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Divergent skull morphology between trophic separated lacustrine forms of Dolly Varden charr from Lake Kronotskoe, Kamchatka, Russia

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Abstract Trophic morphology divergence between two forms of Dolly Varden *Salvelinus malma* from Lake Kronotskoe (Kamchatka Peninsula, Russia): *Salvelinus albus* (omnivorous form) and *Salvelinus schmidti* (benthivorous form) was studied using the skull bone's qualitative and measurable attributes. The strongest morphological differences between these two charr forms were associated with the jaw bone structures (i.e. premaxilla, maxilla, angulo-articular and dentary bones). There was an abrupt shift in bone structures with increasing body length in both morphs. *S. albus* of body length less than 350 mm had wider dentary, maxilla and angulo-articular bones than individuals of similar sized *S. schmidti*. In contrast, large (>350 mm) individuals of *S. albus* had narrower bone structures than individuals of *S. schmidti*. This divergent pattern of the bone structures in the jaws is closely linked with their different trophic niches measured by the contence of stable isotope (δ^{13}C, δ^{15}N) values in the tissues. *S. albus* have a narrow, long jaw bones and a terminal mouth and occupy an omnivorous niche with clear ontogenetic niche-shifts (i.e. benthivore and piscivore) by increasing in size. In contrast, *S. schmidti* have short jaw bones and a low mouth; also *S. schmidti* is exclusively benthivores through their entire ontogeny. These trophic traits indicate clear divergence between two lacustrine forms, which occupy different trophic niches but, at the same time, are not completely reproductively isolated.

Keywords *Salvelinus malma* · Skull bone's morphology · Trophic specialization · Sympatric populations

Introduction

Intraspecific variation in trophic morphology is closely related to patterns of niche specialization and ecological segregation in many different taxa (Conuba and Shiba 2007; Herrel and Holanova 2008). Trophic related traits in the head can be a powerful predictor of feeding performance and habitat preference (Mittelbach et al. 1999; Muschick et al. 2011; Küttner et al. 2013). A characteristic peculiarity of polymorphic fish species, like cichlids, barbs and charrs is the sympatric existence of several forms, or ecotypes, which are traditionally distinguished by standard morphological methods (Behnke 1980, 1989; Savvaitova 1989; Nagelkerke et al. 1994; Alekseev et al. 2000; Mina et al. 2001). Studies of skull morphology in various charrs is one of the important methods, which has been used for many years, particularly in studies of intraspecific relationships between different forms (Medvedeva and Savvaitova 1980; Prokofiev 2007; Pichugin 2009). Charrs of the genus *Salvelinus* are characterized by high level of phenotypic divergence and adaptive plasticity (Jonsson and Jonsson 2001). Divergence of Arctic charr

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is recently described in isolated lakes like Thingvallavatn in Iceland (Jonsson et al. 1988; Skulason et al. 1989; Malmquist et al. 1992), Fjellfrøsvatn in Norway (Klemetsen et al. 2002, 2003; Knudsen et al. 2007, 2011), Elgygytgyn Lake in Central Chukotka, Russia (Chereshnev and Skopetz 1993), lakes of Taimyr Peninsula and Transbaikalian lakes in Russia (Savvaitova 1989; Alekseyev et al. 1999). Several sympatric ecotypes of charrs that were described in these lakes include benthivorous forms, deep-water benthivorous "dwarf" forms, piscivore forms and zooplanktivore forms. One evident example of a unique endemic variety of landlocked Dolly Varden charr [*Salvelinus malma* (Walbaum, 1792)], was found in Lake Kronotskoe, Kamchatka Peninsula (Kurenkov 1972; Viktorovsky 1978). The origin of the Lake Kronotskoe is very special: the lake was formed *'de novo'* by lava flows from volcanic eruptions about 12,000 years ago that have dammed the ancient Paleokronockaya River. In this period of time several postglacial lakes were formed after the ice-retreat in the Northern Hemisphere. Evidently, the ancestral anadromous riverine forms of Dolly Varden have given rise to a variety of endemic lacustrine forms of charr. These forms have trophic related morphological differences (i.e. in skull bone traits). In the present study the two most abundant endemic sympatric forms of charr were explored with a special focus on the development of several skull bone traits, which have increasing fork length.

The forms of Dolly Varden in Lake Kronotskoe were described as a separate species: *S. albus* (Glubokovsky, 1977), *S. schmidti* (Viktorovsky 1978) and *S. kronocius* (Viktorovsky 1978) (Viktorovsky 1978; Glubokovsky 1995), but as they are not reproductively isolated (Ostberg et al. 2009); in this article they will be considered as different "forms". They are divided into different ecological and trophic niches: the omnivorous, the benthivorous and the piscivorous dietary niches, respectively. In recent years a dwarf (the fourth endemic form) have been found (Pavlov et al. 2012). Preliminary studies suggested that these forms of charrs also differ in the structure of their skull and karyotype (Viktorovsky 1978; Chereshnev et al. 2002). *S. schmidti* (benthivorous form) is a bottom-dweller, which occur in shallow waters between big rocks and in sandy areas. The omnivorous form, *S. albus* demonstrates a variable foraging tactic during its life: The juveniles consume different benthic organisms in the coastal area and their gut content is very similar to benthivorous forms. However, with the increase of body length this form shifts to be piscivores and it occupies all habitats throughout the lake. The most profound external differences between these two forms are observed in the head shape: *S. schmidti* usually has small mouth, big «lips» and a short rostrum. In sharp contrast, *S. kronocius* (specialized piscivore form) has a very long head with big mouth and long jaws. The omnivore *S. albus* could be defined as the intermediate variant between the two more trophic specialized forms. Genetic studies of mitochondrial DNA have suggested that the Lake Kronotskoe charr forms represent the northern Dolly Varden subspecies complex (Radchenko et al. 2006; Oleinik and Skurikhina 2010). The mtDNA differences between the three resident charr forms suggest a very recent divergence with ongoing exchange of genes through hybridization and no complete reproductive isolation between the forms (Radchenko et al. 2006; Oleinik and Skurikhina 2010).

