Journal of Ichthyology, Vol. 44, No. 1, 2004, pp. 52–69. Translated from Voprosy Ikhtiologii, Vol. 44, No. 1, 2004, pp. 59–76.

Morphological and Genetic Diversity of Amur Graylings (*Thymallus*, Thymallidae)

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Received October 22, 2002

Abstract—Four forms of graylings are found in the Amur basin, designated conventionally as: Upper–Amur, yellow-spotted, Lower-Amur, and large-scale. The Upper–Amur form is distributed from the upper reaches of the Ingoda and Onon up to the inflow to the Amur of the Bureya River, where its sympatry was observed with the Lower-Amur and large-scale forms. The latter also live together in tributaries of the Amur and in some rivers flowing into the Sea of Japan and the Tatar Strait. The results of the multivariate analysis of morphometric and meristic characters, sequences of mitochondrial DNA, and of body coloration and of patterns of the dorsal fin indicate that all of the aforementioned forms are isolated reproductively. The graylings from the Amur are represented by two phylogenetic lines, the Upper-Amur, yellow-spotted, and Lower-Amur form belonging to the first line and the large-scaled form—to the second line. A high divergence level of the large-scaled form, according to analysis of mitochondrial DNA, is indicated not only for the Amur groups but also for other Siberian graylings. Of all discerned forms only two, the Upper-Amur and yellow-spotted ones, may be attributed to the previously described species by Dybowski *Thymallus grubii*. The Lower-Amur form is also an independent species. The taxonomic status of the large-scale form may be defined by further investigations. The obtained data indicate the urgency of revision of the genus *Thymallus*.

Morphological heterogeneity of graylings *(Thymallus)* populating various parts of the Amur basin was repeatedly noted by many researchers. However, all differences were considered to be the consequences of environmental influence (Tugarina and Khramtsova, 1980, 1981; Zinovyev *et al.*, 1983; Karasev, 1987; Kostitsyn and Zinovyev, 1988). Antonov (1995, 1999, 2000, 2001) arrived at a conclusion on the sympatry in the Levaya Bureya River of three "reproductively isolated" forms. There is a viewpoint implying close relationship of *Th. grubii* from the Amur[1] and the East–Siberian *Th. arcticus pallasi* (Svetovidov, 1936; Zinoviyev *et al.*, 1983; Antonov, 1995; Makoedov and Korotaeva, 1999).

In the list of freshwater fishes of Russia (Bogutskaya and Naseka, 2002) for the Amur, in addition to *Th. grubii* Dybowski, 1869, four forms of graylings are declared: *Thymallus* sp. 1 (the Lower Amur, Primor'e), *Thymallus* sp. 2 (the Lower Amur, Primor'e), *Thymallus* sp. 3 (the Upper Amur, Bureya), and *Thymallus* sp. 4 ("large-scale" form, the Upper Amur), without any information on their morphological differences. Two forms of graylings without species determination are indicated by Shed'ko (2001) as *Thymallus* sp. 1 and *Thymallus* sp. 2 within the family Salmonidae for rivers of Primor'e, however his comparative remarks are superficial.

The systematic status of the Amur grayling *T. grubii* Dybowski as an independent species has already been repeatedly discussed (Svetovidov, 1936; Berg, 1948; Nikolsky, 1956; Pivnicka and Hensel, 1978; Tugarina and Khramtsova, 1980, 1981; Zinovyev *et al.*, 1983; Skurikhina, 1984; Skurikhina *et al.*, 1985; Karasev, 1987). In detail, the history of this problem is described by Tugarina and Khramtsova (1980). Commonly, discussions were concerned with the status of this form. With consideration of the results of the DNA × DNA hybridization analysis it was supposed that the grayling

[1] The Amur grayling was described for the first time by Dybowski (1869) as *Th. grubii*. Later, the taxonomic status and accordingly the name were changed by Berg (1916) into *Th. arcticus grubei*. We use the original name of the Amur grayling given by Dybowski.

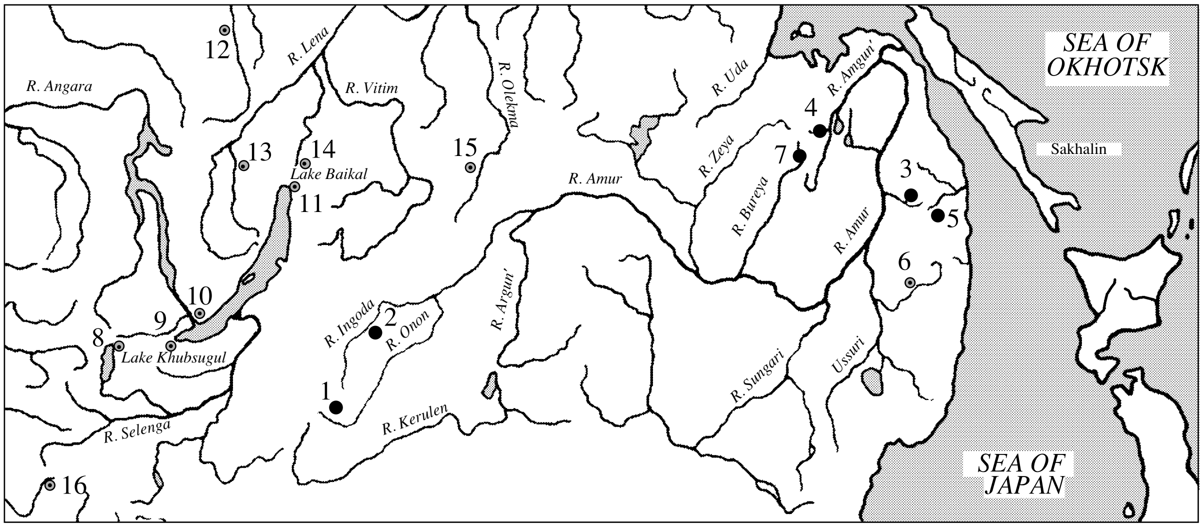

Fig. 1. Schematic map of the area of investigation: (1) R. Onon, (2) R. Ingoda, (3) R. Anyui; (4) R. Merek; (5) R. Buta; (6) R. Khor (Khramtsova and Tugarina, 1980), (7) R. Bureya; (8) Lake Khubsugul; (9) Lake Sobolinoe; (10) Irkutsk Reservoir; (11) Dagar Bay (Lake Baikal); (12) R. Nizhnyaya Tunguska; (13) R. Kutima (the Kirenga); (14) Lake Ogiendo (the Chaya River); (15) the Olongdo (the Olekma); and (16) Lake Khokh Nur (Mongolian People's Republic). Desiguations: ●—Amur graylings; ⊙—Siberian graylings.

from the lower reaches of the Amur is a genetically isolated form which has attained the level of species independence (Skurikhina, 1984; Skurikhina *et al.*, 1985). In spite of the fact that the species level of the Amur grayling is supported by several specialists (Pivnicka and Hensel, 1978; Tugarina and Khramtsova, 1980; Skurikhina, 1984), in recent summaries on freshwater fishes of Russia it is mentioned as a subspecies, thus showing that its position in the genus still requires reconsideration.

In view of contradictory opinions on the diversity of the Amur graylings, additional data on morphology and genetics of populations from different parts of the river are necessary. They would elucidate problems of taxonomy, origin, and distribution of graylings not only in the Amur basin, but also in the entire Palearctic. The present study is aimed at generalization of the original, published data and those obtained from investigations of collections concerning diversity of graylings populating the Amur basin, at determination of their areas and of the level of morphological and genetic divergence.

MATERIAL AND METHODS

The present study is based on collections of graylings in the Amur basin made in 1996–2001, including the basin of its upper reaches—the Ingoda River (the Olengui, the Sypchegur, the Uber-Zhelykhen), the Onon River (the upper reaches, Mongolian People's Republic), the Bureya River; in the lower reaches of the Anyui River and its tributaries (the Gobili, the Ertukuli), in the upper reaches of the Amgun' River (the Merek), and in the upper reaches of the Buta River (the basin of the Tumnin—the Tatar Strait (Figs. 1, 2). The rivers flowing into the Sea of Japan, the Tatar Strait and some rivers flowing into the Sea of Okhotsk are generally supposed to belong to the area of distribution of the Amur grayling (*Annotirovannyi katalog…*, 1998; *Atlas…*, 2002). Accordingly, all considered forms are termed below the Amur graylings.

The fishes were caught with a fishing rod. All fish were preserved in 4% formalin. Coordinates of capture sites are shown in Table 1.

By external characters the graylings were conventionally subdivided into four forms: Upper-Amur (u), Lower-Amur (l), yellow-spotted (y), and large-scale (ls). Altogether, 214 graylings were caught, 119 belonging to the Upper-Amur form, 57—to the Lower-Amur form, 33—to the yellow-spotted form, and 5—to the large–scale form. In ascertaining the geographic areas of these forms, the collections of museums of Moscow State University and of the Zoological Institute of the Russian Academy of Sciences were additionally used.

The fork length, sex, and maturity stage were determined. The age was determined by scales according to Chugunova (1959). The body length and weight of different forms of grayling are shown in Table 2.

We investigated 29 morphometric and 12 meristic characters. Mature specimens and specimens with gonads at maturity stage III were measured according to Pravdin (1966). Tables 3 and 4 demonstrate the data for both sexes. Meristic characters were assessed using a binocular microscope MBS-10. All rakers on the first gill arch were counted, including primordial ones. Only those vertebrae were counted whose spinous processes were not fused.

