

# ZOOSYSTEMATICA ROSSICA

Zoological Institute, Russian Academy of Sciences, St Petersburg • https://www.zin.ru/journals/zsr/ Vol. 33(2): 293–307 • Published online 5 December 2024 • DOI 10.31610/zsr/2024.33.2.293

RESEARCH ARTICLE

# An integrative approach to resolving taxonomic uncertainty in forms of *Triglopsis quadricornis* (Teleostei: Cottidae) from Lake Ladoga and Lake Onega

# Интегративный подход для решения таксономической неопределенности форм *Triglopsis quadricornis* (Teleostei: Cottidae) из Ладожского и Онежского озер

V.G. Sideleva & Z.V. Zhidkov

В.Г. Сиделева, З.В. Жидков

Valentina G. Sideleva, Zoological Institute, Russian Academy of Sciences, 1 Universitetskaya Emb., St Petersburg 199034, Russia. E-mail: vsideleva@gmail.com

Zakhar V. Zhidkov , Zoological Institute, Russian Academy of Sciences, 1 Universitetskaya Emb., St Petersburg 199034, Russia. E-mail: zhidkovz@gmail.com

**Abstract.** According to the results of a comparative analysis of morphological and molecular genetic data, the marine form of the fourhorn sculpin from the Baltic Sea and the two freshwater forms from Lake Ladoga and Lake Onega belong to the nominative subspecies *Triglopsis quadricornis quadricornis* (Linnaeus, 1758). The observed differences are at the intraspecific level.

**Резюме.** По результатам сравнительного анализа морфологических и молекулярно-генетических данных показано, что морская форма четырёхрогого подкаменщика из Балтийского моря и две пресноводных формы из Ладожского и Онежского озер принадлежат к номинативному подвиду *Triglopsis quadricornis quadricornis* (Linnaeus, 1758). Обнаруженные различия находятся на внутривидовом уровне.

Key words: Baltic Sea, freshwater forms, fourhorn sculpin, morphology, mtDNA, cottoid fish

**Ключевые слова:** Балтийское море, пресноводные формы, четырёхрогий подкаменщик, морфология, мтДНК, коттоидные рыбы

ZooBank Article LSID: 0403A2FF-E9B2-4672-936F-94AE37DC7071

## Introduction

The species *Cottus quadricornis* (fourhorn sculpin) was described by Linnaeus in 1758 from the Baltic Sea. Gratzianow (1907) placed *C. quadricornis* in the genus *Myoxocephalus* Tilesius, 1811 (the type species *M. stelleri* Tilesius, 1811). The presence of a transverse dermal fold on the isthmus was indicated as the main diagnostic fea-

ture of this genus. The type locality of *M. stelleri* is Avacha Bay, Kamchatka (Tilesius, 1811). The presence of a transverse fold on the isthmus in *C. quadricornis* prompted the transfer of this Baltic Sea species from the genus *Cottus* Linnaeus, 1758 to the genus *Myoxocephalus*. Additionally, the presence of an opening behind the fourth gill arch and four bony outgrowths on the occiput, originally referred to as "tubercles", were

indicated as diagnostic characters of *M. quadricornis* (Gratzianow, 1907). These features serve to distinguish *M. quadricornis* from all other species within the genus *Myoxocephalus*.

In faunistic studies of the fishes of the Russian Empire (and later the USSR), Berg (1916, 1923, 1949) used the name *Myoxocephalus quadricornis* for the fourhorn sculpin from the Baltic Sea. This systematic position of the species remained unchanged until 1979, when A.V. Neelov examined the structure of the seismosensory system in cottoid fishes of the subfamily Myoxocephalinae. He discovered that the type species, *M. stelleri*, along with other species of the genus *Myoxocephalus*, have a distinctly different pattern of seismosensory system compared to *M. quadricornis* (Neelov, 1979).

All species of *Myoxocephalus*, including *M. stelleri*, have a complex seismosensory system, including narrow sensory canals that feature branched dermal canaliculi containing several hundred small pores. In contrast, the seismosensory system of *M. quadricornis* is composed of sensory canals that resemble interconnected extensive cavities. Each cavity gives rise to a single unbranched dermal canaliculus, which has a very fine pore at its terminus. The total number of pores across all seismosensory canals in the head is approximately 30.