By comparing individuals from different trophic forms of charr from the Lake Kronotskoe, one of the most profound distinctions between them is observed in morphological traits in the skull (Viktorovsky 1978). The osteological analysis of the bones, used in this study, is based on traditional taxonomical and phylogenetic research in many groups of salmon fish (Norden 1961; Vladykov 1963; Medvedeva and Savvaitova 1980; Alekseyev and Power 1995; Savvaitova et al. 2004). Analysis of the bones in the skull allows specifying the features, which characterize these forms and evaluate the morphological development of these features.

In the present study the two most abundant endemic sympatric forms of charr were investigated with a special focus on the development of several skull bone traits with increasing fork length.

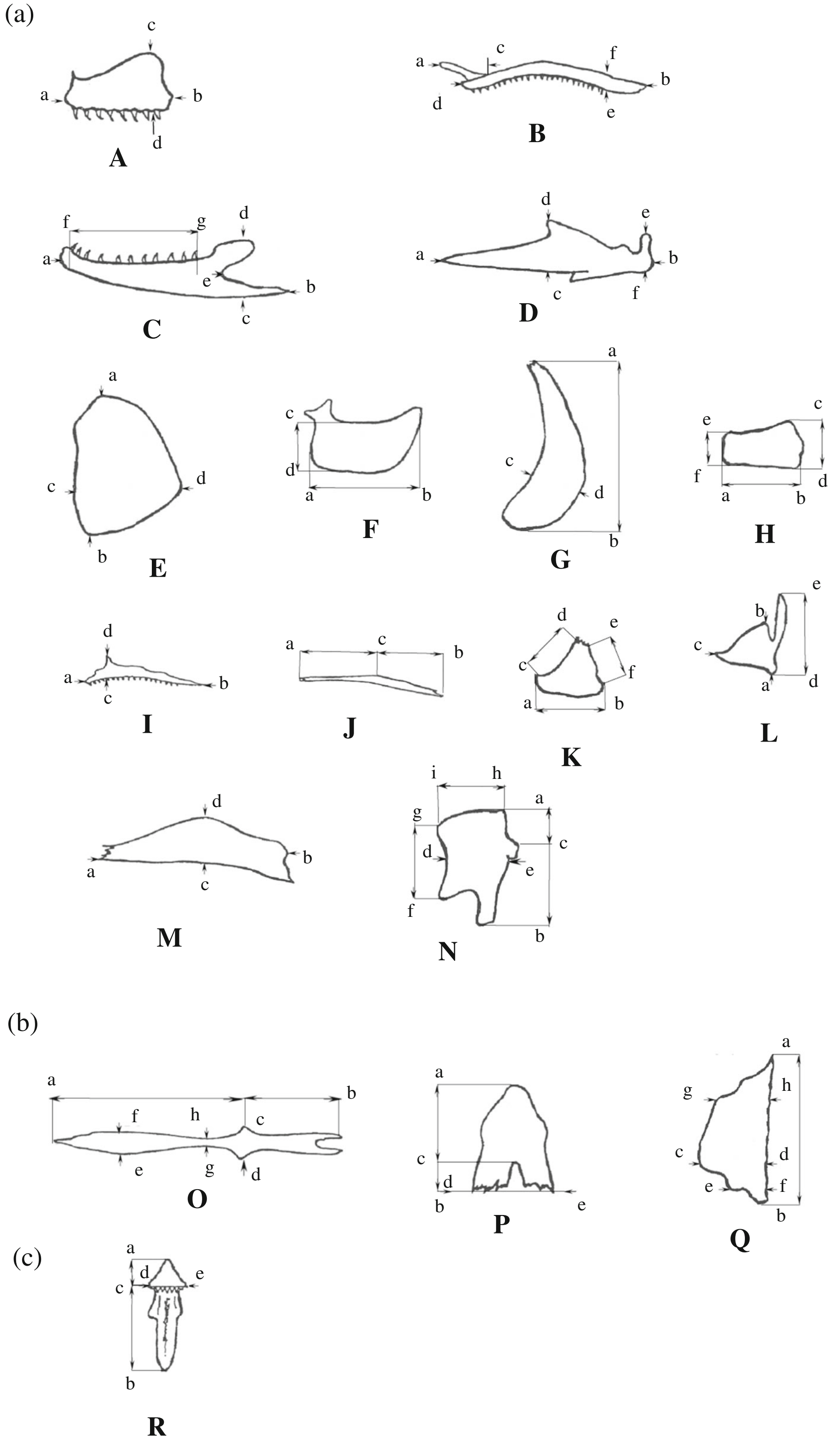
The goal of this study is to compare osteological head bone structures between two charr forms with different trophic niches: one omnivore form (*S. albus*) and one benthivore form (*S. schmidti*). We have focused specifically on describing the development of osteological features of the two forms (*S. albus* and *S. schmidti*) and revealing the possible evolutionary connection of these features with charr's trophic niche (habitat and dietary utilization). We suppose that the highly specialized benthivore *S. schmidti* have relatively higher jaw structures in comparison to the omnivore *S. albus* that utilize a wider dietary niche. Possible divergent trophic niches are measured by using stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values.