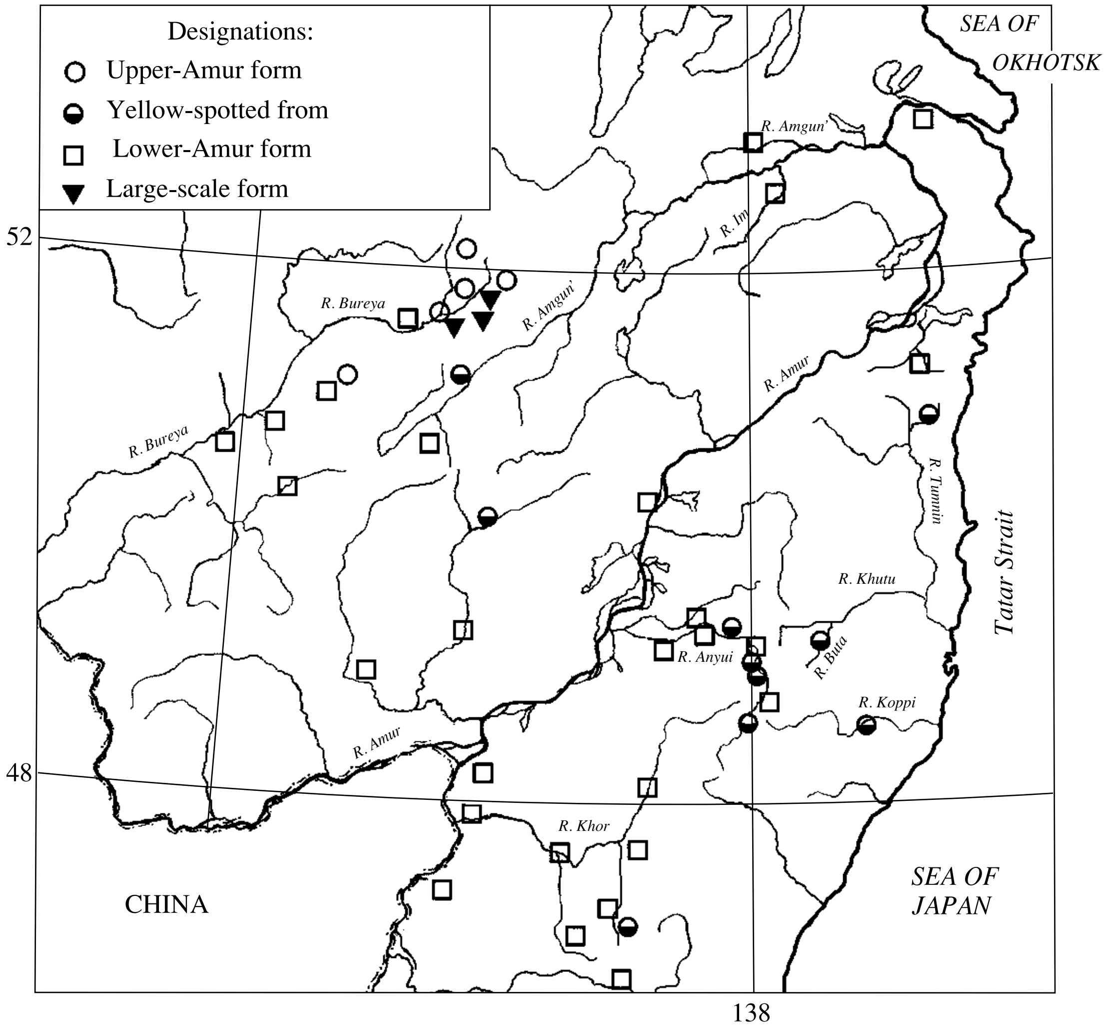

Fig. 2. Distribution of different forms of grayling in the basins of the Middle and Lower reaches of the Amur.

For comparison, the original materials on morphology of graylings were used from Lakes Khokh Nur and Khubsugul (Mongolia), the Kutima and Olongdo rivers, Lakes Ogiendo and Leprindokan (the basins of the upper and middle reaches of the Lena), the Nuzhnyaya Tunguska (the Yenisei basin), the Tal'tsinka (Irkutsk Reservoir), and Lake Baikal. Localities of these samples are shown in Fig. 1. The morphological characteristic of the Upper-Amur form from the Levaya Bureya River is not mentioned, as fishes are in poor condition, only drawings of fins and the results of the molecular-genetic investigation are used.

Statistic analysis of morphological characters, construction of dendrograms and diagrams of scattering were made using software Statistica'99 version 5.5A and SPSS version 8.0, with consideration of recommendations described by Borovikov and Borovikov (1998), Rokitskii (1973), and also available in the Statsoft manual on statistics (www.statsoft.ru). For analysis of morphological characters by the principal component method (PCA) the variation-covariation matrix was used. Significance of differences and their value was determined by *t*-test at the level $p = 0.001$ and by CD coefficient (Mayr, 1969), respectively.

For molecular-genetic analysis the adipose fin or a part of the caudal fin fixed in 96% ethanol was used. In 86 specimens of different forms the total DNA was isolated by high-salt extraction. Some haplotypes of graylings used in comparison of samples from the upper reaches of the Amur, Baikal, the Angara-Yenisei basin, the Lena, and water bodies of Mongolia are represented in the paper by Koskinen *et al.* (2002). Structure of primers (straight LRBT-25: AGA-GCG-CCG-GTG-TTG-TAA-TC and reverse LRBT-1195 GCT-AGC-GGG-ACT-TTC-TAG-GGT-C) localized in the bordering with the control area mitochondrial DNA (mtDNA) genes coding transport RNA is selected by the data published for the first time by Uiblein *et al.* (2001) on DNA sequences of brook trout (GENBANK D83947). Using this pair of primers, the complete control area

Table 1. Geographical coordinates of collection localities and the size of treated material

Locality, river	Basin	Coordinates		Analysis, specimens	
		latitude	longitude	morphological	DNA
Olengui	Ingoda–Shilka–Amur	51°02.′00	112°34.′50	28	–
Sypchegur	″	51°23.′01	113°20.′00	34	5
Uber-Zhelikhen	″	50°48.′64	112°17.′14	32	–
Onon	Shilka–Amur	48°35.′67	110°48.′97	22	5
Anyui*	Amur	49°17.′94	137°55.′85	23	3
Gobili*	Anyui–Amur	49°15.′24	138°19.′45	26	12
Ertukuli*	Anyui–Amur	49°17.′94	137°03.′01	25	2
Merek*	Amgun'–Amur	51°17.′04	134°47.′82	8	7
Levaya Bureya	Bureya–Amur	51°55.′00	134°53.′00	5	8
Buta*	Khutu–Tumnin	49°09.′86	138°56.′89	8	6

* Coordinates are determined by GPS-12 “Garmin,” other coordinates are calculated using the general geographic map of Russia, scale 1 : 1000000.

Table 2. Average values of body length, mm (above the line) and weight, g (under the line) of different forms of the Amur graylings (by the observed data)

River (form)	Age, years						*n*, specimens
	1+	2+	3+	4+	5+	6+	
Ingoda (u)	–	152.05 / 36.22	161.61 / 48.03	186.08 / 79.01	220.0 / 116.0	239.0 / 164.0	94
Onon (u)	123.3 / 18.35	148.71 / 35.92	165.11 / 51.6	194.8 / 109.0	212.5 / 116.0	–	22
Anyui (l)	–	169.01 / 65.07	183.9 / 85.88	210.72 / 129.98	236.62 / 183.28	–	57
Anyui (y)	–	179.7 / 65.0	209.68 / 107.4	218.92 / 129.5		–	17
Merek (y)	–	165.63 / 56.33	204.75 / 106.0	233.15 / 163.5	266.0 / 275.0	–	8
Buta (y)	–	–	–	255.33 / 249.66	267.87 / 281.5	321.3 / 445.0	8
Levaya Bureya (ls)	–	195 / 68.0	246.0 / 150.0	273.4 / 217.3	–	–	5

Note: The forms of graylings: Upper-Amur (u), Lower-Amur (l), yellow-spotted (y), large-scale (ls).

(1002 pairs of bases), 87 pairs of bases of the gene of proline t-RNA, and 11 pairs of bases of the gene of phenylalanine t-RNA were amplified. The conditions of amplification were the following: denaturation at 96°C for 1 min and 25 denaturation cycles at 96°C (1.5 min), annealing at 56°C (1 min), and synthesis at 72°C (1.5 min). For purification of amplification products after electrophoresis in agarose gel a special set of QIAGEN Co. was used. The quantity of the purified product was determined by staining with ethidium bromide in agarose gel. Nucleotide sequences of the 5’ end of the control area, about 600 pair of nucleotides long, were determined using the direct primer for amplification, by means of the set for sequenation Big-Dye^{TM},

Table 3. Morphometric characters of the Amur graylings

Characters	River (form)						
	Ingoda (u) *n* = 85	Onon (u) *n* = 20	Anyui (l) *n* = 36	Anyui (y) *n* = 13	Merek (y) *n* = 5	Buta (y) *n* = 8	Levaya Bureya (ls) *n* = 4
L_{SM}, mm	171.62 139.8–245.5	164.71 140.1–212.5	212.0 178.2–250.5	213.46 197.9–240.5	228.36 202.0–266.0	269.85 250.0–321.3	266.58 246.0–293.0
In % L_{SM}							
l	95.12 ± 0.06 0.55; 93.75–96.61	95.29 ± 0.15 0.67; 94.41–96.92	94.7 ± 0.07 0.4; 93.95–95.79	94.84 ± 0.16 0.59; 94.13–95.8	95.07 ± 0.25 0.55; 94.31–95.63	94.83 ± 0.2 0.57; 94.12–95.63	94.58 ± 0.34 0.67; 93.90–95.28
l_2	77.67 ± 0.13 1.16; 73.54–80.72	76.6 ± 0.23 1.03; 74.86–79.39	78.15 ± 0.23 1.38; 75.73–81.89	78.24 ± 0.18 0.65; 77.3–79.67	77.99 ± 0.45 1.02; 76.78–79.67	77.65 ± 0.32 0.92; 76.78–79.67	78.25 ± 0.22 0.44; 77.53–78.70
r	5.89 ± 0.03 0.27; 5.23–6.62	5.72 ± 0.06 0.29; 4.97–6.29	5.89 ± 0.05 0.27; 5.33–6.37	5.74 ± 0.07 0.27; 5.38–6.27	6.15 ± 0.04 0.1; 6.07–6.32	6.19 ± 0.08 0.22; 5.8–6.52	5.80 ± 0.06 0.11; 5.69–5.97
o	5.04 ± 0.04 0.33; 4.25–5.75	5.28 ± 0.06 0.28; 4.75–6.01	5.18 ± 0.05 0.29; 4.53–5.8	4.64 ± 0.07 0.24; 4.28–5.13	4.8 ± 0.06 0.14; 4.6–5.0	5.05 ± 0.13 0.37; 4.6–5.69	4.32 ± 0.11 0.23; 3.96–4.52
f	9.61 ± 0.04 0.4; 8.27–10.81	9.92 ± 0.09 0.4; 9.21–10.68	9.31 ± 0.06 0.33; 8.59–9.99	9.34 ± 0.06 0.23; 8.93–9.65	9.93 ± 0.13 0.29; 9.58–10.25	9.93 ± 0.17 0.47; 9.04–10.68	9.46 ± 0.04 0.08; 9.38–9.59
c	19.7 ± 0.05 0.45; 18.34–20.68	20.38 ± 0.13 0.6; 19.29–21.45	19.12 ± 0.07 0.42; 18.23–19.98	18.88 ± 0.1 0.37; 18.33–19.71	19.49 ± 0.05 0.12; 19.33–19.66	19.64 ± 0.19 0.53; 18.78–20.56	18.79 ± 0.21 0.42; 18.07–19.11
cH	14.63 ± 0.07 0.61; 13.35–16.2	14.79 ± 0.12 0.53; 13.89–16.61	15.98 ± 0.11 0.68; 14.49–17.42	14.87 ± 0.14 0.5; 13.95–15.7	15.81 ± 0.29 0.64; 15–16.53	15.53 ± 0.28 0.8; 14.14–16.53	14.61 ± 0.54 1.08; 13.01–15.87
ch	10.34 ± 0.06 0.59; 9.14–12.34	10.92 ± 0.08 0.36; 10.49–12.13	11.11 ± 0.1 0.58; 9.58–12.51	10.44 ± 0.15 0.55; 9.22–11.04	11.05 ± 0.22 0.49; 10.42–11.75	10.87 ± 0.19 0.55; 10.16–11.75	9.74 ± 0.25 0.51; 9.07–10.41
k	5.88 ± 0.03 0.3; 5.22–6.45	6.06 ± 0.11 0.47; 5.28–7.35	5.94 ± 0.05 0.27; 5.28–6.7	5.8 ± 0.07 0.26; 5.26–6.12	5.87 ± 0.1 0.23; 5.54–6.19	5.85 ± 0.08 0.21; 5.54–6.19	5.44 ± 0.20 0.39; 5.04–6.09
lmx	6.1 ± 0.04 0.33; 5.34–7.09	6.52 ± 0.05 0.24; 6.02–6.96	6.15 ± 0.05 0.31; 5.51–6.77	5.49 ± 0.07 0.26; 4.95–5.85	5.75 ± 0.07 0.16; 5.52–6.02	5.96 ± 0.11 0.32; 5.52–6.46	5.51 ± 0.11 0.22; 5.15–5.74

Table 3. (Contd.)