Such a contrasting difference in the structure of the seismosensory system provided a rationale for transferring the species M. quadricornis into the genus Triglopsis, with the type species being Triglopsis thompsonii Girard, 1851 (Neelov, 1979). Triglopsis thompsonii has a seismosensory system similar to that of T. quadricornis: the canals are structured as cavities, each containing a single canaliculus and a limited number of pores. This configuration of the seismosensory system is a unique characteristic of the genus Triglopsis Girard, 1851 and is markedly different from the system found in species of the genus Myoxocephalus (Neelov, 1979; Sideleva & Zhidkov, 2022). To date, there is no consensus among ichthyologists regarding the genus to which the species "quadricornis" belongs. According to Eschmeyer's online catalog (Fricke et al., 2024), researchers are divided on this issue: some insist on the genus Myoxocephalus, while others favor Triglopsis. Recent studies have demonstrated that Triglopsis differs from other genera of the subfamily Myoxocephalinae by a set of morphological characters, as well as by genetic isolation (Sideleva & Zhidkov, 2022). The genetic p-distances between *T. quadricornis* and *M. stelleri* were found to be 13.4%, whereas interspecific genetic distances among species within the genus *Myoxocephalus* ranged from 1.8% to 8.8%. Among the morphological characters, the following are diagnostic for this genus: four well-developed spines on the preoperculum; sensory canals of the lateral line that are arranged as sequentially connected cavities; and very small pores located on the dermal tubules that protrude above the surface of the head. Based on these data, we consider the fourhorn sculpin as a member of the genus *Triglopsis*.

In large lakes of glacial genesis, located in Scandinavia and northern European Russia, *T. quadricornis* has developed freshwater relict forms (Berg, 1916, 1949; Lönnberg, 1919; Segerstråle, 1956; Kudersky & Lotarev, 1964). It was shown that the *T. quadricornis* forms from Lake Ladoga and Lake Onega, the largest continental water bodies in Europe, exhibit morphological differences compared to the Baltic fourhorn sculpin. Berg (1916) used these differences as taxonomic characters when describing morphs, which were later raised to the rank of subspecies.

*Myoxocephalus qadricornis lonnbergi* Berg, 1916 was described from Lake Ladoga. In this species, the bony outgrowths on the head are weakly expressed; the eye diametre is 17.5–17.9% of the head length; the interorbital space is greater than or equal to the eye diametre; and the bony plates on the body are less developed compared to the Baltic form (Berg, 1916, 1949).

In 1916, Berg found two specimens of the fourhorn sculpin from Lake Onega in the collection of the Zoological Museum of the Imperial Academy of Sciences (currently ZIN) (ZIN Nos. 9026a and 3842). He identified them as *M. quadricornis relictus. Cottus quadricornis* var. *relicta* was described by Liljeborg (1891) from lakes Vättern and Vänern (Sweden). In "A review of the forms of Myoxocephalus quadricornis (L.)", published in 1932, Berg and Popov described a new subspecies, *Myoxocephalus quadricornis onegensis*, based on the two aforementioned specimens from Lake Onega. The following distinctive morphological characters were observed in the fish from Lake Onega: the absence of outgrowths on the head; the large eyes, with a longitudinal diametre measuring 24–26% of the head length; a flat interorbital space; and either the absence (No. 9026a) or vestigial presence (No. 3842) of bony plates on the body (Berg & Popov, 1932; Berg, 1949).

For a long time, the freshwater subspecies from Lakes Ladoga and Onega were considered distinct taxa. In 1997, M. Kottelat synonymised the subspecies *M. q. lonnbergi* and *M. q. onegensis* with the nominative subspecies, adopting the name *Triglopsis quadricornis quadricornis* (Kottelat, 1997). However, he emphasised the necessity for additional research on these lake forms to ascertain their taxonomic status.

Molecular genetic studies based on the nucleotide sequences of the mitochondrial *cytb* and *ATPase* 8+6 genes have shown that the haplotypes of *T. quadricornis* from the Baltic Sea and the Onega Lake system belong to the same clade, which is distinct from the haplotypes of individuals inhabiting the Arctic seas. The haplotypes of the Baltic marine form differ from those of Lake Onega by only one substitution in each genetic marker (Kontula & Väinolä, 2003). Additionally, the analysis of the sequences of the control region (mtDNA) and the *RAG1* gene (nDNA) revealed that the Baltic and Ladoga forms also belong to a single isolated clade (Sideleva & Zhidkov, 2022).

The analysis of previous studies clearly indicates significant morphological differences among the Baltic, Ladoga and Onega forms. Meanwhile, initial fragmentary genetic data reveal similarities among these three forms. The discordance between morphological and molecular genetic data creates taxonomic uncertainty, as the degree of divergence among the studied forms of *T. quadricornis* remains unclear.

