

## Phylogenetic position of *Salmo (Platysalmo) platycephalus* Behnke 1968 from south-central Turkey, evidenced by genetic data

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To determine whether the current classification of the flathead trout *Salmo (Platysalmo) platycephalus*, endemic to the upper reaches of the Zamanti River system, Turkey, based solely on morphology, is in congruence with molecular phylogeny, the nucleotide sequence variation in mitochondrial (control region and cytochrome *b* gene) and nuclear (internal transcribed spacer of rRNA genes) DNA for the flathead trout and various representatives of the genus *Salmo* was studied. On the basis of pair-wise genetic distance estimates, the highest differences were found to exist between the flathead trout and *S. salar*, *S. ohridana* and *S. obtusirostris*, whereas the differences between the flathead trout and *S. trutta* were minimal. All the analyses performed firmly positioned the flathead trout within the Adriatic phylogeographic lineage of *S. trutta*; however, the exact position of the flathead trout within the Adriatic cluster was irresolvable. Accordingly, classifying the flathead trout as a subgenus of *Salmo* is unjustifiable and its reclassification in a lower taxonomic category is suggested by the present study. © 2004 The Fisheries Society of the British Isles

Key words: archaic trout; ITS1; mtDNA; *Salmo trutta*; taxonomy; Turkey.

### INTRODUCTION

The Mediterranean–Adriatic region contains the richest source of endemic Salmoninae species as well as the greatest differentiation within the species *Salmo trutta* L. (Behnke, 1968). A group of the so-called archaic trout [*Salmothymus*, *Acantholingua* and *Salmo (Platysalmo)*] is also known to inhabit this basin. As the evolutionary history and classification of *Acantholingua ohridana* (Steindachner) and *Salmothymus obtusirostris* (Heckel) have already been studied and determined (Philips *et al.*, 2000; Snoj *et al.*, 2002; the newly proposed classification, *i.e.* *Salmo ohridana* and *Salmo obtusirostris*, is used hereafter), the flathead trout *Salmo (Platysalmo) platycephalus* Behnke (Fig. 1) is the last in this group whose phylogeny, although vital for understanding comprehensive evolutionary pathways of salmonid fishes, is still controversial and whose status within the Salmoninae has not yet been firmly established.

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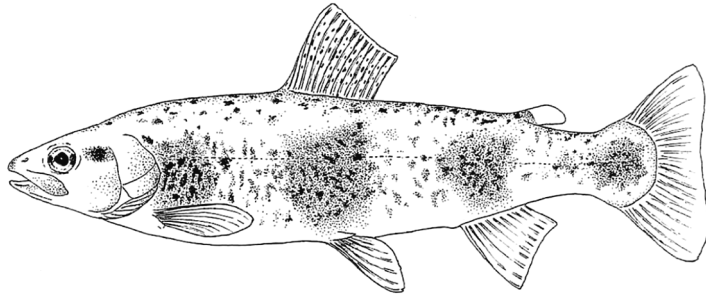


FIG. 1. A mature *Salmo* (*Platysalmo*) *platycephalus* from the River Soguksu, southern Turkey (31 cm fork length).

The flathead trout has a very restricted distribution. So far, it has been observed only in the Rivers Soguksu, Karagöz (Behnke, 1968; pers. obs.) and Uzunyayla (A. Alp pers. comm.; pers. obs.), the tributaries to the River Zamanti which belongs to the Seyhan Basin emptying into the Mediterranean Sea (Fig. 2). According to native fishermen, the flathead trout are also caught in the River Zamanti near the town of Pinarbasi (Fig. 2). There is no salmonid habitat, however, in the River Zamanti north and south of the town. In tributaries of the lower course of the River Zamanti (the Rivers Ecemis and Kapuz; Fig. 2), only brown trout have been observed (Ekingen, 1976; pers. obs.). Four brown trout originating from the Zamanti River system, one from the River Kapuz and three from the River Zamanti itself, have been recently reported and examined on the basis of mitochondrial DNA (Bernatchez, 2001). The latter specimens were recognized as Adriatic whereas the former one as the Danubian phylogeographic lineage of *S. trutta* (Bernatchez, 2001).