Characters	River (form)						
	Ingoda (u) *n* = 85	Onon (u) *n* = 20	Anyui (l) *n* = 36	Anyui (y) *n* = 13	Merek (y) *n* = 5	Buta (y) *n* = 8	Levaya Bureya (ls) *n* = 4
i/lmx	1.77 ± 0.02 0.16; 1.4–2.24	1.93 ± 0.03 0.14; 1.72–2.24	1.93 ± 0.03 0.19; 1.62–2.47	1.88 ± 0.03 0.1; 1.71–2.01	2.0 ± 0.04 0.08; 1.88–2.12	1.96 ± 0.05 0.13; 1.74–2.14	1.90 ± 0.05 0.11; 1.81–2.08
lmd	10.02 ± 0.05 0.42; 8.71–10.91	10.63 ± 0.08 0.36; 9.97–11.33	10.06 ± 0.07 0.44; 9.18–11.05	9.58 ± 0.08 0.29; 8.98–10.16	9.86 ± 0.09 0.21; 9.6–10.15	10.05 ± 0.11 0.32; 9.6–10.56	9.39 ± 0.05 0.11; 9.22–9.49
H	19.33 ± 0.1 0.88; 16.85–21.34	19.2 ± 0.19 0.84; 17.95–21.14	22.96 ± 0.22 1.31; 19.77–25.81	20.52 ± 0.29 1.04; 18.97–22.83	21.76 ± 0.6 1.35; 19.46–23.46	20.87 ± 0.57 1.6; 18.73–23.46	23.37 ± 0.74 1.48; 21.46–25.58
h	6.54 ± 0.03 0.24; 6.01–7.1	6.36 ± 0.06 0.27; 5.95–7.25	7.56 ± 0.05 0.27; 7.02–8.26	6.97 ± 0.08 0.3; 6.45–7.61	7.28 ± 0.11 0.25; 6.93–7.63	7.15 ± 0.1 0.28; 6.73–7.63	7.62 ± 0.21 0.42; 6.95–8.03
w	12.39 ± 0.09 0.87; 10.33–13.84	12.23 ± 0.22 0.99; 10.98–15.4	12.73 ± 0.15 0.87; 10.62–14.09	11.87 ± 0.23 0.81; 10.15–13.1	12.55 ± 0.33 0.74; 11.68–13.53	12.45 ± 0.23 0.64; 11.68–13.53	9.41 ± 0.34 0.68; 8.77–10.53
aD	31.4 ± 0.14 1.28; 27.68–38.27	32.92 ± 0.27 1.19; 30.28–36.24	28.74 ± 0.18 1.08; 27.1–31.86	28.97 ± 0.18 0.65; 27.87–30.32	29.42 ± 0.41 0.92; 28.27–30.38	29.67 ± 0.33 0.93; 28.27–30.84	31.65 ± 0.27 0.53; 30.89–32.22
pD	42.99 ± 0.14 1.33; 39.43–46.88	42.73 ± 0.26 1.17; 40.7–44.86	40.4 ± 0.2 1.22; 37.7–43.13	43.78 ± 0.46 1.65; 40.87–48.16	41.74 ± 0.58 1.3; 39.36–43.26	42.46 ± 0.57 1.61; 39.36–45.29	42.16 ± 0.83 1.65; 39.59–44.20
aA	69.65 ± 0.13 1.16; 65.97–72.55	70.41 ± 0.21 0.93; 69.08–73.12	70.55 ± 0.19 1.12; 68.6–73.04	68.78 ± 0.21 0.75; 67.37–69.98	69.84 ± 0.71 1.58; 67.9–72.37	69.45 ± 0.54 1.52; 67.32–72.37	68.93 ± 0.54 1.07; 67.58–70.45
aV	45.48 ± 0.13 1.19; 42.37–47.98	46.04 ± 0.19 0.87; 44.65–47.82	45.15 ± 0.19 1.15; 42.56–46.96	44 ± 0.17 0.62; 42.44–44.93	45.1 ± 0.5 1.12; 43.96–47.18	44.76 ± 0.35 1.0; 43.94–47.18	44.45 ± 0.69 1.38; 42.32–46.17
lp	17.5 ± 0.09 0.81; 15.71–19.59	17.69 ± 0.16 0.72; 16.16–19.13	17.32 ± 0.13 0.77; 15.75–19.07	18.68 ± 0.2 0.72; 17.23–19.71	17.73 ± 0.58 1.29; 15.64–18.86	18.1 ± 0.41 1.17; 15.64–19.28	16.46 ± 0.40 0.79; 15.29–17.45
P–V	27.1 ± 0.15 1.4; 22.99–30.6	26.85 ± 0.2 0.87; 24.82–28.37	28.27 ± 0.16 0.98; 26.21–30.33	26.71 ± 0.27 0.99; 25.41–28.77	28.31 ± 0.59 1.31; 26.83–30.05	27.69 ± 0.5 1.4; 25.89–30.05	28.19 ± 0.28 0.55; 27.64–28.88

Table 3. (Contd.)

Characters	River (form)						
	Ingoda (u) *n* = 85	Onon (u) *n* = 20	Anyui (l) *n* = 36	Anyui (y) *n* = 13	Merek (y) *n* = 5	Buta (y) *n* = 8	Levaya Bureya (ls) *n* = 4
V–A	25.35 ± 0.13 1.22; 21.76–28.39	25.24 ± 0.26 1.16; 23.09–27.06	26.09 ± 0.23 1.38; 22.62–29.09	25.49 ± 0.26 0.95; 23.98–27.31	25.29 ± 0.48 1.08; 23.86–26.69	25.08 ± 0.41 1.16; 23.56–26.69	24.65 ± 0.56 1.13; 22.79–25.77
lD	22.05 ± 0.17 1.52; 18.23–25.54	21.36 ± 0.26 1.14; 18.88–23.45	28.39 ± 0.22 1.33; 25.35–32.26	24.3 ± 0.35 1.28; 22.37–26.96	25.81 ± 0.6 1.33; 24.5–28.23	25.02 ± 0.53 1.51; 23.19–28.23	26.70 ± 0.19 0.37; 26.18–27.13
hD_1	11.17 ± 0.14 1.28; 8.85–16.17	11.36 ± 0.2 0.89; 9.21–12.7	11.64 ± 0.13 0.78; 10.36–13.11	10.9 ± 0.21 0.74; 9.51–11.91	11.71 ± 0.19 0.33; 11.25–12	8.68 ± 0.19 1.35; 7.5–10.5	10.79 ± 0.55 1.10; 9.51–12.10
hD_2	10.68 ± 0.28 2.59; 7.09–21.06	9.85 ± 0.59 2.66; 6.74–16	16.62 ± 0.4 2.33; 12.24–21.31	13.99 ± 0.55 1.97; 10.78–18.3	17.1 ± 1.12 1.94; 14.85–19.58	15.26 ± 0.26 2.60; 11.0–24.0	15.56 ± 0.81 1.63; 13.11–17.45
lA	9.45 ± 0.08 0.7; 8.16–10.97	8.76 ± 0.16 0.7; 7.86–10.54	9.42 ± 0.1 0.6; 8.09–10.59	9.11 ± 0.16 0.58; 8.19–10.48	10.26 ± 0.46 1.04; 9.26–12.22	9.47 ± 0.15 1.52; 6.5–14.5	9.70 ± 0.12 0.24; 9.31–9.90
hA	12.3 ± 0.13 1.17; 9.56–16.13	11.79 ± 0.24 1.05; 10.15–13.89	13.17 ± 0.17 1.02; 11.27–14.71	12.31 ± 0.17 0.62; 11.3–13.79	12.21 ± 0.17 0.37; 11.84–12.92	14.25 ± 0.12 1.29; 10.5–16.5	12.93 ± 0.57 1.13; 11.14–14.26
lP	15.25 ± 0.06 0.59; 14.26–17.14	14.99 ± 0.13 0.57; 13.85–16.43	16.63 ± 0.09 0.52; 15.57–17.73	15.67 ± 0.13 0.48; 14.71–16.2	16.25 ± 0.29 0.64; 15.56–17.45	17.3 ± 0.08 0.87; 16.0–20.0	16.97 ± 0.80 1.60; 14.78–18.88
lV	15.11 ± 0.12 1.08; 13.51–18.95	14.57 ± 0.24 1.07; 12.86–16.72	17.17 ± 0.19 1.15; 14.64–20.4	16.32 ± 0.2 0.74; 15.02–17.47	18.23 ± 0.46 1.03; 16.53–19.44	18.19 ± 0.14 1.42; 16.0–21.5	17.47 ± 0.36 0.72; 16.31–18.19

Note: Note for tables 3, 4, 5. Above the line—average value and error, under the line—standard deviation and limits; (L_{SM}) fork length; (l) trunk length; (l_2) length up to the end of scales; (r) snout length; (o) horizontal eye diameter; (f) postorbital part of head; (c) head length; (cH) head length at occiput; (ch) head length at eye; (k) forehead width; (lmx) length of upper jaw; (i/lmx) width of upper jaw; (lmd) length of lower jaw; (H) the greatest body depth; (h) the least body depth; (w) body thickness; (aD) antedorsal distance; (pD) postdorsal distance; (aA) anteanal distance; (aV) anteventral distance); (lp) caudal peduncle length; (P–V) pectoventral distance; (V–A) ventroanal; distance; (lD) length of dorsal fin base; (hD_1) depth of anterior part of dorsal fin); (hD_2) depth of posterior part of dorsal fin; (lA) length of anal fin base; (hA) anal fin depth; (lP) pectoral fin length; (lV) ventral fin length; (ll) number of perforated scales in lateral line; (D_1) number of non-branched rays in dorsal fin; (D_2) number of branched rays in dorsal fin; (D) total number of rays in dorsal fin; (P) number of branched rays in pectoral fin; (V) number of branched rays in ventral fin; (A_1) number of nonbranched rays in anal fin; (A_2) number of branched rays in anal fin; (sb) number of gill rakers; (rb) number of branchiostegal rays; (vt) number of vertebrae; and (pc) number of pyloric caeca.

