



General patterns of sexual dimorphism in graylings (*Thymallus*), with a comparison to other salmonid species

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Abstract Among fishes, salmonids (family Salmonidae) have attracted a great deal of research attention focused on sexual dimorphism and associated selective forces. Most of this research has been directed toward anadromous and mostly semelparous salmon and trout (*Oncorhynchus*, *Salmo*), and comparatively little is known about intersexual variability in strictly iteroparous freshwater salmonids. We examined a comprehensive data set of 28 linear morphometric characters in 11 of 15 currently recognised species of grayling (Thymallinae, *Thymallus*), a genus consisting of iteroparous species only, to identify general patterns of intersexual morphological variability. Overall, we found that all grayling species show common sex-specific traits particularly relating to size dimensions of the dorsal, anal, pelvic and pectoral fins. Although the magnitude of sexual dimorphism differed among species, there was no significant phylogenetic signal associated with these

differences across the genus. These results are discussed in terms of the assumed selection pressures driving sexual dimorphism in graylings and are compared to existing knowledge in Salmonidae as a whole where similarities and differences with both Salmoninae and Coregoninae exist. The present study provides the first detailed genus-wide comparison of sexually dimorphic phenotypic characters in graylings, and highlights the need for more large-scale comparative studies in multiple salmonid species to better understand general macroevolutionary trends among this important group of freshwater fishes.

Keywords Salmonidae · Sexual selection · Dorsal fin · Anal fin · Morphology · Secondary sexual characters

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Introduction

In many animal taxa, a key aspect of intraspecific variability is associated with sexual dimorphism (Andersson 1994), the differences in physiology, morphology and behaviour of conspecific males and females (sensu Punzalan and Hosken 2010). By introducing the idea of sexual selection (Darwin 1871), Darwin set the stage for the general recognition of sex-specific roles in shaping organismal diversity. Although both empirical and theoretical studies

suggest that the evolution and maintenance of sex-specific traits is more complex, also involving other selection mechanisms (e.g. Hedrick and Temeles 1989; Cooper 2010), the theory of sexual selection is still fundamental to a general understanding of intersexual variability (Clutton-Brock 2007).

The general mechanisms that drive the evolution of sexual dimorphism are well studied in several taxonomic groups including insects (Wilhelm et al. 2011), birds (Berns and Adams 2012), mammals (Swanson et al. 2013), reptiles (Agha et al. 2017), fishes (Oke et al. 2019) and amphibians (Pincheira-Donoso et al. 2021). Among fishes, salmonids (family Salmonidae) have become one of the most frequently studied groups of species used to address questions on the evolution of sexual dimorphism and associated selective forces (Fleming and Reynolds 2004). Salmonids are a diverse group of cold-water adapted fishes in the northern hemisphere and include salmon and trout (*Oncorhynchus*, *Salmo*), lenok (*Brachymystax*), taimen (*Hucho*), Sakhalin taimen (*Parahucho*), char (*Salvelinus*), whitefish and cisco (*Coregonus*), Round whitefish (*Prosopium*), inconnu (*Stenodus*), and grayling (*Thymallus*). Many salmonid species, particularly semelparous Pacific salmon (*Oncorhynchus*), undergo dramatic phenotypic change during the reproductive period, which includes the development of an elongated snout, enlarged teeth, hooked jaws, dorsal hump, elongated fins, thickened skin, and bright colouration (Fleming and Gross 1994; Quinn and Foote 1994). These exaggerated traits are usually male-biased (i.e. larger, thicker or more pronounced in males) and are assumed to have evolved as a consequence of sexual selection where males compete for fertilization opportunities (Fleming and Reynolds 2004). The presence and degree of sexual dimorphism in these traits differ between taxonomic groups and show great intraspecific variability, which often is habitat associated (Johnson et al. 2006; Oke et al. 2019).

Despite extensive research on sexually dimorphic characters in salmonids, the generality of these traits remains poorly investigated. So far, most attention has been given to large anadromous and semelparous species, with few studies addressing sexual dimorphism in iteroparous and/or freshwater salmonids. Graylings (subfamily Thymallinae) are freshwater resident iteroparous species with a suite of distinctive morphological traits potentially relevant for the study of sexual dimorphism (Fig. 1). In comparison to other

salmonids, graylings are easily characterised by their greatly enlarged dorsal fin, which often has a species-specific coloration pattern, that is not, at least not overtly, sex-specific (Knizhin 2009) and is known to undergo secondary sexual development (Ward 1951). While taxonomy and species level phylogeny in graylings are becoming well-resolved in recent years (Knizhin 2009; Weiss et al. 2021), the evolution of sexual dimorphism remains poorly studied, having only been addressed at all in a few species (e.g. Mikheev 2009). Increasing knowledge on sympatric occurrence of multiple grayling species (Shubin and Zakharov 1984; Weiss et al. 2007, 2020, 2021), with little evidence of hybridization and introgression (Froufe et al. 2003; Weiss et al. 2007, 2020; Persat et al. 2016), has drawn increasing attention to elucidating the mechanisms that might support reproductive isolation.

Graylings are a monophyletic sister clade to Coregoninae (Campbell et al. 2020) and are widespread across most of Europe, Siberia, the Russian Far East and some parts of North America (Weiss et al. 2021). They are typical riverine fish, but also occur in many lacustrine habitats across their range, and spawn in spring or early summer after short or medium distanced potamodromous migrations (usually from lakes to rivers and within rivers). Graylings are gravel spawning salmonids, where both males and females are promiscuous with multiple spawning acts, usually involving different mates (Beauchamp 1990). In contrast to most other river spawning salmonids, dominant males occupy and defend spawning territories prior to the arrival of females (Fabricius and Gustafson 1955; Bishop 1971). Observations of frequent territorial contests and the generally increased aggressive behaviour during spawning season (Fabricius and Gustafson 1955) may suggest strong intra-sexual (male-male) competition for territories and access to mates. Likewise, territorial contests as well as courtship and spawning include characteristic behaviours such as the specific display of the colourful dorsal and pelvic fins (Fabricius and Gustafson 1955; Kratt and Smith 1980). Intraspecific competition, however, is not restricted to the spawning season. Similar to other drift-feeding stream salmonids (Fausch and White 1981), feeding positions among graylings are established in dominance hierarchies (Hughes and Dill 1990; Hughes 1992) where the characteristic display of the dorsal and pelvic fins is an



Fig. 1 Phenotypic comparison of female (above) and male (below) graylings in **A** *T. grubii*, during spawning season, both female and male from the Bureya River, Russia; **B** *T. baicalensis*, outside spawning season, both female and male from the Delger mörön River, Mongolia; **C** *T. flavomaculatus*,

during spawning season, female from the Pody River and male from the Gobilly River, Russia; **D** *T. svetovidovi*, outside spawning season, both female and male from the Sharga Gol, Mongolia. Photos by A. Antonov (**A**, **C**) and C. Ratschan (**B**, **D**)

integral behavioural element (Fabricius and Gustafson 1955; Tack 1973).

Given this general behavioural framework and the assumption that selective forces shaping sexually dimorphic phenotypic characters are closely linked to the reproductive behaviour in salmonids (Fleming and Reynolds 2004), we hypothesize that the extent and direction of sexual dimorphism in graylings might be consistent across different species. Thus, we analysed a comprehensive data set of linear morphometric traits to identify general trends of sexual

dimorphism in graylings. Furthermore, we reviewed external morphometric characters subject to sexual dimorphism among salmonids (Salmoninae, Coregoninae, Thymallinae) in order to place our results in a broader phylogenetic context. Finally, by summarizing areas of potential future studies, we hope to foster cross-disciplinary research in ecology and evolution of graylings, which may aid future conservation and management efforts targeting this group of freshwater fishes.