The flathead trout was discovered by Behnke in 1968, on the basis of three specimens, originating from the River Soguksu. The specimens were morphologically examined and compared with various representatives of the Salmoninae. On the basis of external appearance, they were found to be distinct from other species of the genus *Salmo* 'by complete absence of spots or pronounced markings on body or fins' (Behnke, 1968). Based primarily on the dentition and number of pyloric caeca (15, 16) and gill rakers (23, 24), Behnke (1968) recognized the newly discovered salmonid as a new species. He considered it an early branch within the Salmoninae having diverged long before differentiation of modern *Salmo salar* L. and *S. trutta*. But nevertheless, he assigned flathead trout to the genus *Salmo* and placed it in a new monotypic subgenus *S. (Pl.) platycephalus*, 'to emphasize its uncertain affinities'. Cladistic analysis of morphological characters performed by Stearley & Smith (1993) supported Behnke's (1968) hypothesis of the flathead trout as an early branch within Salmoninae and denoted it as a sister species of *S. obtusirostris*. On the basis of the latter evidence, Stearley & Smith (1993) reclassified *S. (Pl.) platycephalus* (*S. platycephalus* in the following text) in the genus *Salmothymus* Berg 1908 as *Salmothymus platycephalus* (Behnke). The common name for the flathead trout in Turkey is 'alabalik', however, this designation also describes other native trout of Turkey.

Due to fishing pressure and introduction of non-native, competitive species [e.g. rainbow trout *Oncorhynchus mykiss* (Walbaum)], *S. platycephalus* became

