ecological Society

Key words: *Salvelinus leuiscus*, *Hucho hucho*, *Parachanna percyi*, phylogeography, Siberian taimen.





Haplotype network for *Hucho taimen* across all of Russia

Three clades are evident, with significant basin haplotype sharing

Ob, Enisey, and Volga

Some presumably ancestral haplotypes in the upper Enisey Basin near Baikal

and

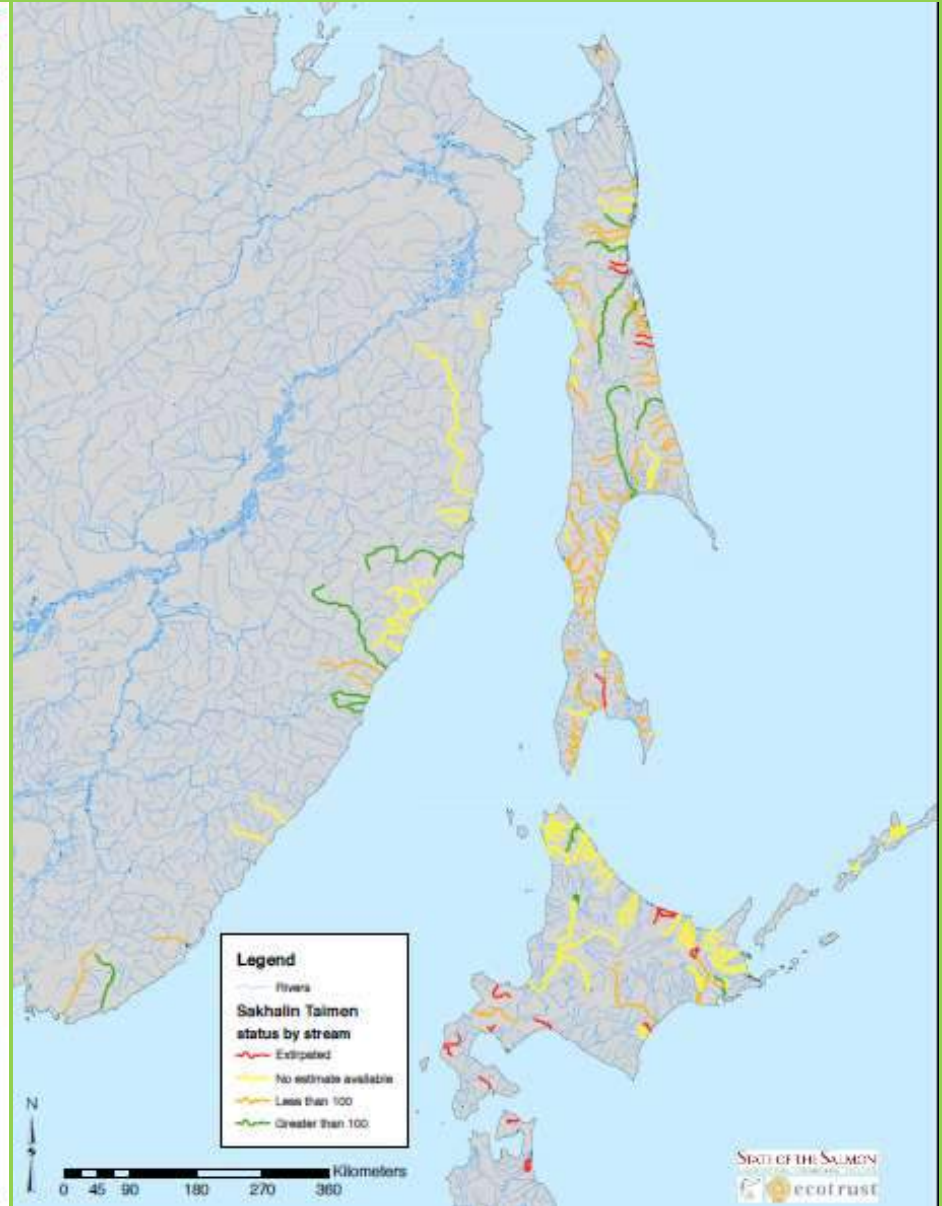
The Amur and Lena basins

Haplotype divergence spans only approximately 1.5% and clades are separated by a maximum of 0.5%.