Materials and methods

The Lake Kronotskoe has a surface area of 242 km^2 and a drainage area of 2,330 km^2, and it is the largest freshwater lake in Kamchatka Peninsula (Russia). The Lake was formed by damming of the ancient Paleokronotskaya River by lava deposits (up to 200 m high) from volcanic eruptions of the Krashennikov and Kronotsky volcanoes. The lake has an average depth of about 58 m, with maximum depth of about 136 m and has a volume of about 12 km^3 (Arakeliantz and Tkachenko 2012). It has five big tributaries: Unana, Listvenichnaya, Uzon, Severnaya and Perevalnaya rivers, as well as many small rivers and creeks. The only outlet from the lake is the Kronotskaya River (44 km total length). It has several rapids that make the lake unreachable for anadromous salmonid species into the lake (Viktorovsky 1978). The ichthyofauna of Lake Kronotskoe is characterized by high level of endemism, as there are four ecologically different forms of Dolly Varden charr of the genus *Salvelinus* (Walbaum, 1792) (Kurenkov 1972; Viktorovsky 1978; Pavlov et al. 2012). Individuals related to these forms are preserved in a research collection in the Zoological Institute of the Russian Academy of Sciences (Universitetskaya emb., 1, St.-Petersburg, 199034, Russia).

The Dolly Varden charr was sampled by multi-mesh gill-nets (mesh size 12, 20, 30, 40, 50 mm; knot to knot) from the littoral (benthic gill-nets, 0–10 m) and pelagic zones (offshore floating gill-nets, near 20 m depth) in Lake Kronotskoe from July to October for a period of 3 years (2010–2012). In the present study two charr samples were used. The sample of *S. albus* consisted of 120 individuals and the sample of *S. schmidti* consisted of 95 individuals. Each individual was tentatively identified in the field as either *S. albus* or *S. schmidti* according to the appearance of the head and mouth, length and height of the body and coloration (Viktorovsky 1978): *S. albus* has a terminal mouth; the upper jaw goes far beyond the edge of the eye; fins and lips are grey; the body is relatively slender. *S. schmidti* has a low “lipped” mouth, the upper jaw does not extend beyond the back edge of the eye; the fins and the lips are usually red or pink; the body is deep.

Only individuals that manifested characteristic external morphology and could be assigned to one of the studied forms (fork length up to 200 mm) were used for the study. The first step was to estimate sexual dimorphism in samples of mature (i.e. spawned at least one time) individuals of *S. albus* and *S. schmidti*, 18 cranial bones were compared between males and females in each sample (Fig. 1). Only big individuals were included in analysis (fork length more than 350 mm in the *S. schmidti* sample and more than 450 mm in the *S. albus* sample). The second step was to define bones that have the greatest differences between *S.albus* and *S. schmidti* samples, the same 18 cranial bones were compared between the forms in all mature individuals (without distinction between males and females as the level of sexual dimorphism was very low). For the first and second steps only big individuals were included in the analysis (the fork length more than 350 mm in the *S. schmidti* sample and more than 450 mm for the *S. albus* sample). The third step: changes in bone structures with the growth of fish were studied by a cross sectional analysis among individuals. The samples of charrs (mature and immature individuals together) were divided into 7 size groups: *S. albus* (A) and *S. schmidti* (S) with fork length less than 250 mm (A1: $n=14$; S1: $n=8$ individuals); fork length 251–350 mm (A2: $n=20$; S2: $n=41$); fork length 351–450 mm (A3: $n=29$; S3: $n=44$) and fork length larger than 451 mm (A4: $n=45$). There were no *S. schmidti* individuals with fork length more than 450 mm. In order to evaluate the changes in the proportion of the bones with the growth of fish in *S.albus* and *S. schmidti* samples, each size group was compared with the next bigger size group. To compare osteological traits between forms, each size group in *S. albus* sample was compared with the corresponding size group in *S. schmidti* sample. To analyze the shape of the bone structures the absolute values of the bone features (i.e. the linear measurements) were divided by the greatest length of the bone. All paired comparisons were made with Mann–Whitney U-tests. Relationships between absolute values of the bone features and the fork length of the individual were analyzed to reveal the minimum size of the fish at which the divergence between charr forms occurs. Both absolute and relative measurements of a specific bone structure (i.e. normalized for length of the bone) were analyzed. The relation between the linear measurements of bone structures and fork length was done by using regression analysis: scatterplots were analyzed and the curve fitting by the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◀ **Fig. 1** The scheme of skull bones measuring: in all cases the measurement "ab" is basic and designates the greatest length **a** lateral view; **b** dorsal view; **c** ventral view. *A*—Premaxilla: *cd*—the greatest height (PmxH), *ac*—length of forward part (Pmxlf), *cb*—length of back part (Pmxlb); *B*—Maxilla: *ac*—length of connective part (Mxls), *db*—length of free part (Mxlcb), *de*—length of dental part (Mxld), *ef*—the greatest width (MxH); *C*—Dentary bone: *cd*—the greatest height (DH), *eb*—depth of notch (Ds), *fg*—length of dental part (Dld), *ad*—length of the upper edge (Dl); *D*—Angulo—articular bone: *cd*—the greatest height (AH), *ef*—height of back edge (Ah), *bd*—length of the free part (Alcb), *ad*—length of connective part (Alcf); *E*—Opercular: *cd*—the greatest width (OpW); *F*—Subopercular: *cd*—the greatest width (SopH); *G*—Preopercular: *cd*—the greatest width (PopW); *H*—Interopercular: *cd*—height of back edge (Iophb), *ef*—height of a front edge (Iophf); *I*—Palatinum: *cd*—the greatest height (PltH); *J*—Ectopterygoid: *ac*—length of forward part (Eclf); *K*—Metapterygoid: *cd*—length of forward part (Mtlf), *ef*—length of back part (Mtlb); *L*—Quadratum: *ad*—possess height (Ql.l), *bc*—width (QW); *M*—Hyomandibular: *ac*—height of back edge (Hmlb), *gf*—height of a front edge (Hmlf), *hi*—width of a front edge (HmW), *de*—width of average part (Hmwl); *N* Supraethmoid: *ac*—the smallest length Sethl, *de*—the greatest width (SethW); *O* Frontal: *cd*—the greatest width (FW), *ef*—the smallest width (Fw), *fg*—width before narrowing (Fwl); *P*—Parasphenoid: *ac*—length of forward part (Pslf), *ef*—width of forward part (PsWf), *gh*—interception width (PsWp), *cd*—distance between lateral possesses (PsW); *Q*—Entopterygoid: *cd*—the greatest width (EnW), *ad*—length of forward part of internal edge (Enlf), *db*—length of back part of internal edge (Enlb); *R*—Vomer: *ac*—head length (Vlg), *de*—head width (Vwg)