Table 4. Meristic characters of the Amur graylings

Character	River (form)						
	Ingoda (u) *n* = 94	Onon (u) *n* = 22	Anyui (l) *n* = 57	Anyui (y) *n* = 17	Merek (y) *n* = 8	Buta (y) *n* = 8	Levaya Bureya (ls) *n* = 5
ll	90.38 ± 0.4 3.89; 82–102	92.45 ± 0.76 3.55; 86–100	81.65 ± 0.41 3.08; 75–91	93.41 ± 1.08 4.47; 85–101	88.75 ± 1.5 4.24; 82–94	88.5 ± 0.92 2.6; 85–94	83.60 ± 1.82 4.08; 80–91
D_1	8.16 ± 0.09 0.85; 7–11	8.36 ± 0.12 0.57; 7–9	9.47 ± 0.12 0.89; 8–11	9.94 ± 0.18 0.73; 9–11	9.75 ± 0.15 0.43; 9–10	10.88 ± 0.37 1.05; 10–13	9.80 ± 0.18 0.40; 9–10
D_2	12.73 ± 0.1 0.92; 11–16	12.23 ± 0.18 0.85; 10–13	15.51 ± 0.14 1.02; 13–17	13.59 ± 0.15 0.6; 13–15	13.0 ± 0.18 0.5; 12–14	12.75 ± 0.39 1.09; 11–14	14.60 ± 0.22 0.49; 14–15
D	20.88 ± 0.09 0.91; 19–23	20.59 ± 0.18 0.83; 19–22	24.98 ± 0.1 0.75; 23–26	23.53 ± 0.22 0.92; 22–25	22.75 ± 0.23 0.66; 22–24	23.63 ± 0.39 1.11; 21–25	24.40 ± 0.36 0.80; 23–25
P	13.99 ± 0.06 0.59; 12–16	13.91 ± 0.13 0.6; 13–15	14.23 ± 0.09 0.7; 13–16	14.35 ± 0.17 0.68; 13–15	14.25 ± 0.23 0.66; 13–15	14.75 ± 0.23 0.66; 14–16	14.80 ± 0.33 0.75; 14–16
V	9.12 ± 0.04 0.41; 8–10	9.14 ± 0.1 0.46; 8–10	10.04 ± 0.05 0.37; 9–11	9.41 ± 0.12 0.49; 9–10	9 –	9.0 ± 0.18 0.5; 8–10	9.80 ± 0.18 0.40; 9–10
A_1	4.11 ± 0.05 0.49; 3–5	4.14 ± 0.1 0.46; 3–5	4.21 ± 0.05 0.41; 4–5	4.24 ± 0.1 0.42; 4–5	4.63 ± 0.25 0.7; 4–6	4.5 ± 0.18 0.5; 4–5	4.40 ± 0.22 0.49; 4–5
A_2	9.09 ± 0.07 0.68; 7–11	8.68 ± 0.13 0.63; 7–10	9.05 ± 0.06 0.44; 8–10	9.18 ± 0.12 0.51; 8–10	8.88 ± 0.21 0.6; 8–10	9.5 ± 0.18 0.5; 9–10	9.80 ± 0.33 0.75; 9–11
sb	17.86 ± 0.14 1.37; 15–21	17.86 ± 0.21 0.97; 16–20	18.23 ± 0.15 1.11; 17–22	19.06 ± 0.27 1.11; 18–22	18.63 ± 0.39 1.11; 18–21	19.13 ± 0.28 0.78; 18–20	20.00 ± 0.28 0.63; 19–21
rb	9.79 ± 0.07 0.7; 8–12	9.5 ± 0.11 0.5; 9–10	9.96 ± 0.09 0.67; 8–11	9.41 ± 0.12 0.49; 9–10	10.0 ± 0.25 0.71; 9–11	9.75 ± 0.15 0.43; 9–10	9 –
vt	55.35 ± 0.1 0.96; 53–57	55.45 ± 0.15 0.72; 54–57	53.33 ± 0.11 0.8; 52–55	55.47 ± 0.24 0.98; 53–57	54.63 ± 0.25 0.7; 53–55	54.13 ± 0.28 0.78; 53–55	53.20 ± 0.18 0.40; 53–54
pc	15.04 ± 0.2 1.97; 11–22	14.77 ± 0.43 2.02; 10–18	14.68 ± 0.2 1.48; 12–18	15.71 ± 0.36 1.49; 14–18	17.5 ± 0.68 1.94; 15–21	19.75 ± 0.98 2.77; 16–24	18.00 ± 0.85 1.90; 15–21

Perkin Elmer and automatic sequenators ABI-377 or ABI-310 at the following conditions: initial denaturation at 96°C for 1 min, then 25 cycles at 96°C (15 s), at 56°C (5 s), and 60°C (4 min). Leveling of the obtained sequences was done manually, using the software Sequence Navigator. The leveled sequences were processed in the PAUP program, version 4.0b10 (Swofford, 2000). The model of accumulation of substitutions in DNA is selected by means of software Modeltester version 3.04 (Posada and Crandall, 1998) following recommendations of Huelsenbeck and Crandall (1997). On the basis of the selected model of DNA evolution, the phylogenetic distances were calculated and the NJ-tree was constructed. For comparison, the unweighted method of maximum parsimony (MP) was used. In the analysis of deletion-insertion, the 5th character is coded and the consensus phylogenetic tree is constructed in which every node of ramification is present not less than in 50% of the initial trees. The values of support of nodes of ramification for the consensus MP (the tree is not shown) and NJ of trees for comparison are shown in the last tree. The average paired values of divergence in the principal clusters and among them were calculated as "p" distances in the Mega program version 2.1 (Kumar *et al.*, 2001). Average genetic distances between clades are calculated from paired distances between haplotypes on the basis of the model of evolution of sequences used in construction of the NJ tree. Methods of molecular-genetic investigations are described in more detail in the publication by Froufe *et al.* (2003).

RESULTS

Description of the Forms and Their Distribution

The Upper-Amur form. Fishes not more than 250 mm long. Body elongated, cylindrical. Sides silvery. Numerous small black spots along lateral line, with the highest concentration in anterior body part. A

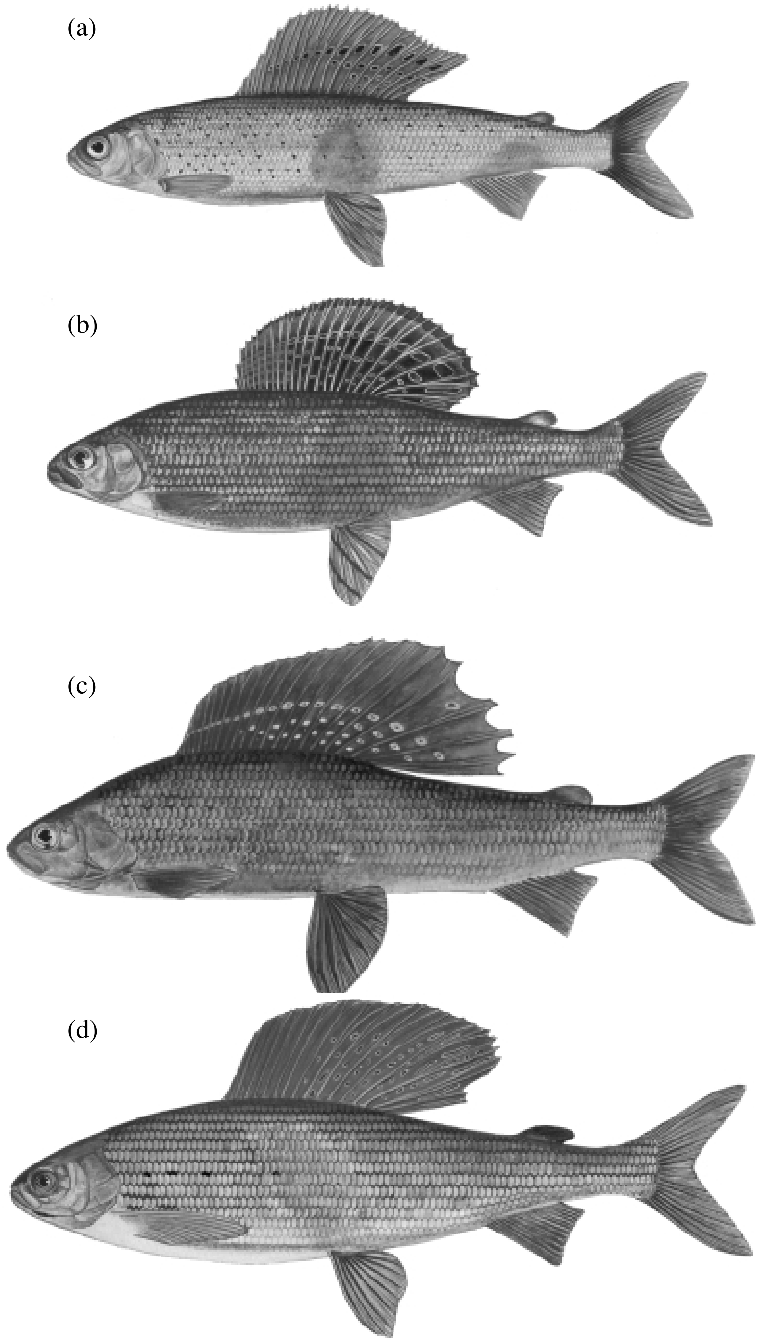

Fig. 3. The Amur graylings: (a) the Upper-Amur form, (b) the Lower-Amur form, (c) the yellow-spotted form, and (d) the large-scale form.

large pallid crimson spot above ventral fins. Two parallel yellowish brown stripes along belly, from branchiostegal rays to ventral fins. Ventral, anal, and caudal fins orange or yellow–gray. Dorsal fin low, its end does not reach adipose fin and is pointed in males. There is a narrow reddish fringe along its upper margin. Several rows of gradually increasing round red-claret spots along this fin. The smallest spots of upper row start from base of first rays and at a narrow angle extend to other rows to upper edge of its posterior part, not fusing with the fringe. Other rows of spots start slightly farther from anterior part of dorsal fin and are almost parallel to each other. In mature specimens, membranes between rays in middle and upper parts of last two–three branched rays with yellowish orange shade (Figs. 3a, 4a, 4b).