Distribution of *Parahucho perryi*

Sakhalin Taimen Tissue Sample Locations

Hucho perryi

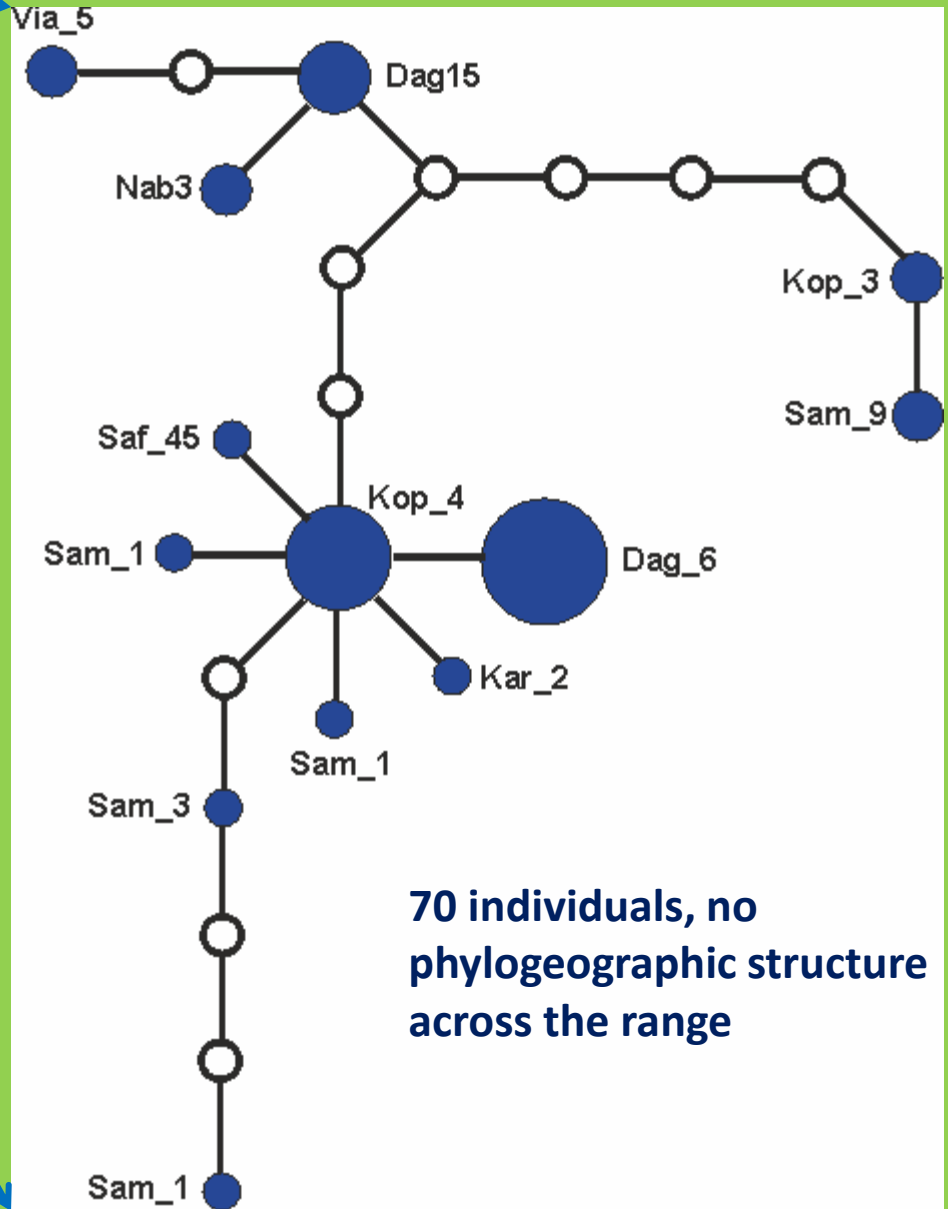
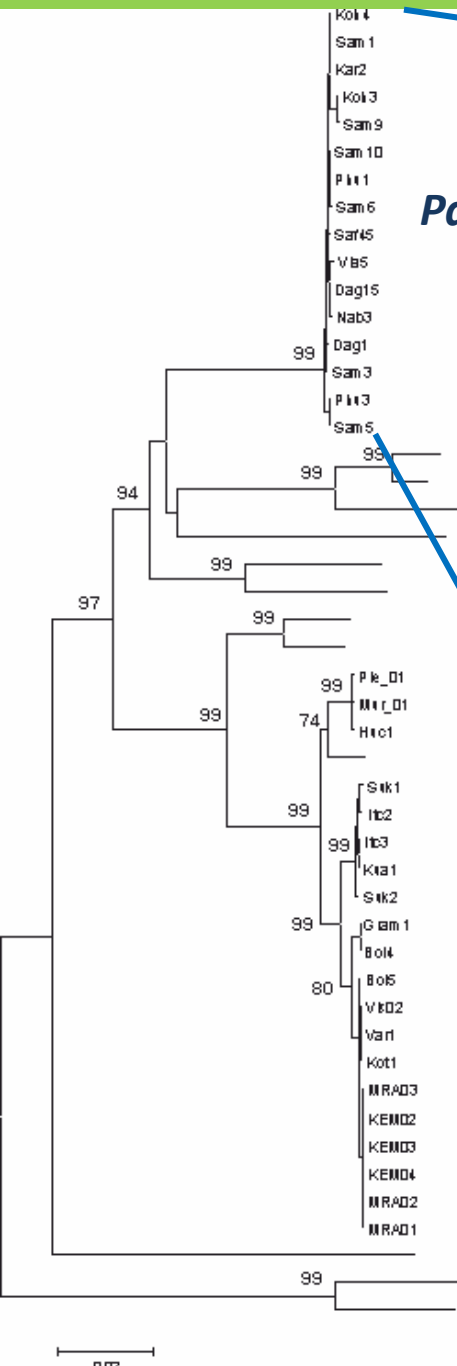