method of least squares (second degree curve) was made. For all statistical calculations Statistica 6.0 was used.

For separating the bones from the flesh and from each other the heads were placed for a few seconds into boiling water. Bones from the left side of the head of each individual were used for analysis. Each bone was photographed with a digital camera Canon 1100 under identical light conditions and standardized scaling. The bones were measured in accordance with previously published procedures (Pavlov et al. 2001). The linear measurements of individual bones were carried out using the digital image analysis program AxioVision.

Stable isotope analysis (SIA) was performed on muscle tissue sampled from the back, in front of the dorsal fin from each individual fish. The tissue was dried at 50–60 °C for 48 h and pulverised before weighing 3–4 mg into a tin case. Analyses of nitrogen (δ^{15}N) and carbon (δ^{13}C) stable isotope ratios were performed using an elemental analyser (Flash 1112, Thermo Electron, Bremen, Germany) coupled to a mass spectrometer (DeltaV Plus, Thermo Electron) at the A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Science. The natural abundances of δ^{15}N and δ^{13}C were expressed in per mil (‰) deviation from international standards:

$$\delta^{15}\mathrm{N}\ \text{or}\ \delta^{13}\mathrm{C} = \left(R_{\mathrm{sample}}/R_{\mathrm{standard}}-1\right) \times 1000,$$

where R is ^{15}N/^{14}N or ^{13}C/^{12}C, respectively.

Stable nitrogen and carbon isotope ratios are expressed as delta values (δ^{15}N and δ^{13}C, respectively) relative to the international standards for carbon (Vienna PeeDee Belemnite) and nitrogen (atmospheric nitrogen). Standard deviation of the internal standard was less than 0.03 ‰ for δ^{13}C and δ^{15}N in each run (Tiunov and Kirillova 2010).

All individuals without division on size groups were included in comparison between values of stable isotopes in *S. albus* and *S. schmidti* samples (Mann–Whitney *U*-test).

Results

Stable isotopes

Individuals of the benthivore specialist *S. schmidti* have significantly ($p<0.001$) lower carbon and nitrogen values compared with the omnivore *S. albus* (Table 1, Fig. 2). This confirms the considerable differences in food specialization between these forms.

The main differences in head bone structures

The samples of mature individuals consisted of 33 individuals of mature *S. albus* and 18 individuals of mature *S. schmidti* and 18 cranial bone structures of the skull were measured from each individual fish (see Fig. 1). In the samples of *S. albus* there were only 5 bone structures that differed significantly between females and males ($P<0.05$, Table Sm1, see supplementary material) and in the sample of *S. schmidti* only 3 bone structures differed (Table Sm2, see supplementary material). Therefore the following analysis was done without gender distinctions.

Significant differences between *S. albus* and *S. schmidti* mature individuals were revealed for 10

Table 1 Amount of stable isotopes (carbon δ^{13}C and nitrogen δ^{15}N) in muscles of *S. albus* and *S. schmidti*

	δ^{13}C		δ^{15}N	
	S. albus	*S. schmidti*	*S. albus*	*S.schmidti*
Med (Lq-Uq)	−24.17* −24.93 – −22.71	−16.89 −19.76 – −15.17	10.01* 8.32–10.75	8.42 7.51–9.12

Med median, *Lq* low quartile, *Uq* upper quartile; significance level: *$p<0.05$

bone structures ($P<0.05$; Table Sm3, see supplementary material); the majority of the differences in the bone structures were revealed for jaw bones: premaxilla, maxilla, dentary bone and angulo-articular bone. Therefore, only these bone structures were included in the following analysis.