It populates rivers of the upper and middle reaches of the Amur from its sources to the Bureya, inclusively,

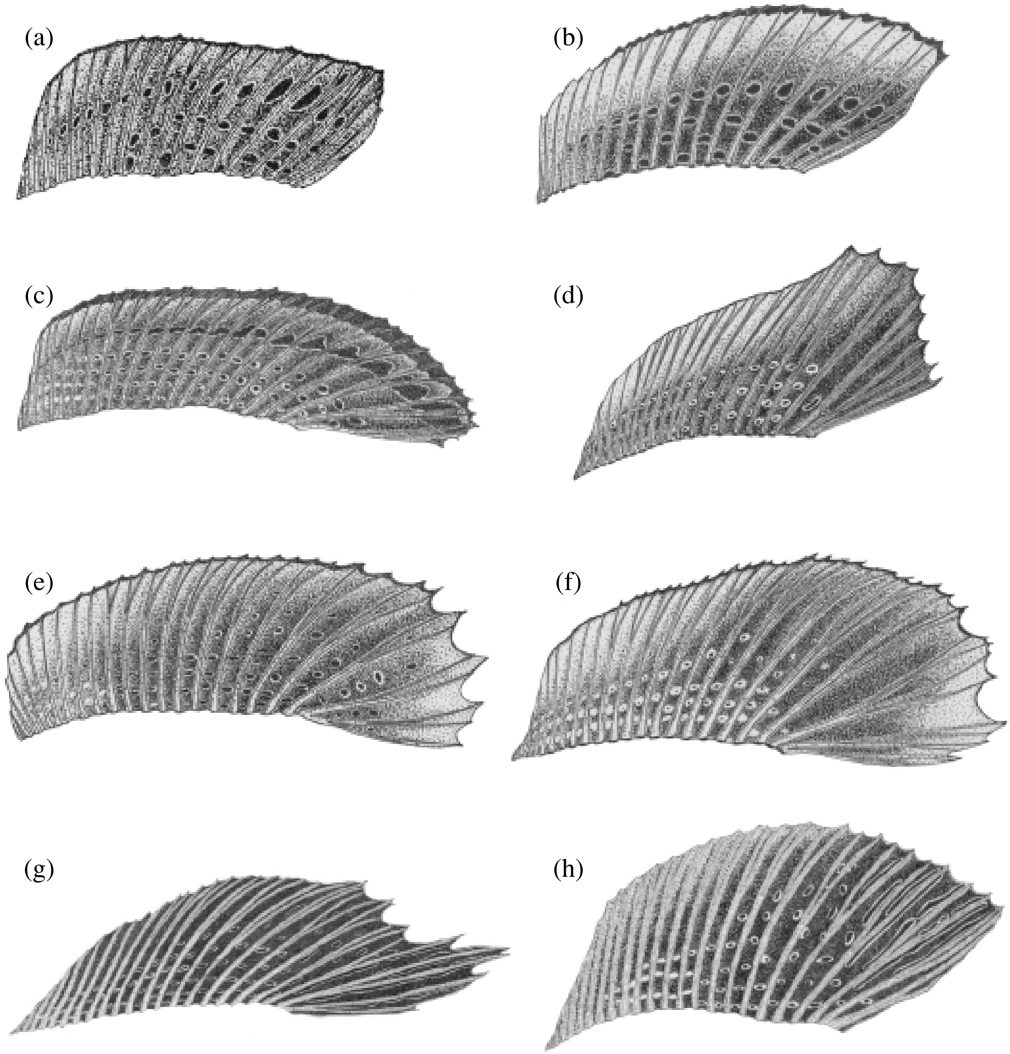

Fig. 4. Variants of pattern of dorsal fin of the Amur grayling. The Upper-Amur form: (a) the Ingoda, (b) the Bureya. The Lower-Amur form: (c) the Anyui. The yellow-spotted form: (d) the Anyui, (e) the Merek, (f) the Buta. The large-scale form: (g, h) the Bureya.

more frequent in the middle and upper reaches of tributaries of the second–third order (Figs. 1, 2).

Lower-Amur form. Body length up to 350 mm. Body deep, compressed, with slightly pendulous ventral area. Lower jaw slightly prominent compared to upper jaw. No dark spots on body. Two parallel yellow–brown stripes from branchiostegal rays to ventral fins. There are meandering yellow–orange bands between rows of scales. Claret red spot above ventral fins, caudal peduncle of similar coloration. Ventral, anal, and caudal fins with greenish turquoise shade. Dorsal fin with large base. In males, its posterior end reaches adipose fin or surpasses it. Along dorsal fin four–five stripes consisting of round and slightly elongated spots increasing in size. Upper row of large spots exceeding in diameter lower spots by two–three times. Lower rows often incomplete. Claret fringe of dorsal fin widening to its posterior end (Figs. 3b, 4c).

It populates rivers of the middle and lower reaches of the Amur: the Zeya, Bureya, Anyui, Khor, Amgun', and small tributaries below the Bureya, as well as the Sungari with its tributaries. Upstream of the Amur it was recorded up to the Bolshoi Never (ZMMGU, no. R-8671, settlement Dzhalinda). It occurs in the lower, middle, and, more rarely, upper parts of these tributaries. It is present in rivers draining into the southern part of the Sea of Okhotsk and into the Sea of Japan (the Botchi, Tumnin, Tugur, and Koppi) and in rivers of the north-western part pf Sakhalin (A.I. Zhul'kov, personal communication). Sympatry of this form with the Upper-Amur form in the Bureya was reported (Antonov, 1995) and in the Zeya. Probably, the zone of cohabitation of the aforementioned forms comprises the basins of all tributaries of the Amur from the Bol'shoi Never to the Bureya (Fig. 2).

Yellow-spotted form. Length up to 500 mm. Body oval, elongated. Scattered black spots on the head in most specimens. Between rows of scales the orange spots are grouped into meandering lines, as in the Lower-Amur form. Irregular crimson red spot above ventral fins. All fins, except pectoral and dorsal fin, of the same coloration. Very narrow vermilion fringe along upper edge of dorsal fin. Spots on the fin small, round, surrounded by a wide dull-colored ring. Some of them may be larger and slightly elongated. Upper row of spots starts at base of the third or fourth unbranched rays and extends almost to upper part pf the fin not fusing with the fringe. The other three–five rows start approximately from beginning of branched rays and proceed almost parallel to fin base. Coloration of posterior edge in middle and lower part intensively yellow or brown–yellow. In adult males, end of the fin may be irregular (Figs. 3c, 4d, 4e, 4f).

It populates only the upper reaches of some tributaries of the Amur in its lower reaches, the upper reaches of rivers draining into the Sea of Japan, the Sea of Okhotsk, and in the Tatar Strait: the Uda, Tugur, Koppi (M.B. Skopets, personal communication), Nemui, Tumnin. Sympatry with the Lower-Amur form is reported in tributaries of the Amur—the upper reaches of the Anyui, Khor, Gur, and Amgun' (Fig. 2). The area of sympatry of these forms is probably wider and comprises other large tributaries of the lower reaches of the Amur and some rivers draining into the Sea of Japan and the Sea of Okhotsk.

The large-scale form. Body length up to 500 mm. Body deep, massive. Weight may attain 1000 g. Scales large. Snout oval. Black spot on lower jaw, absent in other Amur forms. Body with dark blue shade against greenish turquoise background. Several small black spots near head. Meandering yellowish brown bands on sides between rows of scales. In males, dorsal fin large, often with irregular posterior edge, extending beyond adipose fin. Fringe on dorsal fin not discernible. Caudal peduncle, anal and caudal fin claret red. Several rows of spots differing in form against the almost black background of dorsal fin. In anterior part, they are small and round with light periphery in three–four rows. Closer to posterior part of the fin the number of rows increases and spots gradually become elongated. Bands appear on last three–four membranes between rays, in addition to round spots (Figs. 3d, 4g, 4h).

Graylings of this form are found only in the upper reaches of the Levaya Bureya (Antonov, 2001) (Figs. 1, 2).

Morphology

Characteristics of morphometric and meristic characters of different forms of the Amur graylings are shown in Tables 3 and 4.

Comparison of the studied forms by *t*-test revealed significant differences with the significance level p = 0.001 by most morphometric and meristic characters in the large-scale form from all analyzed samples; in the Upper-Amur form and the yellow-spotted form from the Lower-Amur form. The smallest number of differences is found between the yellow-spotted form and the Upper-Amur form. In populations of the yellow-spotted form from the Merek, Anyui, and Buta, and in fishes from the Onon and Ingoda, significant differences are almost absent. The exception is only the number of pyloric caeca, in graylings from the Anyui and Buta the differences were significant (t = 3.87; p = 0.001).

Significant differences are noted by the Mayr coefficient of difference CD by morphometric characters between the Upper-Amur and Lower-Amur forms in the dorsal fin base length, the highest and smallest body depth, smaller in fishes from the Onon and Ingoda. In graylings from the Lower Amur the antedorsal distance is smaller, the depth of the posterior part of the dorsal fin and the length of pectoral fins are greater. There are differences between the aforementioned forms also by meristic characters, namely by the number of scales in the lateral line and of vertebrae, less numerous in the Lower-Amur grayling, while the number of branched rays in the total number of rays in the dorsal fin is higher (Table 5).

Comparison of the yellow-spotted form with the Lower-Amur form revealed some distinctions manifesting themselves less in morphometric characters and more in meristic characters. The yellow-spotted graylings from the Anyui River in comparison with the Lower–Amur form have a longer dorsal fin base, more numerous scales in the lateral line and total number of rays in the dorsal fin, and more numerous vertebrae. In the Merek and Byta rivers the yellow-spotted form differs from the Lower-Amur form in the number of branched rays in the dorsal fin, in the Buta River—in more numerous perforated scales in the lateral line and in fewer branched rays in ventral fins.

The Upper-Amur form in comparison with all samples of the yellow-spotted form has a longer antedorsal distance. The graylings from the Ingoda and Onon noticeably differ from the fishes from the Merek and Buta in a less deep body, shorter dorsal fin base, less deep posterior part of the dorsal fin, and shorter ventral fins. There are also noticeable differences in the Upper-Amur form from all samples of the yellow-spotted form in the number of nonbranched rays and in the total number of rays in the dorsal fin.

Comparison of the large-scale form with all other forms revealed significant differences in most meristic and morphometric characters (Table 5).

Application of the method of principal components separately by meristic and plastic characters demonstrated that in the Amur basin three groups are discerned. One is formed by the Lower-Amur form from the Anyui River, another—by the Upper-Amur and yellow-spotted forms, and the third—by the large-scale form (Fig. 5).