Parahucho perryi – Dagi River expedition

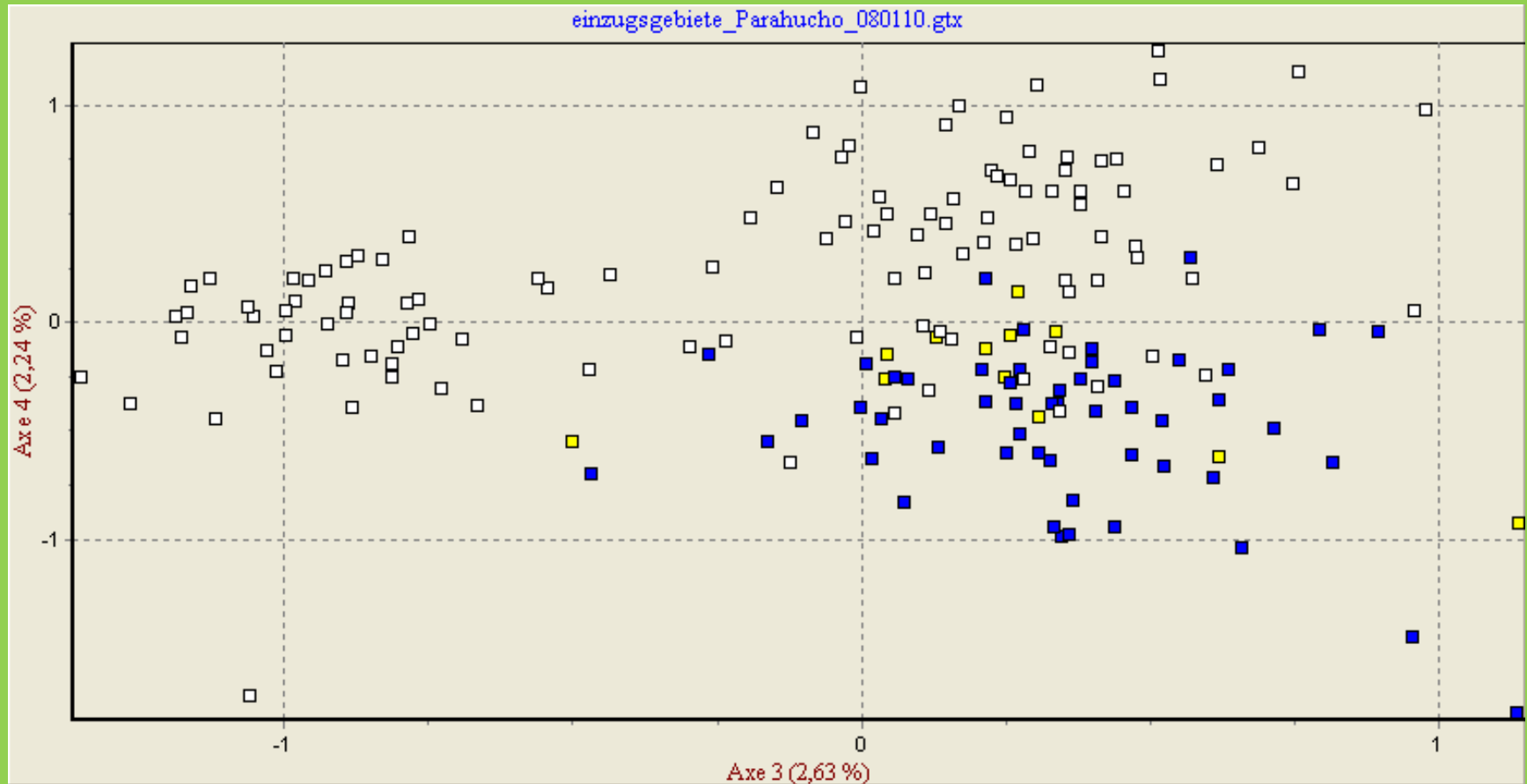


Haplotype network for *P. perryi* (< 1% divergence)