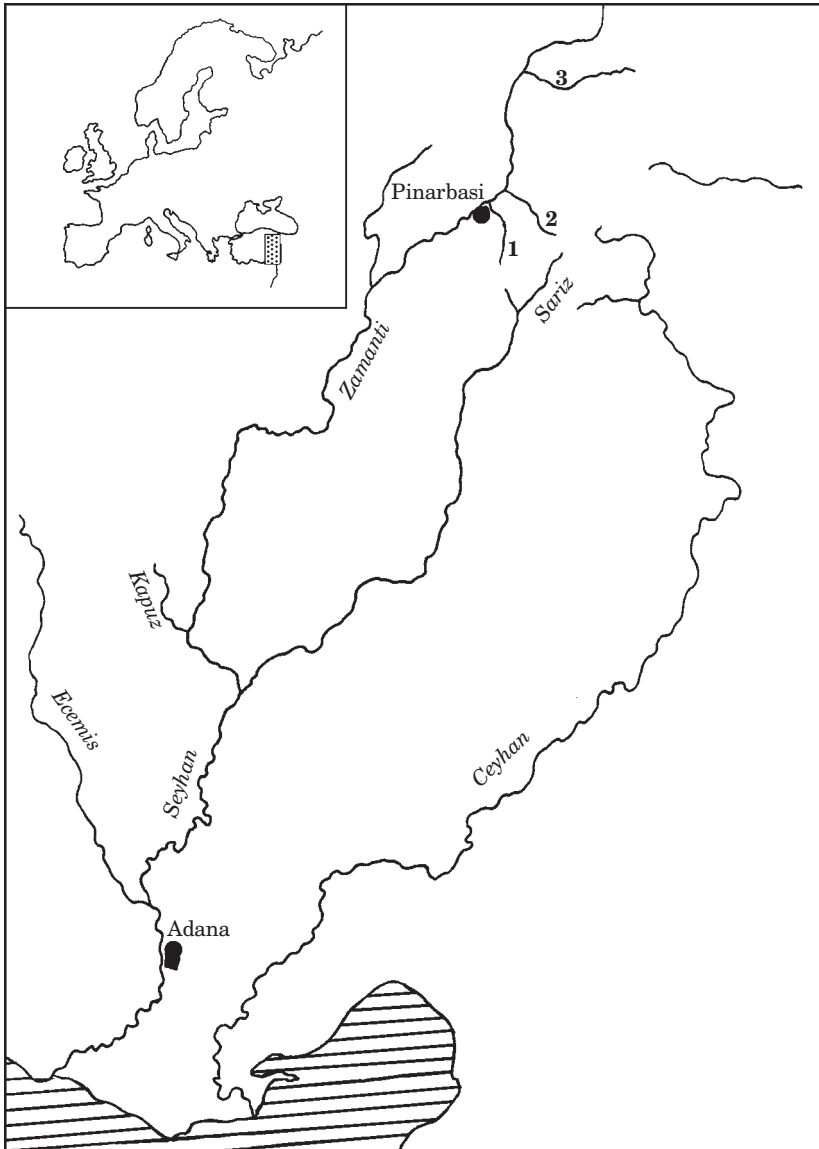


FIG. 2. The distribution of *Salmo platycephalus* in the upper reaches of the Zamanti River system: Rivers (1) Soguksu, (2) Karagöz and (3) Uzunyayla. In the Rives Sariz, Kapuz and Ecemis, only *Salmo trutta* were found.

critically endangered, and is as such listed under the IUCN red list (IUCN, 2003). Furthermore, in 1997 the upper reach of the River Soguksu was polluted by sediments and over-fertilization due to road construction. Salmonids could only survive in the lower part of the stream, or in the River Zamanti. Since the whole stream has now been restored, the population of *S. platycephalus* has become well established again. Recent observation indicates that *S. platycephalus*

is the most numerous in the River Uzunyayla (pers. obs.). In the River Karagöz, this fish has become scarce, probably due to overfishing.

To date, information about the flathead trout are based primarily on the study of Behnke (1968). Additional information, based on the first field observations and describing mainly its habitats and distribution, is given by Schöffmann (1992). Despite the flathead trout's unusual appearance, uncertain phylogeny and 'vague' classification, it has not attracted any further scientific attention. The objective of the present study was to investigate the phylogeny of the flathead trout in order to examine whether its current classification, established on morphology, is supported also by molecular data. To obtain a comprehensive picture of the evolutionary history of the flathead trout, mitochondrial DNA (the control region and cytochrome *b* gene) and nuclear DNA (internal transcribed spacer of ribosomal RNA genes; ITS1) were employed in this study.

## MATERIALS AND METHODS

### SAMPLES AND DNA ISOLATION

Overall, nine mature individuals of the flathead trout were collected, five from the type locality in the River Soguksu, and four from the River Uzunyayla, in 2002 and 2003, respectively (Fig. 2). Additional samples representing five phylogeographic lineages of *S. trutta*, (Table I) and *S. ohridana*, *S. obtusirostris* and *S. salar* were also included.

Comparative mtDNA control region analysis was based on the regions which were according to previous studies (Snoj *et al.*, 2002; unpubl. data) recognized as informative (*i.e.* between the tRNA<sup>Pro</sup> gene and poly T block and *c.* 190 bp at the 3'-end of the control region, altogether 740 bp). Cytochrome *b* gene and ITS1 were inspected in the regions which were previously reported as informative (Giuffra *et al.*, 1994; Patarnello *et al.*, 1994; Phillips *et al.*, 2000; Presa *et al.*, 2002). Total DNA was isolated from fin clips, preserved in 96% ethanol, using the Wizard Genomic DNA Purification Kit (Promega), following the manufacturer's instructions.

### DNA AMPLIFICATION AND SEQUENCING

PCR amplification of an *c.* 2400 bp mtDNA fragment composed of cytochrome *b* gene and control region was performed using primers HN20 (Bernatchez & Danzmann, 1993) and C-Glu (Cronin *et al.*, 1993). The conditions of PCR were: initial denaturation (95° C, 3 min) followed by 30 cycles of strand denaturation (94° C, 45 s), primer annealing (52° C, 45 s) and DNA extension (72° C, 2 min). Primers KP2 and 5.8S were utilized for DNA amplification of the ITS1 region as described by Phillips *et al.* (2000).