The aim of this study is to determine the taxonomic status of the Baltic, Ladoga and Onega forms of *T. quadricornis* through a comparative analysis of their morphological and molecular genetic differences.

# Material and methods

*Material*. The material for this study were collected between 2020 and 2023. Specimens of *T. quadricornis* were sampled from Lakes Ladoga and Onega, as well as from the Gulf of Finland in the Baltic Sea. In the Gulf of Finland, fish were

captured using gill nets at depths ranging from 24 to 31 metres. In Lake Ladoga, bottom trawls were used to catch fish at depths of 30 to 35 metres. A trammel net was utilised for sampling fish in Lake Onega. The captured fish were placed in a jar containing water and clove oil as an anesthetic. Subsequently, the samples were preserved in 96% ethanol. All material is stored in the ichthyological collection of the Zoological Institute of the Russian Academy of Sciences, St Petersburg, Russia (ZIN). In addition to the captured fish, historical fund collections of ZIN, as well as photographs and X-rays from the Swedish Museum of Natural History, Stockholm, Sweden (NRM), were examined. A total of 111 specimens of T. quadricornis were analysed, comprising 13 specimens from the Baltic Sea, 68 specimens from Lake Ladoga, and 30 specimens from Lake Onega.

The following material have been examined. **Baltic Sea:** 

*Cottus quadricornis*, NRM 2491, syntype, Baltic Sea, without data about place, date and collector;

Triglopsis quadricornis: ZIN 48658, 1 specimen, Gulf of Finland, Luga Bay, near Novye Ruchyi Vill., depth 15 m, 2 Apr. 1988, coll. G.P. Rudenko; ZIN 56885, 3 specimens, Gulf of Finland, Samoed Bank, 15 km from Kurgalskiv Peninsula towards Seskar I., depth 24-31 m, 1 Apr. 2022, coll. V. Morev; ZIN 37824, 1 specimen, Gulf of Finland, near Zelenogorsk, 28 March 1965, coll. A.V. Neelov; ZIN 1496, 1 specimen, Baltic Sea, Revel Bay (Tallinn Bay), 1856, coll. V. Zatsepin; ZIN 14125, 2 specimens, Baltic Sea, Aland Is., Svibyviken Bay, near Mariehamn, Kagno I., 31 July 1907, coll. S. Pavlovich; ZIN 26934, 1 specimen, Baltic Sea, Aland Is., near Mariehamn, near Lokskar I., 20 July 1907, coll. S. Pavlovich; ZIN 26936, 3 specimens, Baltic Sea, Aland Is., Svibyviken Bay, near Mariehamn, near Lagneskar I., 17 Aug. 1897, coll. S. Pavlovich; ZIN 26935, 1 specimen, Aland Is., Svibvviken Bay, near Mariehamn, near Lagneskar I., depth 6-7 m, 7 July 1897, coll. S. Pavlovich.

#### Lake Ladoga:

*Myoxocephalus quadricornis lonnbergi*, ZIN 20691, holotype, 45 km north from Konevets I., 26 July 1905, coll. A.S. Skorikov;

*Triglopsis quadricornis*: ZIN 56996, 22 specimens, near Morye Vill., depth 30–35 m, bottom trawl, 2 Dec. 2021, coll. I. Tyrkin; ZIN 56993, 30 specimens, near Nikulyasy, depth 24–26 m, bottom trawl, start of trawling 60°25.01N 31°03.139E, end of trawling 60°27.789N 30°59.113E, 6 Sept. 2000, coll. A.A. Teslya; ZIN 56994, 8 specimens, near Nikulyasy, depth

26–36 m, bottom trawl, start of trawling 60°27.447N 31°02.125E, end of trawling 60°32.015N 31°09.869E, 7 Nov., 2000, coll. A.A. Teslya; ZIN 56005, 7 specimens, depth 25–35 m, bottom trawl, start of trawling 60°28.662N 30°59.882E, end of trawling 60°30.981N 31°07.633E, 19 Nov. 2000, coll. A.A. Teslya.

#### Lake Onega:

*Myoxocephalus quadricornis onegensis*, ZIN 9026a, holotype, Petrozavodsk, 1887, coll. Günter; ZIN 3842, paratype, 1875, coll. I.S. Polyakov;

*Triglosis quadricornis*: ZIN 25535, 2 specimens, at Palselga Vill., 27 June 1935, coll. V. Egoshin & V. Pokrovsky; ZIN 57053, 8 specimens, Megostrov I., depth 6–28 m, stationary net, 16 Nov. 2022, coll. A.V. Detsko; ZIN 57052, 18 specimens, Megostrov I., depth 6–28 m, stationary net, 20 Nov. 2023, coll. A.V. Detsko & Z.V. Zhidkov.