Materials and methods

Morphological data set

To test for general patterns of sexual dimorphism among graylings, we analysed a large morphological data set established over a period of more than 10 years and spanning > 1500 individual specimens from 11 species across the whole distribution range of the genus (Table 1). Many subsets of the data set have been used in a range of taxonomic, systematic, and evolutionary studies to date (e.g. Froufe et al. 2003; Knizhin et al. 2004, 2006a, b, c, d, 2007, 2008a, b; Knizhin and Weiss 2009; Knizhin 2009; Weiss et al. 2006, 2020), but the data have never been investigated as a whole nor in the context of sexual dimorphism. Twenty-eight linear measurements following those introduced by Svetovidov (1936), Pravdin (1966), and Knizhin et al. (2004) were made point to point or as a projection to midline using a caliper to the nearest 0.1 mm (Fig. 2, Table S1). Measurements were taken from formalin (4%) preserved specimens.

Sex and stage of maturity were determined by visual examination of gonads following the classification of ovarian reproductive stages by Sakun and Butskaya (1968). Fishes of stage I (oogonia and oocytes did not yet start protoplasmic growth, immature condition) were excluded and only fishes between stage II (previtellogenic condition) and stage VI (postspawning condition, before returning to stage II) were included in the analysis. Most fishes were sampled after spawning season (July–October). Only

few specimens of *T. arcticus* (n = 23), *T. baicalensis* (n = 60) and *T. thymallus* (n = 65) were taken before or during spawning season in spring or early summer.

Data transformation

In order to emphasize general trends in the data set as well as equalize variances among groups, we excluded measurements showing extreme values. These were defined as the deviation of $3 * IQR$ (inter quartile range) from the 25th ($Q_1 - 3 * IQR$) and 75th ($Q_3 + 3 * IQR$) percentile respectively, calculated using raw measurements relative to body length (referring to fork length). We excluded extreme values for each species and sex separately, rather than the entire data set, to retain the natural species-specific variability. In total, 52 specimens (3.3% of the entire data set) were excluded (19 males, 33 females). The final data set consisted of measurements for 1539 fish (806 males and 733 females) (Table 1). For a few individuals, some measurements were not obtained due to damage or poor preservation condition. To retain these specimens in the analyses, predicted values from linear regression models (per sex, species and trait) were used to substitute missing data. Such cases account for 0.6% of the entire data (0.7% of males and 0.6% of females).

All morphometric measurements were converted to their base 10 logarithm to linearize allometry and equalize variances (Sidlauskas et al. 2011). We evaluated potential allometric scaling among species by comparing species-specific slopes of reduced-

Table 1 Number of male and female specimens used in the analysis and mean fork length (Lsm \pm SD) for each species

Species	Male		Female	
	N	Lsm (mm)	N	Lsm (mm)
<i>T. arcticus</i>	102	280.4 \pm 63.7	55	244.2 \pm 48.7
<i>T. baicalensis</i>	266	282.2 \pm 74.2	265	297.2 \pm 60.5
<i>T. baicalolenensis</i>	158	213.6 \pm 38.6	172	194.5 \pm 35.6
<i>T. brevirostris</i>	24	324.1 \pm 125.2	22	345.9 \pm 120.5
<i>T. burejensis</i>	32	300.5 \pm 66.5	35	288.7 \pm 59.1
<i>T. flavomaculatus</i>	25	233.2 \pm 29.8	23	221.3 \pm 33.5
<i>T. grubii</i>	75	199.0 \pm 40.8	50	173.4 \pm 27.0
<i>T. nigrescens</i>	15	280.9 \pm 25.0	15	268.7 \pm 20.5
<i>T. svetovidovi</i>	11	370.7 \pm 13.9	12	368.1 \pm 12.9
<i>T. thymallus</i>	66	330.5 \pm 44.6	58	300.3 \pm 37.4
<i>T. tugarinae</i>	32	215.9 \pm 21.0	26	204.6 \pm 24.9

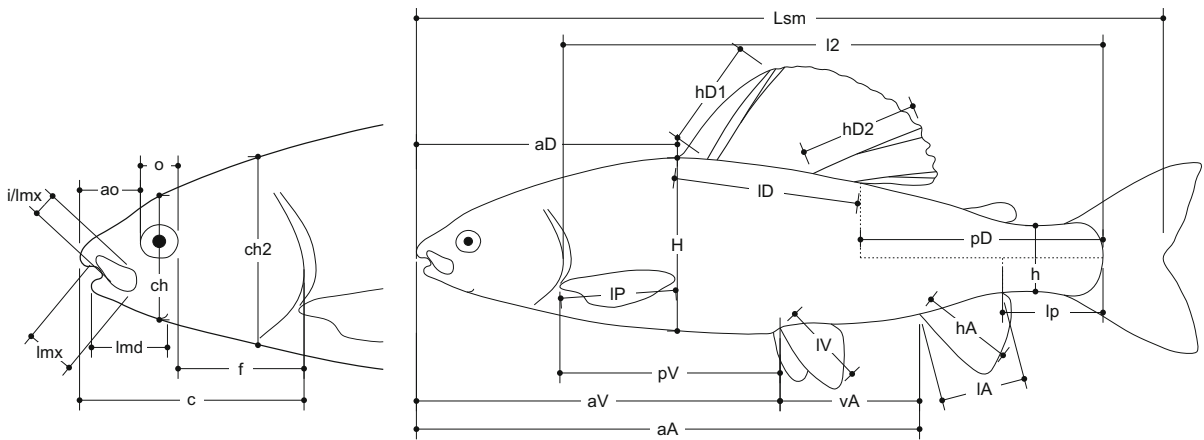


Fig. 2 Schematic illustration of morphometric characters used in the present study. For a detailed description of each measurement see Table S1. Lsm, fork length; l2, trunk length; ao, snout length; o, horizontal eye diameter; f, postorbital length; c, head length; ch2, head depth at nape; ch, head depth through the eye; lmx, upper jaw length; i/lmx, upper jaw depth; lmd, lower jaw length; H, maximum body depth; h, minimal caudal peduncle depth; aD, predorsal length; pD, postdorsal

length; aA, preanal length; aV, prepelvic length; lp, caudal peduncle length; pV, pectoral-pelvic distance; vA, pelvic-anal distance; ID, length of dorsal fin base; hD1, height of anterior part of dorsal fin; hD2, height of posterior part of dorsal fin; IA, length of anal fin base; hA, height of anal fin; IV, length of pelvic fin; IP, length of pectoral fin. Not illustrated are: k, forehead width; w, maximum width of body

major axis regression lines as outlined in Sidlauskas et al. (2011) and implemented in the R package ‘smatr’ (Warton et al. 2006) in R v.4.1.0 (R Core Team 2021). Some statistically significant differences among species were found (14 of 55 pairwise comparisons; Table S2). These differences, however, appeared related to sample size and body-size differences (Table 1), and thus we chose to apply a common slope in the following data transformation. To control for the effects of variation in body size and body size scaling, all morphometric traits were scaled to a common mean fork length using an allometric growth formula commonly applied in Salmonidae (Siwertsson et al. 2013; Jacobs et al. 2020): $\log_{10} Y_{std} = \log_{10} Y_{obs} + b * (\log_{10} L_{std} - \log_{10} L_{obs})$; where Y_{std} is the corrected trait value, Y_{obs} is the measured trait value, b is the slope of the regression of each (\log_{10}) trait against (\log_{10}) fork length, L_{std} is the mean fork length of all specimens (mm), and L_{obs} is the individual fork length. Terminology of these variables follows Siwertsson et al. (2013). The common slope b for each trait was derived from ANCOVA models using species and sex as factors while controlling for body length.

Comparison of sexual dimorphism among graylings

We determined morphological characters contributing to the divergence between males and females, across the whole genus, using a two-way Analysis of Variance (ANOVA). ANOVA models (trait ~ sex + species + species * sex) were implemented in IBM SPSS Statistics v.26. A series of one-way ANOVA analyses with simple effects was additionally used to more precisely evaluate species-specific trends of sexual dimorphism. The assumptions of homoscedasticity and normal distribution of (unstandardized) residuals were not met in all cases. However, visual examination of residuals in histograms and normal Q–Q plots showed them to be approximately normally distributed. Overall, ANOVA analyses on large samples sizes (> 500 observations) are known to be robust against minor deviations of normality (e.g. Johnson 1998). To corroborate two-way ANOVA results and account for different sample sizes in sub-groups (i.e. species), we examined Welch’s tests (unequal variance t-test) in cases where homogeneity of variances was not met (Ruxton 2006), and performed separate non-parametric Kruskal–Wallis tests to support one-way ANOVA results of species-specific trends where normality of residuals was violated.