All PCR amplifications were performed in a programmable thermocycler GeneAmp<sup>®</sup> PCR System 9700 (PE Applied Biosystems). A total volume of 30 µl contained 1 µM of each primer, 0.2 mM dNTP, 1.5 mM MgCl<sub>2</sub>, 1 × PCR buffer, 1 U of *Taq* polymerase (PE Applied Biosystems) and 100 ng of genomic DNA. Amplified DNA fragments were run on a 1.5% agarose gel and were isolated from the gel using the QIAEX II Gel Extraction Kit (QIAGEN).

All sequencing reactions were prepared using BigDye Terminator Ready Reaction Mix (PE Applied Biosystems) according to the manufacturer's instructions. The control region fragment between the tRNA<sup>Pro</sup> gene and poly T block was sequenced using primers 28RIBa (Snoj *et al.*, 2000) and vHF-F. The sequencing primer at the 3'-end of the control region was HN20 (Snoj *et al.*, 2002). The 5'-end of the cytochrome *b* gene was sequenced using the primers C-Glu. For sequence determination of the ribosomal ITS1, the primers KP2 and 5.8S were used. Termination PCR reactions were performed in a

TABLE I. Sample description: species name, number of individuals analysed (*n*) and GenBank accession numbers for mtDNA control region (5'- and 3'-end), cytochrome *b* gene and ITS1 region

Species/lineage (haplotypes)	<i>n</i>	GenBank accession number				Cytochrome <i>b</i> gene	Ribosomal ITS1
		MtDNA control region		3'-end	5'-end		
		3'-end	5'-end				
<i>Salmo platycephalus</i>	9	AY260514*	AY260515*	AY260506*	AY260508*	AY260508*	
<i>Salmo trutta</i>	1	AY260516*	AY260517*	X76250	AY260507*	AY260507*	
	1	AY260518*	AY260519*	X76251	/	/	
	2	AY260520*	AY260521*	X76251	AY260507*	AY260507*	
	5	AY260522*	AY260523*	X76251	AY260511*	AY260511*	
	1	AF253550	AF253550	X76248	AF434217	AF434217	
	1	AY260524*	AY260525*	X76252	AY260510*	AY260510*	
	1	X93586	X93586	X76254	AF434205	AF434205	
<i>Salmo salar</i>	1	U12143	U12143	X76253	AF201312	AF201312	
<i>Salmo obtusirostris</i>	10	AF488535	AF488535	AF488535	AF488534	AY260509*	
<i>Salmo ohridana</i>	2	AY260512*	AY260513*	AF202033	AF202033	AF201313	

\*Sequences obtained in this study.

programmable thermocycler Gene Amp<sup>®</sup> PCR System 9700 (PE Applied Biosystems) under the following conditions: 10 s denaturation at 96° C, 5 s annealing at 50° C and 4 min extension at 60° C, repeated for 30 cycles. The amplified, fluorescently labelled and terminated DNA was salt-precipitated and analysed on the ABI PRISM 310 automated sequencer.

## DATA ANALYSIS

The computer programme ClustalX (Thompson *et al.*, 1994) was used to align sequences. Sequencing data were subjected to a distance analysis using the PHYLIP computer package (Version 3.5c; Felsenstein, 1993). Sequence divergences were calculated with the DNADIST programme, applying the Kimura-two-parameter model (Kimura, 1980) and with a transition : transversion ratio = 2.

A phylogenetic tree was generated from the aligned sequences using quartet-puzzling, maximum likelihood procedure in the PUZZLE programme, version 5.0 (Strimmer & von Haeseler, 1996). It was performed under the HKY model of sequence evolution (Hasegawa *et al.*, 1985).

## RESULTS

### MITOCHONDRIAL DNA

For all the specimens of *S. platycephalus* analysed, a whole mtDNA control region and a 276 bp at the 5'-end of the cytochrome *b* gene were determined. No variation was observed among them.