Parahucho perryi

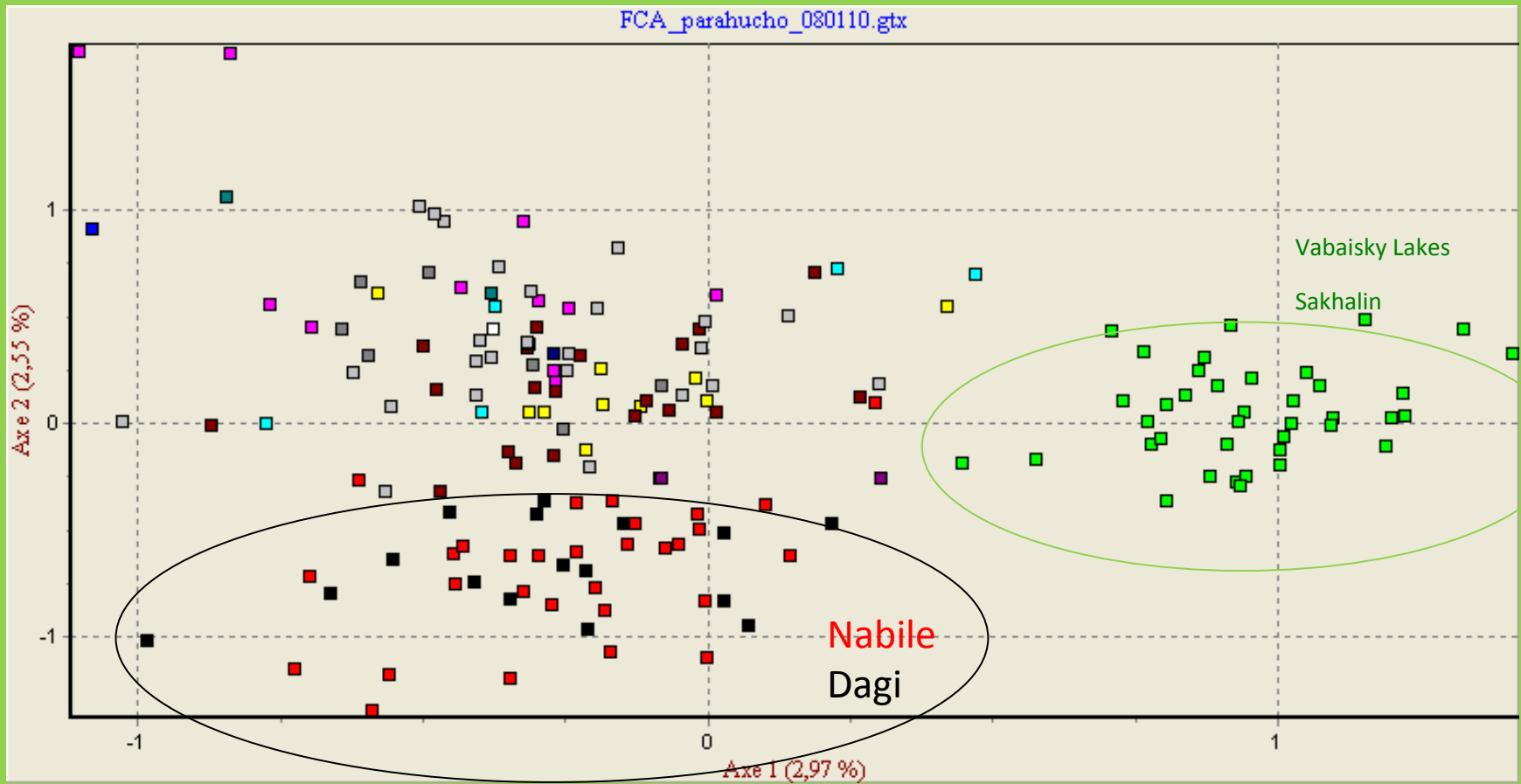


Factorial Correspondence Analysis based on 10 microsatellite loci (Outliers removed, coded by major drainage)



- Sakhalin
- Russian mainland
- Hokkaido

Factorial Correspondence Analysis based on 10 microsatellite loci (coded by population)



Summary

Hucho hucho – limited „endemic“ distribution, extremely low levels of mtDNA variation, self-reproducing populations still in decline. Where investigated, moderate levels of variation at the population level, very little regional data on self-reproducing status of populations

Hucho bleekeri – more or less equally diverged from *H. hucho*, and *H. taimen*, extremely limited distribution, no further genetic information available.

Hucho taimen – very large distribution, limited mtDNA variation for such a large range, some lineage diversification across major drainages, potentially relevant for conservation purposes, Ural specimens clearly *H. taimen*.

Hucho ishikawae – no data

Parahucho perryi – clearly distinct genus, limited distribution, mtDNA variation moderate, but no sign of phylogeographic structure with the exception of a few isolated populations, limited population genetic structure, some signs of inbreeding.

THANK YOU!

(and special thanks to Pete Rand of the Wild Salmon Center)



Mur River, City Center Graz, Austria