Phylogenetic comparative analysis

Because species are not independent and patterns of sexual dimorphism could have a phylogenetic component, we estimated the ancestral state of sexual dimorphism for each character and tested for a phylogenetic signal across species. The phylogenetic reconstruction is based on complete mitochondrial genomes of 15 *Thymallus* species (considering the current taxonomy presented in Weiss et al. (2021)) and two *Coregonus* species as outgroup with the following GenBank accession numbers: MT063012 (*T. arcticus*), MT063023 (*T. baicalensis*), MT063019 (*T. baicalolenensis*), MT063036 (*T. brevicephalus*), MT063033 (*T. brevirostris*), MT063037 (*T. burejensis*), MT063039 (*T. flavomaculatus*), MT063038 (*T. grubii*), MT063028 (*T. nigrescens*), MT063030 (*T. nikolskyi*), MT063004 (*T. thymallus*), KJ866485 (*T. tugarinae*), CM031715 (*C. clupeaformis*), and NC_025576 (*C. peled*). Alignment and analyses of mitochondrial data follow Weiss et al. (2021). All parts of the analysis were performed in PhyloSuite v.1.2.2 (Zhang et al. 2020) and IQ-TREE (Nguyen et al. 2015) was used for maximum likelihood (ML) analysis. The computed ML tree was visualised and edited with FigTree v.1.4.4 (Rambaut 2018) and CoreDRAW 2019.

For phylogenetic comparison, we excluded those species where morphological data were absent in our data set (*T. aeliani*, *T. brevicephalus*, *T. ligericus*, *T. nikolskyi*). We estimated and visualised the degree of sexual dimorphism in morphometric traits (based on mean pairwise differences (least square means) from linear two-way ANOVA models) at each node in the mtDNA phylogeny using the ‘fastAnc’ and ‘contMap’ functions in the R package ‘phytools’ (Revell 2012) in R v.4.1.0 (R Core Team 2021). To test for a phylogenetic signal of sexual dimorphism for each character, we estimated Pagel’s λ (Pagel 1999) and Blomberg’s K (Blomberg et al. 2003) using the ‘phylosig’ function in ‘phytools’.

Literature review on sexually dimorphic traits in Salmonidae

To evaluate our observations in graylings in a broader phylogenetic context, we synthesized current knowledge of sexual dimorphism in external morphometric traits across Salmonidae. We included characters that

are reversible and temporally linked to the breeding season as well as those that undergo a non-reversible change starting usually at the onset of sexual maturity. Sexual size dimorphism was not considered due to its high intraspecific variability (e.g. Jonsson and Jonsson 2015). Literature searches were performed in Web of Science, Scopus and Google Scholar (key words in different combinations: sexual dimorphism, secondary sexual character(s), morphometric character(s), female, male, salmonids, *subfamily*, *genus*, *trait*). The results were augmented with additional studies, especially from Russia, that were not found with these search engines. A morphometric trait was considered sexually dimorphic if a statistical analysis was performed to test for intersexual variability or the morphological characters were described as explicit sexual dimorphism.

Results

Comparison of sexual dimorphism among graylings

The average fork length among all individuals was 260.5 ± 73.6 mm. Males were larger than females in the global data set (263.7 ± 73.7 mm vs. 256.9 ± 73.2 mm; two-way ANOVA, $F_{1,1517} = 8.208$, $P = 0.004$; Welch, $F_{1, 1513.03} = 3.938$, $P = 0.047$), but this pattern was not consistent across all species reflected by the significant species \times sex interaction (Table 2). At the species-specific level, only *T. arcticus*, *T. baicalolenensis*, *T. grubii* and *T. thymallus* exhibited a significant male-biased size dimorphism (one-way ANOVA, $F < 22.590$, $P < 0.001$; Kruskal Wallis H, $X^2 < 20.770$, $P < 0.001$).

Two-way ANOVA analyses on morphometric characters showed that significant species \times sex interactions were present in 21 out of 28 traits. Significant differences between the sexes were found in 18 characters, 12 of which remained significant after table-wide Bonferroni correction (Table 2). When species-specific pairwise differences were examined, predorsal length, length of dorsal-fin base, height of anterior part of dorsal fin, height of posterior part of dorsal fin, height of anal fin, and length of the pelvic and pectoral fins showed a uniform pattern of sexual dimorphism (Figs. 3, S1), although these differences were not statistically significant for all

Table 2 Two-way ANOVA results of fork length and 28 standardized morphometric characters. Least square means (LSM) for each trait and sex are given with their standard errors

Variables	Sex					Species			Species x Sex		
	LSM male	LSM female	df	F	P-value	df	F	P-value	df	F	P-value
<i>Body length</i>											
Fork length (Lsm)	2.424 ± 0.005	2.404 ± 0.005	1	8.208	0.004	10	115.861	0.000	10	5.661	0.000
<i>Morphometric charaters</i>											
Trunk length (l2)	2.306 ± 0.000	2.306 ± 0.001	1	0.004	0.953	10	26.847	0.000	10	2.897	0.001
Snout length (ao)	1.198 ± 0.002	1.193 ± 0.002	1	3.071	0.080	10	92.154	0.000	10	4.078	0.000
Horizontal eye diameter (o)	1.066 ± 0.002	1.068 ± 0.002	1	1.126	0.289	10	40.328	0.000	10	3.100	0.001
Postorbital length (f)	1.405 ± 0.001	1.404 ± 0.001	1	1.091	0.296	10	62.643	0.000	10	4.430	0.000
Head length (c)	1.701 ± 0.001	1.698 ± 0.001	1	5.135	0.024	10	76.975	0.000	10	4.682	0.000
Head depth at nape (ch2)	1.583 ± 0.001	1.580 ± 0.001	1	2.528	0.112	10	92.117	0.000	10	3.184	0.001
Head depth through the eye (ch)	1.425 ± 0.002	1.419 ± 0.002	1	6.571	0.011	10	65.819	0.000	10	3.365	0.000
Forehead width (k)	1.181 ± 0.002	1.174 ± 0.002	1	5.104	0.024	10	26.916	0.000	10	2.174	0.017
Upper jaw length (lmx)	1.164 ± 0.002	1.167 ± 0.002	1	1.168	0.280	10	72.336	0.000	10	5.296	0.000
Upper jaw depth (i/lmx)	0.698 ± 0.003	0.694 ± 0.003	1	1.545	0.214	10	29.787	0.000	10	2.888	0.001
Lower jaw length (lmd)	1.398 ± 0.002	1.397 ± 0.002	1	0.611	0.435	10	93.422	0.000	10	3.898	0.000
Maximum body depth (H)	1.739 ± 0.002	1.740 ± 0.002	1	0.750	0.387	10	128.123	0.000	10	0.681	0.743
Minimal caudal peduncle depth (h)*	1.269 ± 0.001	1.262 ± 0.001	1	12.646	0.000	10	201.091	0.000	10	0.892	0.540
Maximum width of body (w)	1.479 ± 0.003	1.491 ± 0.003	1	9.439	0.002	10	17.921	0.000	10	2.771	0.002
Predorsal length (aD)*	1.922 ± 0.001	1.930 ± 0.001	1	42.958	0.000	10	357.146	0.000	10	3.269	0.000
Postdorsal length (pD)	2.037 ± 0.001	2.042 ± 0.000	1	9.758	0.002	10	66.577	0.000	10	2.416	0.008
Preanal length (aA)*	2.262 ± 0.000	2.266 ± 0.001	1	36.540	0.000	10	22.651	0.000	10	2.949	0.001
Prepelvic length (aV)	2.076 ± 0.001	2.079 ± 0.001	1	8.548	0.004	10	38.705	0.000	10	2.179	0.017
Caudal peduncle length (lp)	1.634 ± 0.001	1.635 ± 0.001	1	0.113	0.737	10	53.547	0.000	10	0.518	0.878
Pectoral-pelvic distance (pV)*	1.864 ± 0.001	1.872 ± 0.001	1	24.976	0.000	10	18.483	0.000	10	1.213	0.277
Pelvic-anal distance (vA)*	1.819 ± 0.001	1.825 ± 0.001	1	10.487	0.001	10	18.789	0.000	10	1.445	0.155
Length of dorsal fin base (ID)*	1.780 ± 0.002	1.759 ± 0.002	1	69.945	0.000	10	316.737	0.000	10	1.966	0.033
Height of anterior part of dorsal fin (hD1)*	1.486 ± 0.002	1.455 ± 0.003	1	85.915	0.000	10	56.664	0.000	10	1.770	0.061
Height of posterior part of dorsal fin (hD2)*	1.585 ± 0.005	1.473 ± 0.005	1	257.779	0.000	10	147.709	0.000	10	8.347	0.000
Length of anal fin base (IA)*	1.391 ± 0.002	1.369 ± 0.002	1	57.600	0.000	10	53.979	0.000	10	2.858	0.002
Height of anal fin (hA)*	1.470 ± 0.002	1.512 ± 0.002	1	209.443	0.000	10	36.577	0.000	10	5.838	0.000
Length of pelvic fin (IV)*	1.633 ± 0.002	1.595 ± 0.002	1	150.800	0.000	10	93.433	0.000	10	2.478	0.006
Length of pectoral fin (IP)*	1.622 ± 0.002	1.612 ± 0.002	1	23.019	0.000	10	59.127	0.000	10	1.298	0.226