Nucleotide identity test of the mtDNA control region between *S. platycephalus* and the other representatives of the genus *Salmo* revealed the highest similarity between *S. platycephalus* and the Ad1, Ad3 and Ad4 individuals (the Adriatic lineage of the brown trout; Bernatchez, 2001). The *S. platycephalus* haplotype differed from each of the three Adriatic ones in only two mutational events (all of them were transitions), located at the positions 57, 277, 542 and 997 (Table II). The latter mutation, characterized by thymine, was so far found only in *S. platycephalus*. Pair-wise genetic distances for these three haplotypes were 0.27% (Table III), whereas the overall span of pair-wise genetic distances within the *S. trutta* complex observed in this study ranged from 0.27 to 1.78% (mean  $\pm$  s.d.,  $0.92 \pm 0.49$ ). Other pair-wise relations were comparable to those previously reported (Bernatchez *et al.*, 1992).

In the cytochrome *b* gene region, a novel haplotype, unique to the flathead trout, was also detected. It was characterized by a mutation event at site 7 (Table II, numbering as in GenBank) where thymine was found to be characteristic for the flathead trout, and cytosine for all the other *Salmo* representatives studied. This single nucleotide mutation event occurred at the third place of a codon and did not involve amino acid change. Other mutations, found in this study, have already been described (Giuffra *et al.*, 1994; Snoj *et al.*, 2002) and were not informative in this context. On the basis of the pair-wise genetic distances, the flathead trout was most related to the Adriatic or *marmoratus* lineage of *S. trutta* (Table III; Giuffra *et al.*, 1994). The genetic distances between *S. platycephalus* and Ad3, Ad4 and Ma1-haplotype individuals (they share the same cytochrome *b* gene haplotype) and *S. platycephalus* and Ad1-haplotype individuals were 0.36 and 0.72%, respectively, whereas the overall

TABLE II. (a) Variable nucleotide positions of the informative part of mtDNA control region (for the variable positions of the 5'-end, see Bernatchez, 2001) and cytochrome *b* gene, and (b) the ribosomal ITS1 region. Numbers above the nucleotides refer to the variable positions according to the successive nucleotide in the sequences under the Genbank accession number AF488535 (mtDNA control region), AY260506 (cytochrome *b* gene) and AY260508 (ITS1 region). The nucleotide for each position is given for *S. platycephalus*, for other haplotypes variable nucleotides are entered, the identity (-) and deletion (/) are indicated

	Variable sites																															
	mtDNA control region										Cytochrome <i>b</i> gene																					
Species/lineage (haplotypes)	4	5	5	5	8	8	9	9	9	9	9	9	9	9	1	7	2	2	2	2	2	4	6	7	7	8	1	1	1	2	2	
<i>S. platycephalus</i>	A	T	G	G	C	C	T	G	A	C	T	T	C	C	A	T	C	T	T	G	C	A	C	C	T	A	T	C	A	T	G	
<i>S. trutta</i>	-	C	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	
Ad3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Ad4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Ma	-	-	-	-	-	T	-	-	-	-	-	-	-	-	T	C	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	
Me	-	-	-	-	-	-	-	-	-	-	/	-	-	-	-	C	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	
Da	-	C	A	C	T	-	-	-	-	-	/	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	
At	-	-	-	-	-	-	A	-	A	/	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	
<i>S. obtusirostris</i>	-	C	-	-	-	-	-	-	-	/	-	-	-	-	T	C	G	C	-	-	T	G	-	-	C	-	C	T	-	-	C	A
<i>S. ohridana</i>	G	-	-	-	-	-	-	G	-	-	/	-	-	-	-	C	-	C	-	-	T	G	-	-	C	C	T	G	-	-	C	A



TABLE III. Pair-wise genetic distance estimates under the Kimura-two-parameter method for mtDNA control region, cytochrome *b* gene and ITS1 region between *S. platycephalus* and the other *Salmo* representatives studied