Table 5. Coefficient of difference (CD) of forms of the Amur grayling by morphometric and meristic characters

Character	River (form)																
	Ingoda (u)	Onon (u)	Ingoda (u)	Onon (u)	Ingoda (u)	Onon (u)	Ingoda (u)	Onon (u)	Anyui (y)	Merek (y)	Buta (y)	Ingoda (u)	Onon (u)	Anyui (l)	Anyui (y)	Merek (y)	Buta (y)
	Anyui (l)		Anyui (y)		Buta (y)		Merek (y)		Anyui (l)			Bureya (ls)					
r																1.67	1.18
o												1.29	1.88	1.65		1.3	1.22
f																1.27	
c				1.55								1.05	1.56			1.3	
ch													1.36	1.26		1.31	
lmx				2.06				1.93				1.07	2.20	1.21			
lmd				1.62				1.35	1.16			1.19	2.64	1.22		1.47	1.53
H	1.66	1.75										1.71	1.8		1.13		
h	2.00	2.22			1.17	1.44	1.51	1.77				1.64	1.83				
w												1.92	1.69	2.14	1.65	2.21	2.3
aD	1.13	1.84	1.26	2.15		1.53		1.66						1.81	2.27	1.54	1.36
aV				1.37													
lp															1.47		
lD	2.22	2.85				1.38	1.32	1.80	1.57		1.19	2.46	3.54		1.45		
hD2	1.21	1.36					1.42	1.58				1.16	1.33				
lP	1.24	1.50															
lV							1.48	1.74				1.31	1.62				
ll	1.25	1.63							1.56		1.21	1.70	1.76	1.51	1.75	1.64	1.70
D1			1.13	1.22	1.43	1.56	1.24	1.39						1.19	1.34	1.39	1.45
D2	1.43	1.75							1.19	1.65	1.31	1.04		1.41	1.21	1.14	
D	2.47	2.78	1.45	1.68	1.36	1.57	1.19	1.45		1.58		1.18	1.16	1.57	1.42	1.38	1.40
P												1.46	1.44	1.47	1.49	1.48	1.56
V	1.18	1.08								2.81	1.20	1.24	1.23	1.47	1.28	1.34	1.18
A2												1.22	1.14	1.28	1.29	1.20	1.37
sp. br.												1.38	1.45	1.47	1.57	1.51	1.64
r. br.												1.23	1.22	1.28	1.21	1.28	1.30
vert.	1.15	1.39							1.20			1.54	1.56	1.47	1.54	1.53	1.50
p. c.												1.31	1.26	1.35	1.49	1.64	1.75

Note: Note as for table 2.

The first and second principal components explain 43.7% of the total dispersion of meristic and 65.5% of morphometric characters. The greatest loads on the first and second principal components by meristic and morphometric characters are shown in Tables 6 and 7 respectively.

The results of cluster analysis of samples of the Amur graylings in comparison with populations of graylings from eastern Siberia and Mongolia by merisitic characters are represented in Fig. 6. In the dendrogram, the cluster is separated comprising the fishes of the Lower-Amur form from the Anyui and Khor rivers and the fishes of the large-scale form from the Levaya Bureya. There is a combination at a comparatively low level of the yellow-spotted form from the Gobili and Ertukuli rivers (the Anyui basin), the Merek, the Buta, and the graylings of the Upper-Amur form from the Onon and Ingoda together with the populations of Siberian grayling *T. arcticus* ssp. From rivers of basins of the upper and middle reaches of the Lena—the Kirenga, Chaya, Chara, and Olekma. Separate branches are formed by the Mongolian grayling *T. brevirostris* from Lake Khokh Nur and the Kosogol grayling *T. arcticus nigrescens* from Lake Khubsugul. The total cluster is formed by populations of the west Siberian grayling *T. arcticus arcticus* from the Nuzh-

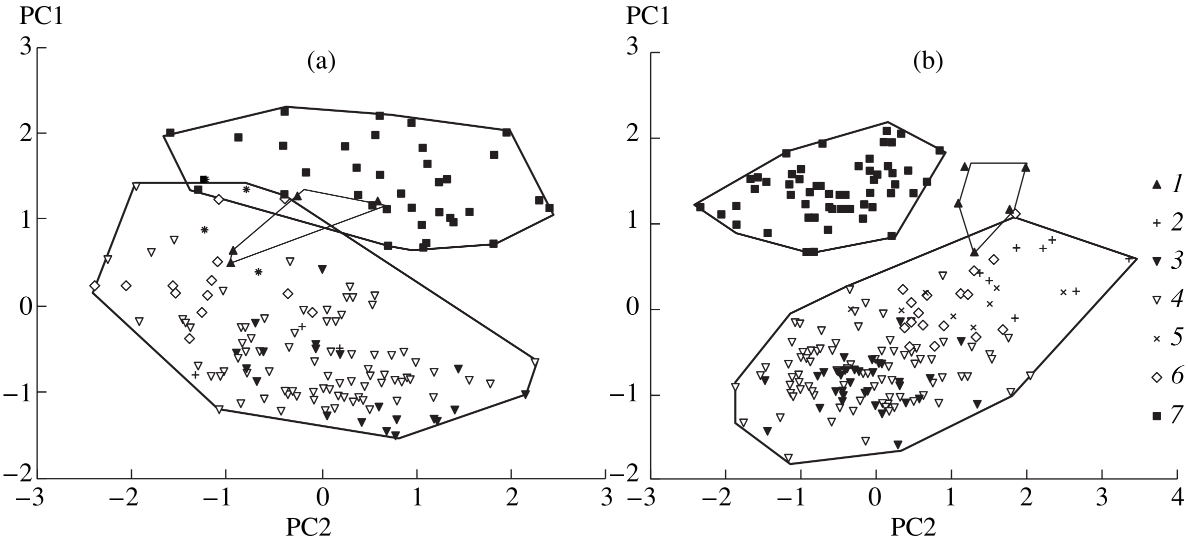

Fig. 5. Distribution of different forms of the Amur grayling in the space of principal components: (a) by 29 morphometric characters; (b) by 12 meristic characters. (*1*) the Levaya Bureya (ls), (*2*) the Buta (y), (*3*) the Onon (u), (*4*) the Ingoda (u), (*5*) the Merek (y), (*6*) the Anyui (y), (*7*) the Anyui (l).

nyaya Tunguska and Taltsinka (Irkutsk Reservoir) with the white and black Baikal graylings *T. arcticus baicalensis* from Dagar Bay (northern Baikal) and also from Lake Sobolinoe (southern Baikal).

Molecular-genetic analysis

In the obtained nucleotid sequences, 600 pairs of bases in size, and 63 phylogenetically informative positions total are found. Of 56 tested, the most probable model of evolution of sequences was the model of Hasegava-Kishino-Yano (HKY) (Hasegava *et al.*, 1985).

Table 6. Loads of eigenvectors on the first two principal components for 12 meristic characters

Character	Principal components	
	1	2
ll	–0.201	0.171
D1	0.153	0.245
D2	0.221	–0.149
D	0.253	0.025
P	0.084	0.202
V	0.199	–0.132
A1	0.057	0.287
A2	0.035	0.143
sb	0.068	0.382
rb	0.052	–0.134
vt	–0.207	0.058
pc	0.012	0.406

Application of this model revealed in the phylogenetic NJ-tree eight clusters with a high probability of support reflecting the divergence level of the analyzed populations (70–100% bootstrap values for the NJ-tree and 99–100% for MP consensus tree), characterizing the diversity of graylings of large water basins of eastern Siberia and of the Amur basin in particular (Fig. 7). Individual branches comprise: (a) graylings of the Upper-Amur form from the Ingoda, Onon, and Bureya; (b) the yellow-spotted form represented by the grayling from the Buta; (c) the graylings of the same form from the Merek, Gobili, and Ertukuli (the upper reaches of the Anyui); (d) the fishes of the Lower-Amur form from the Anyui; (e) the graylings from the Baikal and Angara-Yenisei basins; (f) the graylings from rivers of the basins of the upper and middle reaches of the Lena (the Kutima, Kuanda, and Olongdo); (g) the Mongolian grayling from the Central Asiatic basin (Lake Khokh Nur); and (h) the graylings of the large-scale from the Bureya.

The diagram demonstrates that the graylings from the Levaya Bureya are represented by two groups. One of them has no genetic distinctions from the Upper-Amur form. Another (the large-scale form) is significantly divergent from all samples and is represented by the basal cluster in relation to populations of the Siberian and Monglian graylings.

The maximum value of divergence level between clusters (by noncorrected p-distances), representing different forms within the Amur basin, attains 4.4% (Table 8). This exceeds 4% of divergence between sequences of the control area of mitochondrial DNA of populations of the European grayling *T. thymallus*, revealed by analysis of genetic material from the entire geographic range of this species (Weiss *et al.*, 2002).

The yellow-spotted form of grayling from tributaries of the Amur forms a monophyletic cluster of two sister groups (1% of divergence). One of these groups consists only of fishes from the Buta River, which formerly might belong to the Amur basin. In their turn, the graylings of this form from tributaries of the lower reaches of the Amur are most similar to the Upper-Amur form from the Ingoda, Onon, and Bureya rivers, but are remote haplotypes discovered in graylings of the Lower-Amur form from the Anyui. Generally, all haplotypes of fishes of the Amur basin, except the large-scale form from the Byreya and the Lower-Amur form, form a monophyletic group (98–100% bootstrap support).

The maximum value of divergence between monophyletic clusters with a high probabilistic support is revealed in the pair formed by populations from the upper reaches of the Lena, on the one hand, and by those from the upper reaches of the Amur (the Ingoda—Onon), on the other hand—5.4%. Haplotypoes of graylings from the Baikal and Angara-Yenisei basins are the closest (2.4% divergence) to the haplotypes from the upper reaches of the Lena. These two clusters form a group, which compared with other groups are more closely related to the Mongolian grayling *T. brevirostris* from Lake Khokh Nur (Table 8).

In construction of the consensus tree by the method of maximum parsimony (MP) only the polychotomy with three lines is represented by: (1) the Upper-Amur and yellow-spotted forms; (2) the Lower-Amur form; and (3) all other considered forms, including the large-scale form.