Traits that remained significant after table-wide Bonferroni correction (significance level at $\alpha = 0.05/28 = 0.0018$) are marked with an *

species (Table S2). The magnitude of sexual dimorphism showed considerable variation in some

characters, exemplified by a sevenfold difference in magnitude of the posterior part of dorsal fin between *T.*

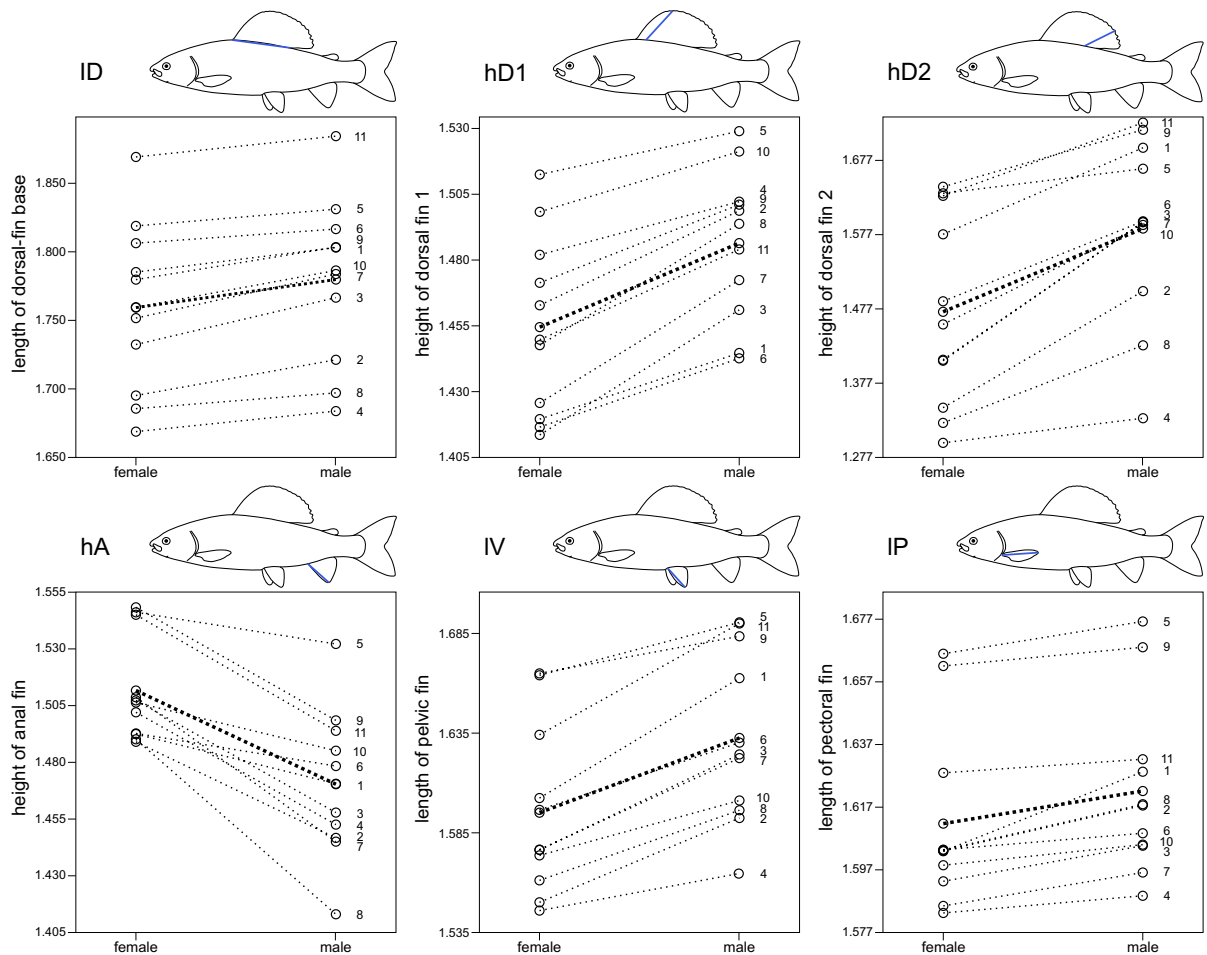


Fig. 3 Comparison of pairwise differences (least square means) for the most uniform sexually dimorphic traits of the dorsal, anal, pelvic and pectoral fins (two-way ANOVA, significant after table-wide Bonferroni correction). Bold lines represent mean values for all species. Height of dorsal fin 1 refers to height of anterior part of dorsal fin and height of dorsal fin 2 refers to

height of posterior part of dorsal fin. 1 = *T. arcticus*, 2 = *T. baicalensis*, 3 = *T. baicalolenensis*, 4 = *T. brevirostris*, 5 = *T. burejensis*, 6 = *T. flavomaculatus*, 7 = *T. grubii*, 8 = *T. nigrescens*, 9 = *T. svetovidovi*, 10 = *T. thymallus*, 11 = *T. tugarinae*

baicalolenensis (0.187, most dimorphic) and *T. burejensis* (0.033, least dimorphic).

In general, male graylings had significantly greater length and height dimensions of dorsal, pelvic and pectoral fins, a longer base of the anal fin, and a deeper caudal peduncle than conspecific females. In contrast, female-biased traits were related to the height of the anal fin, length dimensions of the abdomen, and a greater distance between the fins. Deviations from these general trends in *T. brevirostris*, *T. burejensis*, *T. flavomaculatus*, *T. svetovidovi* and *T. tugarinae* (Fig. S1), were not statistically significant (one-way

ANOVA, $F < 0.809$, $P > 0.370$; Kruskal Wallis H, $X^2 < 0.510$, $P > 0.470$).

While some attributes of the head and abdomen were non-significant in the global two-way ANOVA analyses, non-parametric Welch's tests suggested a significantly greater postorbital length (Welch, $F_{1,1518.36} = 13.347$, $P < 0.001$) and head length (Welch, $F_{1,1525.56} = 10.651$, $P = 0.001$) in males, and a greater body width (Welch, $F_{1,1236.93} = 11.917$, $P < 0.001$), postdorsal length (Welch, $F_{1,1526.86} = 48.073$, $P < 0.001$) and preventral length (Welch, $F_{1,1536.71} = 27.415$, $P < 0.001$) in females. These traits were found significant for sex-

differentiation only in a few species (Table S2), although the direction of sexual dimorphism was similar for most species.