	Control region	Cytochrome <i>b</i> gene	Ribosomal ITS1
<i>S. platycephalus</i> / <i>S. trutta</i> Ad1	0.27	0.73	0.18
<i>S. platycephalus</i> / <i>S. trutta</i> Ad3	0.27	0.36	No data
<i>S. platycephalus</i> / <i>S. trutta</i> Ad4	0.27	0.36	0.18
<i>S. platycephalus</i> / <i>S. trutta</i> Ma	0.54	0.36	1.23
<i>S. platycephalus</i> / <i>S. trutta</i> Me	0.82	1.09	0.53
<i>S. platycephalus</i> / <i>S. trutta</i> At	1.09	1.09	0.70
<i>S. platycephalus</i> / <i>S. trutta</i> Da	1.50	1.09	1.23
<i>S. platycephalus</i> / <i>S. obtusirostris</i>	1.37	2.95	1.23
<i>S. platycephalus</i> / <i>S. ohridana</i>	1.23	3.70	1.59
<i>S. platycephalus</i> / <i>S. salar</i>	7.34	6.01	1.60
<i>S. trutta</i> / <i>S. salar</i>	7.04–7.94	4.86–6.01	1.60–1.96

span of pair-wise genetic distances within *S. trutta* complex ranged from 0.36 to 1.45% (mean  $\pm$  s.d.,  $0.78 \pm 0.39$ ).

According to the Kimura-two-parameter distance calculation, using the combined data set of the control region and the cytochrome *b* gene, the sequence for *S. platycephalus* was most related to the sequences of *S. trutta* Adriatic lineage. The phylogenetic tree based on these data clearly showed that the *S. platycephalus* belongs to the Adriatic cluster, however, the branching within the cluster was not resolved (unpubl. data).

#### RIBOSOMAL INTERNAL TRANSCRIBED SPACER 1

The 574 bp sequence of the ITS1 was identical in all the specimens of *S. platycephalus* analysed. In comparison to the other *Salmo* representatives studied, this sequence was most similar to that for the brown trout of the Adriatic lineage, differing from it in only one transition at the position 19. Genetic distance between these two sequences was 0.18, whereas the overall pair-wise genetic distance span within the *S. trutta* complex ranged from 0.00 to 1.41% (mean  $\pm$  s.d.,  $0.798 \pm 0.53$ ). The ITS1 sequence of *S. platycephalus* was most related to the sequences of Ad1 and Ad4 (sharing the same ITS1 sequence) and Me individuals. Contrary to the results obtained by mtDNA analysis, no considerable relationship was observed between the ITS1 sequences of *S. platycephalus* and *marmoratus* phylogeographic lineage (Tables II and III).

Among the samples sequenced, no heterozygotes were found in the ITS1 region.

#### COMBINED ANALYSIS OF MITOCHONDRIAL AND NUCLEAR DATA

A combined analysis of mitochondrial control region, cytochrome *b* gene and ITS1 region, performed for *S. salar*, *S. obtusirostris*, *S. ohridana* and five phylogeographic lineages of *S. trutta*, produced a phylogenetic tree which represents relationships among the taxa as shown in Fig. 3. A close relationship of the

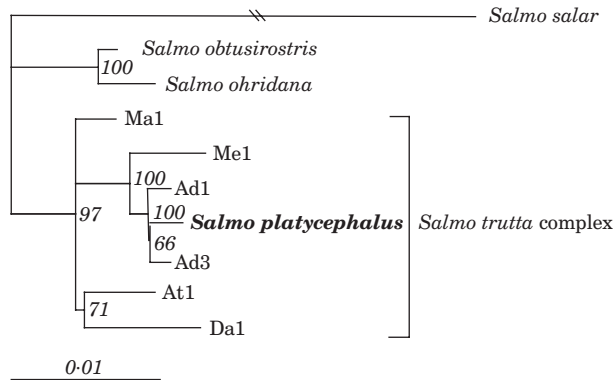


FIG. 3. Maximum likelihood tree, based on combined data set of mtDNA control region, cytochrome *b* gene and ITS1 region, relating the *Salmo obtusirostris*, *Salmo ohridana*, *Salmo platycephalus* and *Salmo trutta* complex (abbreviations Da, At, Me, Ad, and Ma refer to main phylogenetic lineages). *Salmo salar* represents the outgroups of the tree. Confidence statements (%) estimated from 10 000 puzzling steps are shown between the nodes.