Differences in jaw bones structures of *S. albus* and *S. schmidti* with increasing fork length

Relative values

Relative values of jaw bones morphological traits, results of paired comparison between neighbor size groups in each sample and results of paired comparison between corresponding size groups in *S. albus* and *S. schmidti* samples are shown in Table 2.

In *S. albus* sample the comparison between size groups A1 and A2 have revealed the significant differences for only two features (Mxlcb, Pmxlb). After reaching a fork length of 350 mm (comparison between A2 and A3; A3 and A4 size groups) several additional differences were revealed: extension of the dental edge of dentary bone and the general length of maxilla; decrease in the height of maxilla and premaxilla; decrease in length of back part of premaxilla and angulo-articular bone. These changes in proportions indicate that, in general, with increasing fork length these jaw bones became more elongated.

In the jaw structures of *S. schmidti* there were no significant differences between S1 and S2 size groups (Table 2). After achievement of 350 mm fork length some changes in bones shape were revealed: in general, the bones of the lower jaw (angulo-articular and dentary bones) became broader, and all jaw bone structures became relatively longer.

Fig. 2 The variation in δ^{13}C and δ^{15}N values between *S. albus* and *S. schmidti* samples

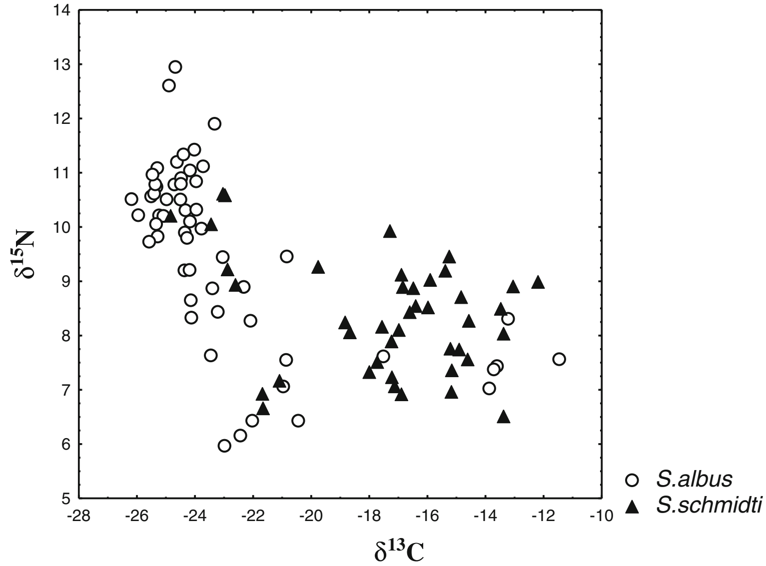

Table 2 Jaw bone morphological traits of *S. albus* (A) and *S. schmidti* (S) in different size groups (relative values)

1 Feature	2 A1 Med (Lq-Uq)	3 A2 Med (Lq-Uq)	4 A3 Med (Lq-Uq)	5 A4 Med (Lq-Uq)	6 S1 Med (Lq-Uq)	7 S2 Med (Lq-Uq)	8 S3 Med (Lq-Uq)
PmxH	95	81†	73*†	63**	89	90	80**
	(78–148)	(76–84)	(66–83)	(57–66)	(88–94)	(82–100)	(74–87)
Pmxlf	131	88	90	91	87	98	89
	(82–134)	(82–98)	(86–96)	(85–95)	(84–97)	(87–105)	(85–99)
Pmxlb	94	79 *	60 **	50 **	81	80	64 **
	(84–100)	(74–88)	(54–71)	(44–57)	(76–85)	(72–83)	(55–73)
Mxls	17	16	17	16	16	16	16
	(15–18)	(14–17)	(14–18)	(14–17)	(14–17)	(15–18)	(14–18)
Mxlcb	83†	86*†	88**	87	88	87	86
	(82–85)	(85–87)	(86–90)	(85–88)	(85–89)	(85–90)	(85–89)
Mxld	59	63	68**†	68	62	64	63
	(55–64)	(59–65)	(65–70)	(65–71)	(60–63)	(58–67)	(59–66)
MxH	12	10†	9†	7 **	14	13	13
	(11–13)	(9–12)	(8–10)	(7–9)	(13–15)	(12–15)	(11–14)
DH	31	29	28†	26	33	30	35 **
	(28–34)	(27–31)	(26–30)	(24–28)	(28–35)	(28–33)	(32–37)
Ds	47	45†	39 **†	34 **	48	50	43 **
	(41–47)	(40–49)	(37–41)	(29–37)	(44–51)	(45–52)	(41–46)
Dld	45	48†	53 **	58 **	40	42	51 **
	(40–52)	(44–51)	(49–58)	(55–62)	(39–43)	(39–49)	(47–54)
Dl	69	69	73**†	77*	67	66	71**
	(66–71)	(65–72)	(70–75)	(73–80)	(65–70)	(62–70)	(67–72)
AH	31	27†	29†	27	30	31	33 **
	(26–33)	(26–30)	(27–31)	(25–29)	(29–32)	(28–33)	(30–35)
Ah	21	19†	19†	18	22	22	22
	(19–24)	(17–21)	(18–21)	(17–20)	(21–22)	(21–24)	(20–24)
Alcf	47	48†	52	57	43	45	52 **
	(45–51)	(44–53)	(48–58)	(55–61)	(40–51)	(42–47)	(50–54)
Alcb	64	60†	57†	52 **	68	68	62 **
	(61–66)	(54–65)	(55–60)	(49–55)	(66–70)	(65–71)	(59–66)