Phylogenetic comparative analysis

Overall, there was no significant phylogenetic signal of sexual dimorphism among the traits (Pagel’s $\lambda < 0.001$ ($P = 1$), Blomberg’s $K < 0.782$ ($P > 0.160$)) (Table S3). Only the length of the pelvic fin had a low but non-significant phylogenetic signal (Pagel’s $\lambda = 0.951$, $P = 0.543$; Blomberg’s $K = 0.788$, $P = 0.087$). Thus, while ancestral state reconstruction showed that sexual dimorphism in the most general trends (identified by two-way ANOVA analyses) were also present in the most recent common ancestor (Fig. 4, Fig. S2), species-specific patterns

were clearly not related to phylogeny. For example, closely related species often had a clearly different magnitude of sexual dimorphism (e.g. the sister species *T. flavomaculatus* and *T. grubii*, *T. arcticus* and *T. baicalolenensis*). This indicates that trends of sexual dimorphism are not more similar among closely related species than to distantly related relatives. While some species, such as *T. baicalensis* and *T. baicalolenensis* generally tend to have a high degree of intersexual variability, others such as *T. brevirostris* and *T. burejensis*, only show slight differences between the sexes or exhibit contrasting patterns to the general trends observed (Figs. 3, S1).

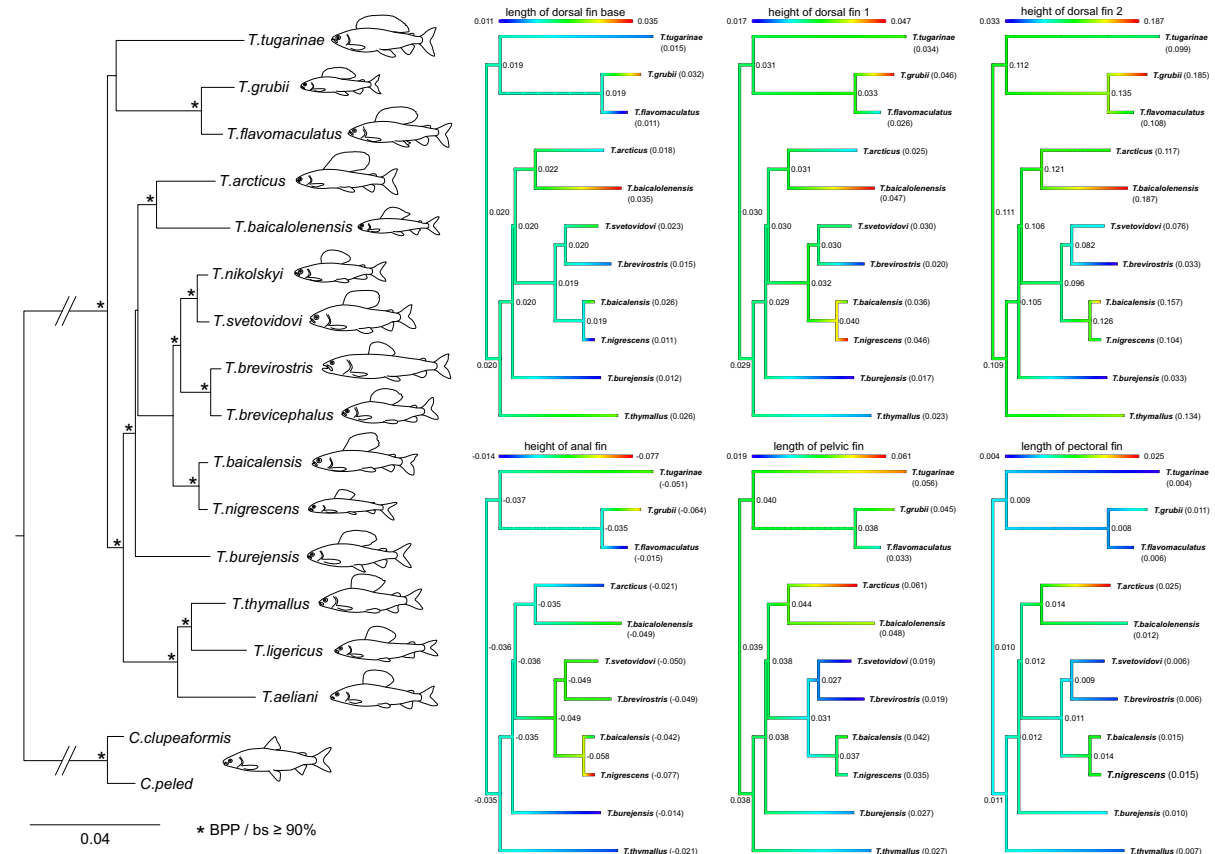


Fig. 4 Phylogenetic reconstruction of *Thymallus* species based on whole mitochondrial genomes, and ancestral state reconstruction of the degree of sexual dimorphism in the most uniform sexually dimorphic traits of the dorsal, anal, pelvic and pectoral fins (red = most dimorphic, blue = least dimorphic).

Thymallus aeliani, *T. brevicephalus*, *T. ligericus*, and *T. nikolskyi* were excluded from ancestral state reconstruction due to missing morphological data. Negative values indicate a female-biased trait

Review of sexual dimorphism in morphometric traits across Salmonidae

We reviewed 56 publications describing sexual dimorphism in morphometric traits among salmonid species (Table 3). The majority of these studies (n = 43) were based on the measurement of linear morphometric traits, while six used geometric morphometrics, and seven studies were descriptive and based on visual examination. A comparatively large number of these studies (n = 19) targeted anadromous (mostly semelparous) species of the genus *Oncorhynchus*. Overall, only a small percentage (11%) of the large number of salmonid species (n = 247), listed as valid species in Fricke et al. (2021), has been explicitly investigated for sexual dimorphism in external morphometric traits. Although this problem may be overstated due to recent taxonomic inflation (e.g. Isaac et al. 2004), whole genera have apparently been ignored as no specific studies on sexual dimorphism in morphometric traits were found for the genera *Brachymystax*, *Hucho*, *Parahucho* and *Stenodus*. Among the species analysed, the most general traits of sexual dimorphism across different genera were:

Length of the jaws and snout

The secondary sexual development of the male jaws (to a lesser extent also present in females), has been reported from several species in the subfamily Salmoninae. The transformation of the jaws (and the elongation of the snout) during the breeding period tends to be most characteristic for semelparous *Oncorhynchus* (upper jaw), and iteroparous *Salmo* and *Salvelinus* (lower jaw) (Table 3). A modification of the upper and/or lower jaw during the reproductive period has not been reported in either Coregoninae or Thymallinae.

Length and depth of the head

The head tends to be generally more robust in male Salmoninae. In Coregoninae and Thymallinae, dimensions of the head appear to be more sexually monomorphic, though observations by Nikulina and Polyayeva (2020) would suggest a larger head in female *Coregonus sardinella*.

Length and height of the dorsal fin

Sexual dimorphism in length and height dimensions of the dorsal fin and its base-length are usually male-biased and reported from species in *Oncorhynchus*, *Salmo*, *Coregonus*, *Prosopium* and *Thymallus*. Exceptions of female-biased dimensions in the dorsal fin may exist such as in *C. sardinella* (Table 3).

Size of the adipose fin

The adipose fin tends to be generally larger (height and length dimensions) in male *Oncorhynchus*, *Salmo*, and *Salvelinus* (Table 3), a trend that appears to be consistent across multiple *Salmo* species and not restricted to the spawning season (compare to data in Delling and Doadio 2005; Turan et al. 2011, 2012). Data of multiple *Coregonus* species in alpine lakes would suggest sexual monomorphism in this trait (Selz et al. 2020). Among Thymallinae, intersexual variability in the size of the adipose fin has not been investigated.