*S. platycephalus* haplotype and the others from the Adriatic cluster of *S. trutta* is evident.

## DISCUSSION

The classification of the flathead trout was until now based only on morphological data. According to this criterion, the flathead trout has been recognized as an archaic trout. It has already been demonstrated, however, that salmonid phylogeny, deduced from morphology may be questionable (Oakley & Phillips, 1999) and as recently reported for *S. ohridana* and *S. obtusirostris* (Philips *et al.*, 2000; Snoj *et al.*, 2002), not necessarily in congruence with phylogeny based on molecular data. The results presented in this study once again demonstrated this discrepancy. Namely, the molecular data obtained and analysed do not support the current classification of the flathead trout. On the basis of pair-wise genetic distance estimates calculated for all the *Salmo* representatives, it is evident that the highest differences exist between the flathead trout and *S. salar*, *S. ohridana* and *S. obtusirostris*, whereas the differences between the flathead trout and *S. trutta* are minimal. From molecular phylogenetic perspective, it is clear that the flathead trout is not only the closest relative to *S. trutta* but may be regarded as a member of the species as well. Furthermore, all the performed analyses of mitochondrial or nuclear DNA sequences firmly positioned the flathead trout within the Adriatic cluster of *S. trutta*. Phylogenetic analysis of the Adriatic cluster based on the combined data set of the mtDNA control region and cytochrome *b* gene, however, produced a tree revealing a polychtonous branching pattern. The unresolved Adriatic clade may represent simultaneous divergence of the taxa within the Adriatic clade including *S. platycephalus*, or, on the other hand, indicate insufficient resolving power of the experimental system to reconstruct the exact splitting of the Adriatic cluster. Since the Adriatic lineage has already been shown as one of late diverged lineages of *S. trutta* (Bernatchez, 2001), it is reasonable to consider that the

flathead trout is not an archaic taxon but rather a relatively recently derived form of the *S. trutta* complex. Although a sympatry of morphologically dissimilar but phylogenetically close populations is not rare and has already been described in *S. trutta* (Ryman *et al.*, 1979; Ferguson & Taggart, 1991; Hynes *et al.*, 1996; Skaala & Solberg, 1997), the flathead and brown trout have never been observed to live sympatrically (pers. obs.). The lack of suitable salmonid habitat in the River Zamanti upstream and downstream of Pinarbasi may prevent natural mixing and hybridization between these two taxa. Nevertheless, the brown trout specimens from the River Zamanti reported by Bernatchez (2001), conflict with this view and indicates a possible overlapping of these two taxa, unless the classification used therein was simplified and the name of 'brown trout' was applied also to *S. platycephalus*. This is likely to be the case, as for other species (*e.g.* *S. marmoratus* and *S. obtusirostris*) studied in this paper, the designation 'brown trout' was used as well. These three Zamanti trout samples were according to mtDNA sequence analysis of the 5'-end control region and RFLP analysis of ND-5/6, cytochrome *b* and the control region, determined as Ad1 individuals (Bernatchez, 2001). Considering nucleotide analysis of the 5'-end of the control region, the same haplotype (*i.e.* Ad1) was found for the flathead trout. Nevertheless, further analyses, which included sequencing of the rest of mtDNA control region, cytochrome *b* gene and ITS1 nuclear region, revealed differences between the flathead trout and Ad1 brown trout individuals.