Significance level in comparison with nearest smaller size group in each sample: *$p<0.05$: **$p<0.01$; significance level in comparison between corresponding size groups: †$p<0.05$. The abbreviations in the column are given in Fig. 1

Med median, *Lq* low quartile, *Uq* upper quartile. L_F<250 mm (A1 and S1), 251–350 mm (A2 and S2), 351–450 mm (A3 and S3), ≥450 mm (A4)

Results of paired comparison between corresponding size groups in *S. albus* and *S. schmidti* samples are shown in Table 2.

In the smallest size groups (A1, S1) significant difference was found for only one feature, in the maxilla bone (Mxlcb). After achieving fork length of 250 mm (A2, S2), several significant differences appeared: *S. albus* had maxilla bone shorter than *S. schmidti* and the angulo-articular, maxilla and premaxilla bones lower than *S. schmidti*. Additionally, *S. albus* had dental edge of the dentary bone longer, than *S. schmidti*.

Some of these tendencies were clearer for the larger fish (A3, S3). In general, *S. albus* had much lower jaw bones with a bigger dental edge than

Table 3 Jaw bone morphological traits of *S. albus* (A) and *S. schmidti* (S) in different size groups (absolute values in mm)

1 Feature	2 A1 Med (Lq-Uq)1	3 S1 Med (Lq-Uq)2	4 A2 Med (Lq-Uq)3	5 S2 Med (Lq-Uq)4	6 A3 Med (Lq-Uq)5	7 S3 Med (Lq-Uq)6
Pmx_ab	3.9	4.2	5.6 *	5	8.5	9
	(3.6–5.4)	(3.4–5)	(4.9–9.7)	(4.5–6)	(7–10.5)	(8–10)
PmxH	3.8	3.8	5.7 **	4.3	6	7.3
	(3.6–4.1)	(3.1–4.5)	(4.7–7.3)	(3.8–5)	(6–8)	(6.1–8)
Pmxlf	3.6	3.9	4.9	5	7	8
	(3.1–4)	(3.5–4.3)	(4.3–9.8)	(4.1–5.8)	(6–9.5)	(7–9)
Pmxlb	3.2	3.4	4.7 *	4	5.5	5.6
	(3.2–3.4)	(3–3.9)	(3.9–6.1)	(3.7–4.6)	(5–6.8)	(5–6.3)
Mx_ab	15.2	15	23.8 **	19.8	30.5	31.9
	(12.5–17.9)	(13.7–18)	(21.5–26.5)	(17.5–23.5)	(26.8–35.5)	(30–34.1)
Mxls	2.4	2.5	3.7	3.2	5	5
	(2–2.7)	(2–2.6)	(3.4–4.1)	(2.6–4)	(4.9–6)	(4.6–6)
Mxlcb	12.9	13.3	20.5 **	17.2	27	28.1
	(10.4–15.9)	(12.1–15.5)	(17.9–22.9)	(15.4–21)	(15.4–21)	(25.5–29.9)
Mxld	9.6	9.2	14.7 **	12.3	21	19.8
	(8.3–10.8)	(8.5–11)	(13–17)	(10.6–14)	(17.3–23.5)	(18.1–22.2)
MxH	2	2.2	2.7	2.7	3 **	4
	(1.7–2.4)	(2–2.4)	(2.4–2.9)	(2.2–3)	(3–3)	(3.5–4.6)
D_ab	16	16.2	24.6 **	20	31.1	31.8
	(14.6–17.6)	(14.9–17.6)	(20.1–27.9)	(17.6–21.9)	(26.8–34.4)	(30.2–35.6)
DH	4.7	5	6.8 *	5.9	9 **	11.4
	(4.5–5.6)	(4.4–5.7)	(6.3–7.9)	(5.3–6.9)	(7.8–10)	(9.7–12.6)
Ds	7.5	7.6	11.5 *	9.5	12.2 *	14
	(6.1–7.9)	(7–8.8)	(9.7–12.6)	(8.4–10.8)	(11.7–13)	(12.7–15.3)
Dld	7.5	6.6	10.9 **	8.5	16.2	16.2
	(5.8–9.1)	(5.9–7.6)	(9–13)	(7.2–11)	(13–20.5)	(14.8–18.3)
Dl	10.8	11	16.8 **	12.6	21.9	22.5
	(9.8–12.4)	(10.6–11.6)	(12.8–18.3)	(11.5–14.6)	(19.1–25)	(21.2–25)
A_ab	15.9	16	23.4 **	19.4	29.2	30.5
	(14–17.1)	(15.6–16.6)	(21.2–27.2)	(17.1–21.5)	(25.5–31.3)	(27.9–32.5)
AH	4.6	4.8	6.5 *	5.6	7.8 **	9.8
	(4.3–5.3)	(4.5–5.6)	(5.8–7.3)	(5–7)	(7.4–9.2)	(8.8–10.9)
Ah	3.4	3.5	4.3	4.1	5.8 **	6.7
	(2.9–3.7)	(3.3–4.2)	(4–5)	(3.7–4.8)	(4.8–6.3)	(5.9–7.3)
Alcf	7.9	6.8	10.9 **	7.9	16.8	15.9
	(6.1–9)	(6.3–8.9)	(9.6–13.1)	(6.8–9.7)	(13.4–19.3)	(15.3–17.5)
Alcb	10.2	10.9	14.8 *	13	16.5 *	18.6
	(10.1–11)	(10.5–11.5)	(13.8–16.1)	(11.6–15)	(15.5–18.7)	(17.3–20.1)