Height of the anal fin

The height of the anal fin was found to be female-biased in several species of *Oncorhynchus*, *Salmo* and *Thymallus* (Table 3). Morphological studies on European, Eurasian, and North African *Salmo* species suggest great interspecific variability in this character and a greater height of the anal fin in males of some species (e.g. Turan et al. 2011, 2012; Doadrio et al. 2015). Observations from *Coregonus* and *Prosopium* may suggest sexual monomorphism in this trait or a greater height of the anal fin in male Coregoninae (Table 3).

Length of the paired pelvic and pectoral fins

The pelvic and pectoral fins were found to be commonly longer in males of species in *Oncorhynchus*, *Salmo*, *Salvelinus*, *Coregonus*, *Prosopium* and *Thymallus*; a trend that appears most consistent in the subfamilies Coregoninae and Thymallinae (Table 3).

Size of breeding tubercles

Breeding tubercles are known only from iteroparous species. They are most characteristic for Coregoninae

Table 3 Sexually dimorphic external morphometric traits in Salmonidae

Subfamily	Genus	Species	Parity	Male-biased	Female-biased	Reference	
Salmoninae	<i>Oncorhynchus</i>	<i>gorbuscha</i> (Pink salmon)	Semelparous	Upper jaw length*, head length*, head width, body depth, caudal peduncle depth*, dorsal fin base length*, adipose fin length*, anal fin base length*	Anal fin height	Davidson (1935), Beacham and Murray (1983, 1985, 1986), Beacham et al. (1988), Zhiotovskiy and Kim (2015)	
		<i>keta</i> (Chum salmon)	Semelparous	Snout length*, upper jaw length*, head length*, postorbital head length*, body depth*, caudal peduncle depth*, prepelvic length*, dorsal fin height*, dorsal fin base length*, adipose fin height*, adipose fin length*	Horizontal eye diameter*, anal fin height*, anal fin base length*	Beacham and Murray (1983, 1985, 1987), Beacham (1984), Myoung et al. (1993) ^a	
		<i>kisutch</i> (Coho salmon)	Semelparous	Snout length*, upper jaw length*, tooth length, body depth*, dorsal fin height*, adipose fin length*, pelvic fin length*, pectoral fin length*	Caudal peduncle depth*, anal fin height*, anal fin base length*	Shapovalov and Taft (1954), Beacham and Murray (1983, 1986), Fleming and Gross (1994)	
		<i>mykiss</i> (Steelhead trout)	Iteroparous	Jaw length, tooth length, body depth			Shapovalov and Taft (1954)
		<i>nerka</i> (Sockeye salmon)	Semelparous	Snout length, upper jaw length*, tooth length, body depth, caudal peduncle depth, adipose fin length*			Beacham and Murray (1983, 1986), Quinn and Foote (1994), Hendry and Berg (1999), Johnson et al. (2006), Oke et al. (2019)
		<i>nerka</i> (Kokanee salmon)	Semelparous	Snout length*, jaw length*, tooth length, body depth*, caudal fin height, pelvic fin length*, pectoral fin length*		Anal fin height*, anal fin base length*	Ricker (1938), Winans et al. (2003), Thorn and Morbey (2016)

Table 3 continued

Subfamily	Genus	Species	Parity	Male-biased	Female-biased	Reference
		<i>tshawytscha</i> (Chinook salmon)	Semelparous	Snout length*, upper jaw length*, head length*, adipose fin length*, adipose fin height*		Beacham and Murray (1983, 1986), Merz and Merz (2004)
	<i>Salmo</i>	<i>fahrettini</i>	Iteroparous	Upper jaw length, mouth gape length, adipose fin base length		Turan et al. (2020)
		<i>kottelati</i>	Iteroparous	Upper jaw length, mouth gape length, mouth gape width, head length		Turan et al. (2014)
		<i>salar</i> (Atlantic salmon)	Iteroparous	Jaw length, adipose fin size*		Tchernavin (1944), Næsje et al. (1988), Järvi (1990)
		<i>trutta</i> (Brown trout)	Iteroparous	Upper jaw length, head length*, body depth, dorsal fin height, adipose fin length	Abdomen length*, predorsal length, pectoral-pelvic distance	Reyes-Gavilán et al. (1997), Monet et al. (2006)
	<i>Salvelinus</i>	<i>alpinus</i> (Arctic char)	Iteroparous	Mouth size*, head length*, head depth*, body depth*, pectoral fin length*		Janhunen et al. (2009)
		<i>confluentus</i> (Bull trout)	Iteroparous	Head length, adipose fin height		McPhail and Murray (1979, seen in McPhail and Baxter (1996)), Nitychoruk et al. (2013)
		<i>fontinalis</i> (Brook trout)	Iteroparous	Snout length, lower jaw length, mouth width, head length, head depth, pelvic fin length, pectoral fin length	Body width	Willson (1997), Proulx and Magnan (2004), Kazyak et al. (2013)
		<i>malma</i> (Dolly Varden trout)	Iteroparous	Snout length, body depth, adipose fin height		McPhail and Murray (1979, seen in Beacham and Murray (1983)), Yamamoto et al. (2017)
Coregoninae	<i>Coregonus</i>	<i>artedi</i> (Cisco)	Iteroparous	Dorsal fin length, pectoral fin length, anal fin length		Jacobson et al. (2020)

Table 3 continued

Subfamily	Genus	Species	Parity	Male-biased	Female-biased	Reference
		<i>clupeiformis</i> (Lake whitefish)	Iteroparous	Upper jaw length*, pelvic fin length*, pectoral fin length*		Casselman and Schulte-Hostedde (2004)
		<i>lavaretus</i> (Lavaret)	Iteroparous	Caudal peduncle width*, anal fin base length*, pelvic fin length*, pectoral fin length*	Predorsal length*, prepelvic length*, pectoral-pelvic distance*, body with	Heese (1987)
		<i>peled</i> (Peled)	Iteroparous	Horizontal eye diameter*, minimum body depth*, dorsal fin height*, dorsal fin length*, anal fin height*	Pectoral-pelvic distance*	Mamcarz and Nowak (1986) ^b
		<i>sardinella</i> (Least cisco)	Iteroparous	Interorbital width*, prepectoral length*	Head length*, head width*, head depth at nape*, body depth*, caudal peduncle length*, dorsal fin base length*, dorsal fin height*, anal fin base length*, pectoral fin base length*	Nikulina and Polyaeva (2020)
	<i>Prosopium</i>	<i>zugensis</i> (Albeli)	Iteroparous	Size of breeding tubercles*		Wedekind et al. (2008)
		<i>coulteri</i> (Pygmy whitefish)	Iteroparous	Dorsal fin height*, anal fin height*, pelvic fin length*, pectoral fin length*		McCart (1965)
		<i>cylindraceum</i> (Round whitefish)	Iteroparous	Size of breeding tubercles	Abdomen length	Normandeau (1963)
Thymallinae	<i>Thymallus</i>	<i>arcticus</i> (Arctic grayling)	Iteroparous	Postorbital length*, head length*, head depth at eye*, body depth*, postdorsal distance*, dorsal fin height*, dorsal fin base length*, anal fin base length*, pelvic fin length*, pectoral fin length*	Horizontal eye diameter*, predorsal length, preanal length*, pectoral-anal distance*, anal fin height*	Rawson (1950), Ward (1951), Bishop (1967, 1971), Tack (1973), Ridder (1989), Zinovjev and Bogdanov (2012), Romanov (2016)

Table 3 continued

Subfamily	Genus	Species	Parity	Male-biased	Female-biased	Reference
		<i>flavomaculatus</i> (Yellow-spotted grayling)	Iteroparous	Lower jaw length, dorsal fin height, dorsal fin base length	Anal fin height	Semenchenko (2005) ^c , Knizhin et al. (2006a)
		<i>thymallus</i> (European grayling)	Iteroparous	Dorsal fin height*, dorsal fin base length*, anal fin base length*, pelvic fin length*, pectoral fin length*	Anal fin height	Magreiter (1951), Persat (1977), Zinovjev (2012), Kucheruk et al. (2015)
		<i>tugarinae</i> (Lower-Amur grayling)	Iteroparous	Dorsal fin height, anal fin base length, pelvic fin length, pectoral fin length	Lower jaw length, preanal length, pectoral-pelvic distance, anal fin height	Mikheev (2009)

Traits reported significant at $P < 0.05$ are marked with an *

^aMyoung et al. (1993) reported a significantly longer postorbital head length in females while Beacham and Murray (1987) reported the character to be male-biased

^bSexual dimorphism in fishes of age 4 + is reported

^cTherein reported as *T. arcticus grubii* (Samarga River, Pymorsky Territory)

but were also found in individual species of Thymallinae (*T. arcticus*, Kratt and Smith (1978); *T. thymallus*, Witkowski (1982)) and Salmoninae (*Salvelinus namaycush*, Muir et al. (2012)). Size and abundance of breeding tubercles were found to be usually male-biased.