Table 7. Loads of eigenvectors on the first two principal components for 29 morphometric characters

Character	Principal components	
	1	2
l	–0.248	–0.056
l_2	0.382	–0.196
r	0.112	0.019
o	–0.198	0.368
f	–0.262	–0.046
c	–0.537	0.140
cH	0.568	0.461
ch	0.336	0.379
k	0.012	0.214
lmx	–0.248	0.324
i/lmx	0.281	0.067
lmd	–0.219	0.333
H	0.800	0.272
h	0.857	0.185
w	0.117	0.310
aD	–0.728	0.120
pD	–0.569	–0.560
aA	0.116	0.757
aV	–0.221	0.605
lp	–0.068	–0.295
P–V	0.354	0.576
V–A	0.219	0.316
lD	0.927	0.120
hD_1	0.300	–0.032
hD_2	0.947	–0.207
lA	0.355	–0.094
hA	0.118	0.483
lP	0.733	0.124
lV	0.857	–0.207

DISCUSSION

The above descriptions of coloration of the Amur graylings demonstrate that they all have external characters easily distinguishing different forms. The principal characters are the form and distribution of spots on the dorsal fin and presence of marks on body sides. The Upper-Amur form differs from other forms in numerous black marks against the background of an even silvery coloration of the body, in small size, and a low dorsal fin, not reaching the adipose fin; bright orange meandering stripes between rows of scales along the body are absent (present in the Lower-Amur form). Representatives of the Upper-Amur form, and all others, in distinction from the Lower-Amur form, do not possess a “dipperlike” mouth, a noticeably compressed body, and pendulous belly.

It should be noted that the original description by Dybowski (1869) of the Amur grayling as “mottled” indicated not a brightness of coloration in the sense of Tugarina and Khramtsova (1980), but just the presence of numerous black spots (mottles) scattered all over the body. In fishes of the Lower-Amur form the mottles occur extremely rarely. A greenish hue of the body, characteristic of the Lower-Amur form, is completely absent in the Upper-Amur form. Thus, the body coloration and form of the Upper-Amur and of the Lower-Amur groups are essentially different.

Analysis of the pattern of dorsal fins reveals similarity in position of spots and in the presence of yellow coloration of the posterior part of the dorsal fin in the Upper-Amur and the yellow-spotted forms. However, in contrast to other groups, in the yellow-spotted form the pattern of the dorsal fin is formed only by small round spots with a dull periphery. Yellow coloration of its posterior part and specific arrangement of the rows of spots are distinctive characters of this form populating the upper reaches of tributaries of the Lower Amur, small rivers draining into the Tatar Strait, the Sea of

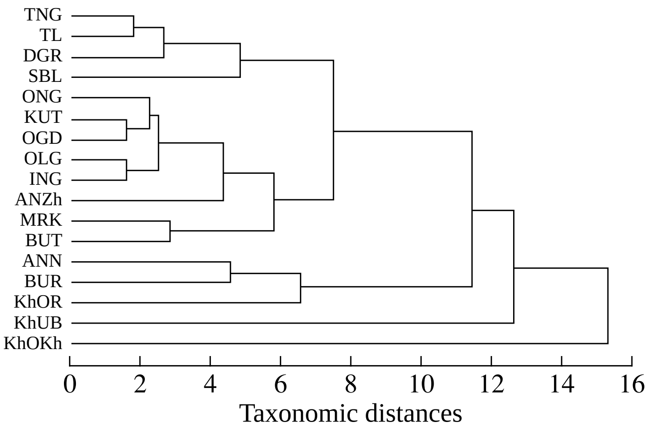

Fig. 6. Dendrogram of differences of the Amur graylings and some populations of graylings from water bodies of eastern Siberia by 12 meristic characters constructed by the UPGMA method. *T. arcticus arcticus*: TNG—the Nizhnyaya Tunguska (the Yenisei), TL—the Taltsinka (Irkutsk Reservoir); *T. arcticus baicalensis* (the white grayling): DGR—Dagary bay (Lake Baikal); *T. arcticus baicalensis* (the black grayling): SBL—Lake Sobolinoe (the Snezhnaya—Southern Baikal); *T. arcticus* ssp.: KUT—the Kutima, OGD—Lake Ogiendo (the Chaya—the Lena), OLG—Lake Olongdo (the Olekma—the Lena); *T. grubii*: ONG—the Onon, ING—the Ingoda, ANZh—the Anyui, MRK—the Merek, BUT—the Buta; *T.* sp. (large-scale): BUR—the Levaya Bureya; *T. arcticus nigrescens*: KhUB—Lake Khubsugul; *T. brevirostris*: KhOKh—Lake Khokh Nur; *T.* sp.: ANN—the Anyui, KhOR—the Khor (the Lower Amur) (Tugarina and Khramtsova, 1980).

Japan, and part of the graylings from the upper reaches of the Amur basin.

The presence of long longitudinal stripes on membranes between the last rays of the dorsal fin in the large-scale form and of a black spot on the lower jaw seems to indicate a closeness by these characters to the East Siberian (Kolyma) grayling *T. arcticus pallasi.* In the large-scale and the yellow-spotted forms there is some similarity in the distribution of spots on the anterior part of the dorsal fin.

The pattern and form of spots on the dorsal fin are supposed by Makoedov (1983, 1987, 1999) to be important characters of different taxa. In their analysis it was noted that in the aforementioned publications on graylings of the whole Amur basin only one pattern is indicated, identical to that published by Tugarinova and Khramtsova (1980). A combination of elements of coloration of fishes from the Onon described by Dybowski (1869) with those of fishes from the Khor (Tugarinova and Khrantsova, 1980) led to contradictions in the description of the Amur grayling.

Clusterization of groups of the Amur graylings by the method of unweighted paired grouped averages (UPGMA) by meristic characters agrees with the results of comparison of samples by the CD coefficient and of the investigation by the method of principal components. It meets our notions of differentiation of the forms by the body coloration and by the pattern of the dorsal fins. The obtained tree shows the similarity of the Amur grayling to the Siberian *T. arcticus* ssp. from the Upper Lena basin, noted by some previous authors. However, the data of molecular-genetic analysis record the greatest differences in the Upper Amur and the Upper Lena fishes. Additional aspects are necessary for explanation of such similarities and differences. Clear genetic differences of the Lower-Amur form from the Upper-Amur and the yellow-spotted forms indicate their reproductive isolation. This fact confirms the opinion of Skurikhina (1984) that the Lower-Amur form attained a species rank. Reproductive isolation of these forms is confirmed by the results of recent investigations of nuclear DNA (Froufe *et al.*, 2003).

It may be supposed that the period of alternating glaciations and transgressions of waters into the continent little influenced the formation of Amur groups of graylings. The Amur basin may be understood as a kind

Table 8. Average paired values of divergence within and between eight main groups of haplotypes. Under the diagonal—uncorrected "p" genetic distances (in %) calculated by means of software MEGA v. 2.1.; above the diagonal—average genetic distances between groups obtained by the maximum likelihood method (ML), based on HKY evolutionary model (Hasegawa *et al.*, 1985)

Populations	1	2	3	4	5	6	7	8
1		0.075	0.080	0.116	0.104	0.020	0.022	0.045
2	3.45		0.023	0.036	0.080	0.071	0.073	0.051
3	4.07	1.98		0.050	0.083	0.081	0.074	0.057
4	5.42	2.42	3.53		0.102	0.108	0.119	0.078
5	4.39	4.10	4.45	4.97		0.081	0.084	0.111
6	1.42	3.22	3.73	5.06	4.10		0.009	0.049
7	1.18	3.41	4.03	5.41	4.37	0.92		0.053
8	2.13	2.15	2.91	4.15	4.29	2.41	2.08	
Intragroup ("p") in %	0.27	0.21	0.20	0.18	0.13	0.34	0.08	1.49

Note: (1) the Upper-Amur form (Ingoda-Onon); (2) graylings from Baikal and the Angara-Yenisei basin; (3) Mongolian grayling; (4) Grayling from the basin of the upper reaches of the Lena; (5) the Lower-Amur form; (6) the yellow-spotted form (the Buta); (7) the yellow-spotted form (the Anyui, the Merek); and (8) the large-scale form (the Bureya).

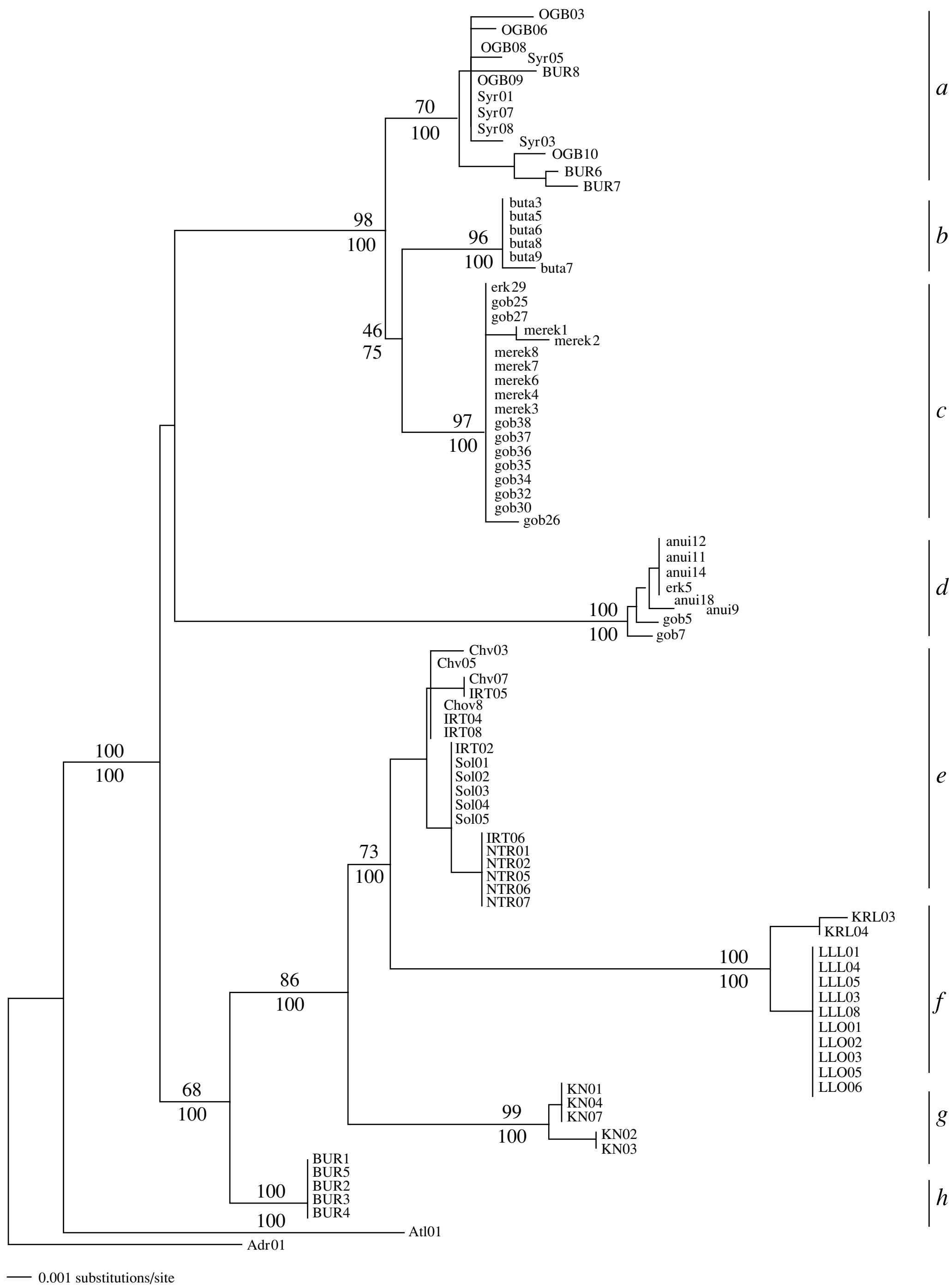

Fig. 7. NJ-phylogenetic tree constructed with consideration of assessment of divergence of nucleotide sequences of the fragment of the control region pf mtDNA (600 pairs of bases in length) calculated according to HKY-model of evolution of sequences. As an external group, haplotypes Atl01 and Adr01 are used. Above branches the values corresponding to bootstrap support of nodes of branching of NJ-tree are indicated, under branches—those of MP-tree (1000 replications).