Whereas the existence of the brown trout in the Zamanti River system has never been questionable, it is not clear, whether the brown trout is native to the Zamanti River system or not. An exotic brown trout haplotype (*i.e.* Da1) was found in the River Kapuz (Bernatchez, 2001), which may indicate that the brown trout is not indigenous in this region. Due to extremely low sample size (one specimen only), however, no firm conclusions can be inferred from this information.

Although the original classification of the flathead trout was performed on the basis of morphology, the measurements themselves, did not show a substantial distinctness of *S. platycephalus* in comparison to *S. trutta*; except for the lower number of pyloric caeca (15–16) and higher number of gill rakers (23–24) reported by Behnke (1968), the meristic characters were typical for *S. trutta*. It is worth mentioning here that the meristic counts performed in the present study revealed 22–25 pyloric caeca and 20–24 gill rakers in the test material. These values considerably differ from the original ones and overlap with values characteristic of brown trout. Presuming that the counts were correct, the most important diagnostic characters for *S. platycephalus* may be considered irrelevant. There is little evidence, mainly limited to a few peculiarities regarding the shape of head bones, dentition and colouration (Stearley & Smith, 1993), which still indicate the distinctness between *S. platycephalus* and the brown trout. On the basis of this assumption, it is plausible that classifying the flathead trout as a subgenus of *Salmo* is questionable and probably unjustifiable also from a morphological point of view. Accordingly, it seems that positioning of *S. platycephalus* in a lower taxonomic category is also much more appropriate from a phenotypic perspective.

Although stemming from a relatively recently diverged Adriatic cluster and despite its presumable short-term evolutionary history, *S. platycephalus* has apparently succeeded in developing a distinct race which according to the

external appearance considerably differs from all the other counterparts of *S. trutta* complex. Several environmental particularities which are characteristic only for *S. platycephalus* habitats may have required specific adaptation and consequently induced its special morphology. For instance, influence of the environment, which may affect feeding habits, may consequently also readily modify morphological characters connected with feeding (e.g. dentary and maxillary bones). Due to a relatively small population size and spatially constrained habitats, *S. platycephalus* has probably been subjected to many stochastic events inducing bottlenecks and genetic drift. In small populations, such factors are expected to be of significance and have a potential to modify original phenotype and rapidly 'fix' newly developed forms (Falconer, 1981). They probably played a major role in the case of *S. platycephalus* evolution.

Several taxa within the genus *Salmo* such as some phylogeographic lineages of *S. trutta* (i.e. Danubian, Adriatic, Atlantic and Mediterranean) are cryptic or so called sibling taxa whose classification rests upon molecular techniques rather than external morphology. On the contrary, *S. platysalmo*, like, for instance, *Salmo marmoratus* Cuvier (marble trout) is an exception: despite its very close relation to the rest of the *S. trutta* complex, it exhibits a pronounced outward appearance. In the case of the marble trout, this taxon has been generally accepted as a distinct species (Kottelat, 1997; Berrebi *et al.*, 2000; Crivelli *et al.*, 2000; Delling *et al.*, 2000; Fumagalli *et al.*, 2002) although it was clearly demonstrated by molecular approach that marble trout is a part of the *S. trutta* complex (Bernatchez *et al.*, 1992; Giufra *et al.*, 1994; Snoj *et al.*, 2000). When nomenclature is influenced more by external morphology than by facts inferred from evolutionary genetics, there is obviously a discrepancy in classification. Nevertheless, the external appearance is with no doubt an important 'anthropocentrically' adjusted criterion, because biodiversity is measured and determined in the field rather than in the laboratory. From this perspective and due to *S. platycephalus* 'diagnosability', reproductive isolation from other *S. trutta* representatives and maintenance of its identity, it should deserve attention in terms of its protection and conservation and should be, analogically to the case of *S. marmoratus*, accepted as a separate species.

A comprehensive study of *Salmo* phylogeny, based on morphology has recently been published (Delling, 2003). In this study, *S. platycephalus* was reported as a sister taxon to the *S. trutta* Adriatic phylogeographic lineage but not closely related to *S. obtusirostris*.

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