Discussion

Sexual dimorphism in graylings

Our analyses revealed a suite of morphometric characters that display significant sexual dimorphism across most grayling species, particularly relating to size dimensions of the dorsal, anal, pelvic and pectoral fins. The differences were pronounced, despite the variation in magnitude and the significant species x sex interaction in our global analysis. Previous studies suggest that the differentiation between the sexes in these characters starts with the beginning of maturity (Ward 1951; Tack 1973; Kratt and Smith 1979) and is then permanently present in adult fishes outside and during the breeding season. Before sexual maturity,

these characters may essentially follow similar growth trajectories in males and females (Kratt and Smith 1979). Yet, our data of fishes at reproductive stage I (immature condition; see Sakun and Butskaya (1968)) indicate a largely similar, but often non-significant pattern of sexual dimorphism for the fins (ANCOVA, $P > 0.05$; $n = 285$, across 6 species; data not shown).

The general predictions on the different reproductive strategies and energy investments suggest differential selection acting on the sexes (Fleming and Gross 1994), and thus the existence of sexual dimorphism in specific morphometric traits in order to increase reproductive success. In male graylings, these predictions are consistent with the male-biased length and height dimensions of the dorsal, pelvic and pectoral fins. The display of the dorsal and pelvic fins is an integral behavioural element in male-male competition and territorial behaviour during spawning season (Fabricius and Gustafson 1955). Sex-specific differences in these characters have already been described for *T. arcticus* (Romanov 2016), *T. flavomaculatus* (Semenchenko 2005), *T. tugarinae* (Mikheev 2009) and *T. thymallus* (Persat 1977; Zinovjev 2012; Kucheruk et al. 2015). The pectoral fin is known to

support swimming stability and manoeuvrability in many fish groups (Bone and Moore 2008) and may be favourable in male-male competition and mate acquisition. However, the most noticeable morphological trait among graylings—the large colourful dorsal fin—may not have evolved as a direct consequence of sex-specific selection as the trait is permanently present in both sexes. Thus, the initial driver of this accentuated character, which does not occur in any other salmonid fish, is most probably rooted in natural selection perhaps in the form of intraspecific competition for position in the typically drift-feeding graylings (Fabricius and Gustafson 1955; Hughes and Dill 1990).

In female graylings, the commonly observed female-biased dimorphism in size among other salmonid species (e.g. Tamate and Maekawa 2004; Morbey 2018) is not supported in our analysis, although length dimensions of the abdomen and distances between fins were generally longer in females. However, these differences may be a secondary effect of the position of the fins perhaps in relation to the extended dorsal and anal fin bases in males. The strongest pattern of female-biased dimorphism was evident for the height of the anal fin. Kratt and Smith (1979) reported that the anal fin is used in lateral display, but the lack of iridescent colouration compared to other fins (see Fig. 1) may indicate a reduced visual function. It is more likely that, similar to other gravel spawning salmonids, the anal fin holds a female-specific (mechanistic) function in reproduction (see Thorn and Morbey 2016), related to the female “probing” behaviour (Groot 1996; Esteve 2005) or oviposition. Compared to other gravel spawning salmonid species, however, female graylings do not construct spawning redds or actively cover the eggs with substrate after fertilization (Fabricius and Gustafson 1955). Instead, the eggs are buried into the substrate by the characteristic spawning behaviour, whereby the caudal region of the female is forced into the porous gravel substrate by vigorous quivering of both sexes and tail flapping of the male (Kratt and Smith 1980). This grayling-specific behaviour may also be key to understanding the observed sexual dimorphism in the caudal region, which includes a deeper caudal peduncle and an elongated length of the anal fin base in males.

An interesting finding of our study is that the extent of sex-specific differences in the general traits is not equal across the genus. The fact that these differences

do not have a significant phylogenetic signal would seem to support a differential strength of intrasexual selection across species. Among the few species that showed weaker or a lack of pronounced sexual dimorphism, *T. brevirostris* from the species poor (i.e. impoverished ichthyofauna; Kottelat (2006)) Altai region of Western Mongolia stands out. This species has comparatively small trait sizes for the fins and generally shows only weak differences in morphometric characters between the sexes. Besides *T. nigrescens*, it is the only species in our data set where in both sexes the greatest height of the dorsal fin is in its anterior part, which does not only affect the shape of the fin (see Knizhin et al. 2008a: Fig. 3), but likely also its display function as the extended posterior part is usually the most colourful region. In contrast, *T. burejensis*, endemic to the Bureya River, a tributary of the Amur River in the Russian Far East, is characterized by having large dorsal, anal, pelvic and pectoral fins in both sexes but significant sexual dimorphism only in the pelvic fin. *Thymallus burejensis* further contrasts with *T. brevirostris* as it occurs in sympatry with up to three other grayling species (*T. baicalolensis*, *T. grubii* and *T. tugarinae*; see Antonov (2004); Knizhin et al. (2004)). This raises the possibility that the unique combination of sex-specific attributes reflects past competition (sensu Connell 1980) among sympatric species, supporting species recognition (i.e. distinction between con- and heterospecific individuals) and thus may help to avoid hybridization.

Review of sexual dimorphism in morphometric traits across Salmonidae

Previous studies have shown that sexual dimorphism in salmonids is primarily driven by breeding competition (Fleming and Reynolds 2004). Other factors such as life history tactics (e.g. early maturation) (Koseki and Maekawa 2000) or habitat characteristics (Oke et al. 2019) can have a profound effect on the development and/or expression of sexually dimorphic traits, which underlines the intraspecific (among-population) variability and facultative nature of sexual dimorphism in this group. By reviewing the collective evidence of the factors responsible for trait-specific sexual dimorphism, patterns emerge supporting links between reproductive behaviour or particular environmental conditions and specific morphometric

character development associated with sex. The reduced taxonomic coverage among existing studies on sexual dimorphism in salmonids limits evolutionary interpretations, but our review nonetheless expands support for a number of assumptions that have been made for single taxa or genera in the past.

Sexual dimorphism is present in all subfamilies of salmonids but the specific traits or patterns of expression differ among groups. In Thymallinae, intersexual variability in morphometric traits is primarily associated with length and height attributes of the fins, although these characters are subject to sexual dimorphism in Coregoninae and Salmoninae as well. The height or length of the dorsal, adipose, pelvic and pectoral fins are male-biased in multiple genera/species, and were found to play a role in behaviours related to display (e.g. Fabricius and Gustafson 1955; Esteve et al. 2009a; Muir et al. 2012) and female choice (Järvi 1990) but are apparently not so energetically costly as to make them facultative and dependent on life history. Interestingly, the height of the anal fin is the only fin-specific character that shows frequent female-biased dimorphism in gravel spawning Thymallinae and Salmoninae but appears to be monomorphic in the open substrate spawning Coregoninae. Thus, this female-biased trait may have evolved in response to selection pressure on females, related to a mechanistic or sensory function involving oviposition or selection of a suitable spawning habitat (Thorn and Morbey 2016) in order to increase offspring survival. However, the height and shape (see Gruchy and Vladykov 1968) of the anal fin have not yet been investigated in several species, and data on *Brachymystax* suggest that both sharp- and blunt-snouted lenok are sexually monomorphic in the height of the anal fin (Alekseyev S., unpublished data).