Grayling of the Upper-Amur form from the Ingoda, onon, and Bureya (*a*); the yellow-spotted form from the Buta (*b*), the Merek, Gobili, and Ertukuli (the upper reaches of the Anyui) (*c*); the Lower-Amur form from the Anyui (*d*); graylings from the Baikal and Angara-Yenisei basins (*e*); graylings from rivers of the basins of the upper and middle reaches of the Lena (the Kutima, Kuanda, and Olongdo) (*f*); the Mongolian grayling from the Central Asian basin (Lake Khokh Nur) (*g*); the large-scale form from the Bureya (*h*).

OGB—the Onon, Syr—the Sypchegur, BUR—the Levaya Bureya, buta—the Buta, merek—the Merek, erk—the Ertukuli, gob—the Gobili, anui—the Anyui, Chv—Lake Khubsugul, IRT—the Tal'tsinka, Sol—Lake Sobolinoe, NTR—the Nizhnyaya Tunguska, KRL—the Kutima, LLL—Lake Leprindokan, LLO—Lake lesha, KN—Lake Khokh Nur, Atl01—the Loire River (France), and Adr01—the Soca River (Republic of Slovenia).

of interglacial refuge. Its nucleus is composed by the Upper-Amur and the yellow-spotted forms, on the one hand, and by the Lower-Amur form, on the other hand. The limited habitat of the large-scale form and absence of specimens with intermediate external morphological characters in other investigated forms also confirm their reproductive isolation.

The aforementioned data suggest the conclusion that the Amur basin is populated by the Amur grayling *T. grubii* with two forms—the Upper-Amur and the yellow-spotted forms, and also by one more independent species—the Lower-Amur grayling *Thymallus* sp. characterized by a high level of morphological and genetic differences from all other Amur forms. As to the large-scale form, its taxonomic status may be defined only after ascertaining its phylogenetic relationships with populations of the East Siberian grayling *T. arcticus pallasi* populating rivers of the coast of the Sea of Okhotsk and rivers of basins of the Laptev and Bering seas.

CONCLUSIONS

With consideration of the obtained results we arrive at the conclusion that all specified forms possess a complex of external diagnostic characters. The results of the molecular-genetic and morphological analyses confirm the outlined groups and their reproductive isolation. The Amur basin is populated by graylings of two phylogenetic lines, one comprising the Upper-Amur, yellow-spotted, and Lower-Amur forms, the other being represented by the large-scale form. Morphologically and genetically, the Upper-Amur and yellow-spotted forms are closely related and make up one species—the Amur grayling *T. grubii* Dybowski. The Lower-Amur form by the level of differences attained the status of a species. The large-scale form differs in essential genetic and morphological differences from all Amur groups, it is genetically closer related to populations of the Siberian grayling *T. arcticus* living in water bodies of eastern Siberia. The obtained data indicate that a revision of the genus *Thymallus* is urgently needed.

ACKNOWLEDGMENTS

We are grateful to S.S. Alekseev, E.A. Antonova†, B.E. Bogdanov, E.A. Vasil'eva, A.V. Lednev, A.N. Matveev, S.S. Samarina, V.P. Samusenk, A.L. Yur'ev for aid in collecting, to M.B. Skopets and A.I. Zhul'kov for the photographs of graylings, to D.V. Kuznetsova for preparation of drawings of fishes, to S.S. Alekseev, L.V. Sukhanova, and P.Ya. Tugarina for valuable advice in the course of preparation of the manuscript, to E.D. Vasil'eva, E.A. Dorofeeva, N.G. Bogutskaya, and A.V. Balushkin for the access to collections of graylings at the museum of Moscow State Univeristy and at the Zoological Institute of the Russian Academy of Sciences.

The study is supported by the Federal Target Program "Integration" (ko 788), the Russian Foundation for Basic Research (project nos. 01-04-49376 and 01-04-96305 "Khabarovsk"), and the Portugese Ministry of Science (FCT no. POCT1/33364/BSE/2000).

REFERENCES

1. *Annotated Catalog of Cyclostomata and Fishes of Continental waters of Russia* Ed. by Yu. S. Reshetnikov, (Nauka, Moscow, 1998), pp. 48–50 [in Russian].
2. A. L. Antonov, Vopr. Ikhtiol. **35** (6), 831 (1995).
3. A. L. Antonov, Tr. Gos. Prirod. Zapoved. "Bureinskii," No. 1, 108 (1999).
4. A. L. Antonov, in *Proc. Biodiversity and Dynamics of Ecosystem in North Eurasia (BDENE), Novosibirsk, August 21–26* (SB RAS, IC & G Novosibirsk, 2000), Vol. 3, Part 1, pp. 120–122.
5. A. L. Antonov, *Lectures in Memoriam of Vladimir Yakovlevich Levanidov (Vladivostok, March 20–22, 2001*, (Dalnauka, Vladivostok, 2001), No. 1, pp. 264–268 [in Russian].
6. *Atlas of Freshwater Fishes of Russia*, Ed by. Yu. S. Reshetnikov (Nauka, Moscow, 2002), Vol. 1 [in Russian].
7. L. S. Berg, *Fishes of Fresh Waters of Russian Empire* (Moscow, 1916).
8. L. S. Berg, *Fishes of Fresh Waters of the USSR and of Adjacent Countries*, 4th ed. (AN SSSR, Moscow, 1948), Part 1 [in Russian].
9. N. G. Bogutskaya and A. M. Naseka, 2002, http://www.zin.ru/.
10. V. P. Borovikov and I. P. Borovikov, *Statistica—Statistical Analysis and Treatment of Statistical Data in Windows* (Filin, Moscow, 1998).
11. N. I. Chugunova, *Handbook on Investigations of Age and Growth of Fishes* (Sovetskaya Nauka, Moscow, 1959) [in Russian].
12. B. Dybowski, Verh. Zool.-Bot. Gesell. Wien **19**, 945 (1869).
13. E. Froufe, I. Knizhin, M. T. Koskinen, *et al.*, Mol. Ecol. **12**, 2345 (2003).
14. M. Hasegava, H. Kishino, and T. Yano, J. Mol. Evol. **22**, 160 (1985).
15. J. P. Hueselbeck and K. A. Crandall, Ann. Rev. Ecol. Syst. **28**, 437 (1997).
16. G. L. Karasev, *Fishes of Transbaikalia* (Nauka, Novosibirsk, 1987).
17. M. T. Koskinen, I. Knizhin, C. R. Primmer, *et al.*, Mol. Ecol. **11**, 2599 (2002).
18. V. G. Kostitsyn and E. A. Zinov'ev, in *Abstracts, III Russian Meeting on Salmonoids, Tolyatti, Mart 1988* (Tolyatti, 1988), p. 162.
19. S. Kumar, K. Tamura, I. B. Jakobsen, and M. Nei, Bioinformatics. Arizona St. Univ. Tempe. **17**, 1244 (2001).
20. A. N. Makoedov, in *Abstracts, X Russian Symposium "Biological problems of the North* (Magadan, 1983), p. 193 [in Russian].
21. A. N. Makoedov, Vopr. Ikhtiol. **27** (6), 906 (1987).
22. A. N. Makoedov, *Relationships of Graylings of Siberia and the Far East* (Moscow, 1999) [in Russian].
23. A. N. Makoedov and O. B. Korotaeva, *Population Phenetics of Fishes* (UMK Psikhologiya, Moscow, 1999) [in Russian].

24. E. Mayr *Principles of Systematic Zoology* (McGraw-Hill, New York, 1969; Moscow: Mir, 1971).
25. G. V. Nikolsky, *Fishes of the Amur Basin* (AN SSSR, Moscow, 1956) [in Russian].
26. K. Pivnicka and K. Hensel, Acta Univ. Carolinae-Biologica 1975–1976 **4**, 37 (1978).
27. D. Posada and K. A. Cransall, Bioinformatics **14**, 817 (1998).
28. I. F. Pravdin, *Handbook on Fish Investigation* (Pishchevaya prom-st', Moscow, 1966) [in Russian].
29. P. F. Rokitskii, *Biological Statistics* (Vysheishaya shkola, Minsk, 1973) [in Russian].
30. S. V. Shed'ko, in *Lectures in Memoriam of Vladimir Yakovlevich Levanidov, Vladivostok, March 20–22, 2001* (Dalnauka, Vladivostok, 2001), No. 1, p. 229 [in Russian].
31. L. A. Skurikhina, Abstract of Candidate's Dissertation in Biology (Moscow, 1984).
32. L. A. Skurikhina, B. M. Mednikov, and P. Ya. Tugarina, Zool. Zh. **64** (2), 245 (1985).
33. A. N. Svetovidov, Tr. Zool. Inst. AN SSSR **3**, 183 (1936).
34. P. Ya. Tugarina and V. S. Khramtsova, Vopr. Ikhtiol. **20** (4), 590 (1980).
35. P. Ya. Tugarina and V. S. Khramtsova, Vopr. Ikhtiol. **21** (2), 209 (1981).
36. F. Uiblein, A. Jagsch, W. Honsig-Erlenburg, and S. Weiss, J. Fish, Biol. **59** Suppl. A, 223 (2001).
37. D. L. Swofford, *PAUP* ver 4.0.b3a. Phylogenetic Analysis Using Parsimony and Other Methods*, (Sinauer Associates, Sunderland, MA, 2000).
38. S. Weiss, H. Persat, R. Eppe, *et al.*, Mol. Ecol. **11**, 1393 (2002).
39. E. A. Zinov'ev, N. S. Romanov, V. S. Russkikh, *et al.*, in *Abstracts, Coordination Meeting on Salmonoids. Morphology, Population Structure, and Problems of Rational Exploitation of Salmonoids, Leningrad, March 1983* (Nauka, Leningrad, 1983), p. 75 [in Russian].

Translated by N.N. Smirnov