Significance level in comparison with corresponding size group: $*p<0.05$: $**p<0.01$. The abbreviations in the column are given in Fig. 1. Size group A4 is not included because there is no corresponding size group in *S. schmidti* sample

Med median, *Lq* low quartile, *Uq* upper quartile. $L_F<250$ mm (A1 and S1), 251–350 mm (A2 and S2), 351–450 mm (A3 and S3)

S. schmidti of a similar size, and the largest size groups (A3, S3) had more clear differences than smaller size groups.

Absolute values

The absolute values of jaw bones structures of *S. albus* and *S. schmidti* changed with increasing fork length (Table 3) from small (<250 mm:A1, S1) to medium-sized (251–350 mm: A2, S2) to the largest charr (351–450 mm: A3, S3). Comparison between the smallest size groups (A1, S1) has not revealed any significant differences in jaw bones parameters. Between the size groups A2 vs. S2 a majority of the parameters related to the jaw structures (angulo-articular, dentary and maxilla bones) was significantly different. Between the size groups A3 and S3 there were significant differences in the heights of the angulo-articular bone, dentary bone and maxilla. Therefore, this study shows the following pattern: among individuals with fork length less than 350 mm the heights of the jaw structures are larger in *S. albus* sample, but among individuals with fork length longer than 350 mm, on the contrary, these features are larger in *S. schmidti* sample. This pattern suggests that for the same increasing fork length, jaw bones grow more in *S. schmidti* individuals than in *S. albus*. To test this hypothesis, dependence of the absolute size of the jaw structures on fork length was analyzed by using regression analysis.

Regression analysis

Regression analysis was done for all the jaw bones features in the samples of *S. albus* and *S. schmidti* separately. Regression coefficients are presented in Table Sm4, see supplementary material. In the sample of *S. albus* a linear trend (only the first degree coefficients differed significantly from zero) was observed for all the features of the angulo-articular bone and for the greatest height of the dentary bone (DH). In the sample of *S. schmidti*, a second-order polynomial trend (the second degree coefficients differ significantly from zero) was observed for these features. Therefore, the relationships between all the features of the angulo-articular bone and the greatest height of the dentary bone (DH) and the fork length were different between *S. albus* and *S. schmidti*. With increasing fork length, these features of the bone structures increase more rapidly in *S. schmidti* compared to *S. albus*. Thus, the fork length near 350 mm is the particular moment of growth (Fig. 3(a), (b), (c)). From this moment in *S. schmidti*