The characteristic transformation of the jaws and snout is limited to Salmoninae, but absent from the basal genera *Brachymystax* and *Hucho* (Esteve and McLennan (2008); Esteve et al. (2009b)), and apparently reduced or absent in *Parahucho* (Esteve et al. 2009a). Thus, these traits may represent a derived set of characters possibly related to the intense breeding competition in Salmoninae. Interestingly, the greatest expression of sexual dimorphism in jaws and snout is exhibited in anadromous and primarily semelparous species (absent in precocious parr; Koseki and Maekawa (2000)), life history strategies that presumably allow more energy to be invested in such

asymmetric growth (Fleming and Reynolds 2004). This contrasts somewhat with anadromous *O. mykiss* (Steelhead trout); a species that shows comparatively little dimorphism in the jaws. However, although the species is iteroparous only a rather small percentage of anadromous individuals manage to breed a second time (10% (0.6–31.3%) in Fleming (1998); 2.4% in Christie et al. (2018)). The fact that another iteroparous anadromous salmonid, *Salmo salar*, exhibits pronounced sexual dimorphism in the lower jaw, as well as a low frequency of repeat spawning with a mean of 11% (0.7–42.5%; Fleming (1998)), suggests a mechanistic relationship between anadromous behaviour or (facultative) semelparity and the development of sexual dimorphism at the level of the individual as opposed to a fixed population or species-specific trait.

Compared to Salmoninae and Thymallinae, species in Coregoninae generally exhibit a low degree of sexual dimorphism (Willson 1997). This may be rooted in the fact that Coregoninae contrast sharply in a range of reproductive behaviours compared to other salmonids. For example, Coregoninae are open substrate spawners, exhibit reduced intrasexual competition for access to mates and commonly spawn at night (Fabricius and Lindroth 1954; Karjalainen and Marjomäki 2018), all behavioural traits that are not common in Salmoninae and Thymallinae (Fabricius and Gustafson 1955; Esteve 2005). Thus, spawning behaviour in Coregoninae may favour a different set of (non-visual) signals such as the development of breeding tubercles, which are commonly male biased in size and abundance (Willson 1997). The lacustrine open substrate spawning *Salvelinus namaycush* (Lake char) with well-described male-biased tubercles (Muir et al. 2012), would support this hypothesis, but there is too little information on tubercles in salmonids in general to draw further conclusions on their prominence and relation to reproductive behaviours and sexual dimorphism.

Overall, it would be revealing to test the effects of environmental conditions vs. common ancestry on trait evolution across a broader phylogenetic range of salmonids with particular focus on species showing diverging life-history tactics or a behavioural repertoire that contrasts with closely related congeners such as observed in *S. namaycush* (Muir et al. (2012)).

Limitations and future research perspectives

While we think that the general trends of sexual dimorphism in graylings reported in this analysis are robust, a few comments on potential caveats and data limitations are warranted. Multiple populations across the range of some species (e.g. *T. arcticus*, *T. baicalensis*), contrast with single populations or low sample sizes of some others (e.g. *T. nigrescens*, *T. svetovidovi*). Thus, some species-specific results may not capture the natural range of variability that is present. Furthermore, the size range analysed does not capture the entire range of sexually mature fish, especially for those species with lower sample sizes. This may be important because the assumed allometric growth component of morphometric traits is likely to lead to a higher degree of dimorphism with age, a variable that we could not assess directly due to the limitation of insufficient sample sizes across multiple age-classes. Lastly, our analyses rely on linear morphometric characters only, without evaluating dichromatism or shape dimorphism, and thus the full scope of sexual dimorphism is assumed to be underestimated. We therefore recognize specific areas of research that could further clarify the patterns and hypotheses concerning the evolution of sexual dimorphism in graylings.

- a. The allometric growth component in morphometric traits could be investigated directly for both males and females, perhaps most simply using hatchery-reared populations and the measurement of individuals across their entire life-cycle (following an ontogenetic approach). These studies could provide direct evidence for the secondary sexual development of specific morphological traits and may also include shape dimorphism (geometric morphometrics) to capture a broader extent of intersexual morphological variability. Moreover, such studies could clarify to what extent sexual dimorphism is related to age and growth.
- b. The large colourful dorsal fin in graylings has long been suggested to play a key role in reproductive behaviour (Fabricius and Gustafson 1955) and its species-specific colouration pattern (Knizhin 2009) raises the question of species recognition. The recognition of potential conspecific mates may be more important for those species living in sympatry with congeners, as this could help maintain reproductive isolation. It is, however, unclear if and to what extent graylings actively choose mates based on visual traits and more generally what mechanisms may underlie species recognition and mate choice in graylings. In other salmonid species, mate choice appears to be quite common where several sexually dimorphic traits such as the adipose fin in *Oncorhynchus* and *Salmo* (Beacham and Murray 1983; Järvi 1990), the kype in *Salmo* (Perry et al. 2019) or the breeding tubercles in *Coregonus* (Wedekind et al. 2008) are thought to be, at least partly, subject to female choice and/or serve a function in displaying status.
- c. The clarification of which morphological characters are more driven by natural vs. sexual selection as well as the relevance of intra- vs. interspecific competition can be investigated by research on contact zones, such as in the Amur drainage, where at least three if not four grayling species can be found in sympatry (Antonov 2004; Knizhin et al. 2004; Weiss et al. 2020, 2021). In these zones, our sample sizes were limited for some species, and the spectrum of investigated characters could be expanded to include both coloration and shape, if not also differential gene expression that may be mechanistically driving these patterns. Such studies would also benefit from more detailed behavioural data, especially on the spatio-temporal distribution of spawning, as well as both the accentuation and display of body and fin colouration during the entire reproduction period. Likewise, genome-wide sequencing and expression studies can help identify sex-biased gene expression (e.g. Sharma et al. 2014) and alleles (Mohammed et al. 2019), which will foster the understanding of the mechanisms that maintain reproductive isolation.
- d. In addition to contact zones, several widespread species, such as *T. arcticus*, *T. baicalensis*, *T. baicalolenensis* and *T. grubii*, which are found in diverse habitats either in sympatry with other grayling species or alone (Weiss et al. 2021), could be investigated specifically for potential morphological (sexually dimorphic) patterns that change based on habitat or the presence or absence of congeners. Thus far, Weiss et al. (2020) touched on the morphological differences within and

between populations of *T. baicalolenensis* across three different major drainage systems (Amur, Lena, Yenisei), and the likelihood that these differences are driven by different selection mechanisms, potentially including ecological niche partitioning in one drainage, but interspecific competition or its avoidance in another drainage.

More generally, some of our observations can be extended to other genera in Salmonidae, where relatively little is known about general patterns of sexual dimorphism across multiple species and different habitats. We assume that both population and species-specific patterns of sexual dimorphism are relevant for long-term population viability. Thus, we should recognize that our increased knowledge of sexual dimorphism and the underlying evolutionary processes clearly contribute to the growing consensus that the management of salmonid fish populations should aim to avoid using artificial rearing and stocking to supplement populations and above all, inter-basin transfers, whether involving conspecific or congeneric material (Laikre et al. 2010; Rand et al. 2012; Weiss et al. 2013). In areas where such cross-basin transfers have already occurred, and extensive hybridization exists between divergent lineages, such as between *T. thymallus* and *T. aeliani* in much of the original range of the latter species (Meraner et al. 2014), it would be revealing to examine sexual dimorphism in populations showing introgression and see whether or not the patterns of trait divergence conform to the general patterns of sexual dimorphism observed in the present study.

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Author contributions Conceived and coordinated the study: GKE, SJW. Analysed the data: GKE. Contributed data and field observations: AA. Wrote the first draft of the paper: GKE, SJW. All three authors contributed equally to the improvement of the manuscript.

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Data availability Data are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval In the present study, a data set of morphological characters was analysed but no direct studies on animals were performed by any of the authors.

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