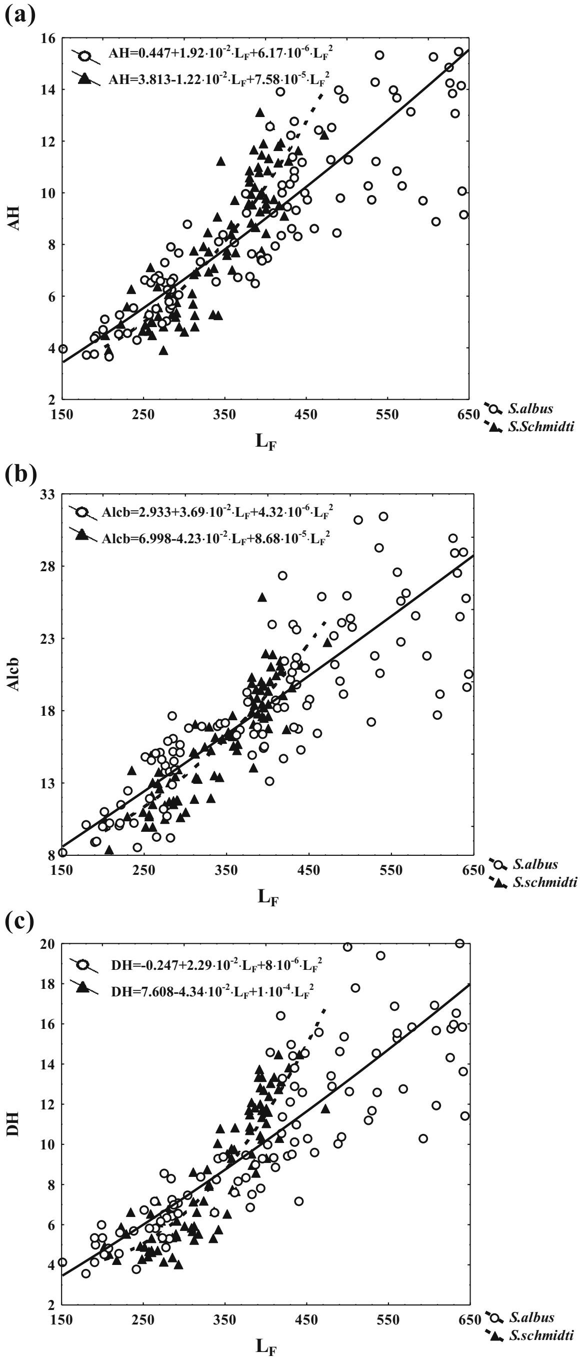

Fig. 3 The scatterplots of the dependences of absolute values of the osteological features (in mm) on Fork Length (L_F) for samples of *S. albus* and *S. schmidti* **a** "the greatest height of angulo-articular bone" (AH); **b** "length of the free part of angulo-articular bone" (Alcb); **c** "the greatest height of dentary bone" (DH)

sample these features increase more rapidly than in *S. albus* sample. Three of these features (the greatest height of angulo-articular bone, length of the free part of angulo-articular bone and the greatest height of dentary bone) significantly differed in A2-S2 and A3-S3 size groups (Table 3): in the A2 size group the value of these parameters were bigger relative to S2 and in the A3 size group they were smaller relative to S3.

Thus, there are two bone structures, the angulo-articular bone and the dentary bone, that showed clear differences between *S. albus* and *S. schmidti* in the rate of development. In general, with the increasing fork length increase the number of osteological features that differed between *S. albus* and *S. schmidti* increases. The jaw bones of *S. albus* became more elongated. On the contrary, the jaw bones of *S. schmidti* increased in height as the fish grew larger. This corresponds to differences between the morphs in stable isotope values.

Discussion

The two most common forms of charr in Lake Kronotskoe have evidently many clear morphological differences, but the level of divergence in *S. albus* and *S. schmidti* is discussed (Viktorovsky 1978; Salmenkova et al. 2005; Ostberg et al. 2009; Oleinik and Skurikhina 2010). Results of this study suggest that the two charr forms in Lake Kronotskoe have a strong morphological differences in jaw bones features, which appear when growth nears 350 mm in fork length. These differences increase with the fish growth. Most likely this is caused by utilization of different habitat and diets (Sibbing et al. 1998) as *S. schmidti* is mainly benthivorous during all its life in the relatively shallow habitat while *S. albus* is omnivorous and occupies different lake habitats (Viktorovsky 1978). This corresponds to different values of stable isotopes between the morphs, and the data available on the external morphology of fishes including charrs from different lakes (McPhail 1984; Skulason et al. 1989; Snorrason et al. 1994; Klemetsen et al. 2002; Kahilainen and Østbye 2006; Knudsen et al. 2007; Alekseyev et al. 2009; Ostberg et al. 2009). In general, benthic morphs are typically characterized by short lower jaws, whereas limnetic or omnivorous morphs typically have long jaws (Ostberg et al. 2009).

Genetic results (Radchenko et al. 2006; Oleinik and Skurikhina 2010) suggest that these two charr forms are not completely reproductively isolated and there seems to be a limited gene flow between them. Thus, these profound jaw distinctions between the forms are mostly epigenetic and most likely explained by their remarkably different trophic preferences. As *S. schmidti* do not change their feeding niche during ontogenesis (Ostberg et al. 2009) they have typically adapted mouthparts that are specialized for benthos consumption on the rocky and pebble bottom substrate (Jonsson et al. 1988; Skulason et al. 1989). This is seen as the increase in the relative height of the angulo-articular bone and the dentary bone of *S. schmidti* creating a large mouth volume that is beneficial for feeding benthos. These morphological features promote a formation of the low «lipped» mouth with the big volume of mouth cavity which is typically observed in *S. schmidti*. Mouth cavity increases the efficiency of consuming benthos organisms from the substratum.

On the contrary, in the population of the omnivore *S. albus* there is a change of prey items. With increasing size of individuals they gradual include larger prey such as fish (Ostberg et al. 2009). They have a typical elongation of most of the jaw bones (premaxilla, maxilla, dentary and angulo-articular bones) with increasing body length. Formation of a terminal mouth increases the efficiency of preying.

Thus, both forms seem to have adapted functional morphological traits that allow them to consume each prey group effectively.

The present study gives an evident example of a unique endemic variety of two landlocked Dolly Varden charr, *S. albus* and *S. schmidti*, with trophic induced traits in the skull. As, apparently, the population of the ancestral anadromous riverine forms of Dolly Varden was isolated in the newly formed lake because of volcanic eruptions, this ancestral population has evolved into a variety of endemic lacustrine forms of charr. Especially important is the large differences in the jaw structures between the two sympatric lacustrine forms with increasing fork length. The strongest differences appear when fork length is near 350 mm and increase with fish growth. This osteological divergence of jaw bone structures is accompanied by clear separation in ecology and trophic niches (stable isotope values) also suggested from other studies (Viktorovsky 1978; Chereshnev et al. 2002), from karyological features (Viktorovsky 1978) and some genetic differentiation at allozyme and microsatellite loci of the nuclear genome and at mitochondrial genes (Salmenkova et al.

2005; Oleinik and Skurikhina 2010) between the two forms. In addition, the osteological method used in the study allows identification of differences related to several jaw bone structures and suggestions of its possible ecological reasons.

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