Morphological methods. To establish morphological differences, we studied features of external morphology, relative body measurements (expressed as a percentage of standard length, or *SL*), and counting characters. A comprehensive set of 27 measurements was employed to analyse the body proportions of the Baltic, Ladoga and Onega forms. These measurements were conducted according to a scheme specifically developed for sculpins (Sideleva et al., 2015). To calculate the average values of the relative measurements and their standard deviations, we utilised Excel 2016 software (Microsoft).

The characters of the external morphology were examined, along with the topography of the sensory canals of the lateral line system, the size of the fontanelles in the bones, and the pores. Among the osteological features of T. quadricormis, the armament of the skull bones, bony outgrowths on the head, and plates on the body were analysed. For a comparative analysis of the three forms of the fourhorn sculpin, eight counts were assessed: the number of rays in five fins, the number of abdominal and caudal vertebrae, and the number of pairs of pleural ribs. For this purpose, digital X-ray images of the entire body were obtained for each specimen. Radiography was conducted using the PRDU X-ray unit (Eltekhmed, St Petersburg) in the Laboratory of Ichthyology of ZIN. To acquire three-dimensional images of the skull bones of the Baltic and lake forms, a Neoscan N80 microtomograph (NEOSCAN BVBA, Belgium) was employed with the following settings: Source Voltage = 58 kV; Source Current =  $68 \mu \text{A}$ ;

Camera Exposure = 127 ms; Filter = Al 0.25 mm; Rotation Step = 0.3°. The 3D reconstructions of the skull were generated using CTVox 3.3 software (Bruker, USA). Tomographic studies were conducted utilising the equipment available at the Core Facilities Centre "Taxon" at ZIN.

Statistical analysis of morphometric data. The study of variability in T. quadricornis was conducted using a set of 27 measurements. For these data, principal component analysis (PCA) based on the correlation matrix was performed using the STATISTICA 10 software package (StatSoft). The obtained principal components are considered as new uncorrelated characters. Most of the original morphometric characters exhibit high and positive factor loadings on the first principal component (PC1). This indicates that PC1 contributes the most to the overall variability and primarily accounts for the size differences among individuals. A scatterplot depicting the second and third components was created to illustrate the differences between samples that are not related to size variability.

To assess the statistical significance of differences among the three samples based on relative measurements (% SL), an analysis of variance (ANOVA) was conducted. This analysis was performed using STATISTICA 10 software.

Molecular genetic methods. For molecular genetic analysis, three specimens of *T. quadricornis* from the Baltic Sea (ZIN 56885), four specimens from Lake Ladoga (ZIN 56996), and three specimens from Lake Onega (ZIN 57053) were examined. As comparative material, tissues from the closely related species *T. thompsonii* (one specimen from Lake Michigan) and *Triglopsis* sp. (four specimens from the White Sea, Chupa Bay) from the genetic collection of the ZIN RAS were utilised.

DNA was isolated from fin-clip tissue samples (100–200 mg) fixed in 96% ethanol using the QIAamp DNA Mini Kit (Quiagen, Germany). The mtDNA control region (*CR*) was amplified using the primers L16638 and H1122 (Kocher et al., 1989), resulting in approximately 1000 bp amplicons that included fragments of flanking tRNA genes. The fragment of the mtDNA cytochrome *b* gene (*cytb*) was amplified using the GluDg and H16460 primers (Palumbi, 1996), resulting in approximately 1100 bp amplicons.

DNA amplification was performed using a BioRad C1000 Touch in a 16 µL reaction volume, which included  $1 \times$  buffer, 1.5 µM MgCl2, 10 µM of each primer, 0.2 µM of each dNTP, 1 µL of template DNA solution, and 1U of HS Tag polymerase (Evrogen, Moscow). The PCR conditions were as follows: for the CR, an initial denaturation step of three minutes at 95 °C was followed by 35 cycles consisting of denaturation at 95 °C for 20 seconds, primer annealing at 59.2 °C for one minute, DNA elongation at 72 °C for 60 seconds, and a final elongation step at 72 °C for ten minutes. For cyt b, the initial denaturation lasted five minutes at 95 °C, followed by 35 cycles of denaturation at 94 °C for 45 seconds, primer annealing at 48°C for one minute, DNA elongation at 72 °C for one minute and 30 seconds, and a final elongation step at 72 °C for five minutes. The PCR products were purified using ethanol and ammonium acetate (3 M) precipitation.

The isolation and amplification of DNA were conducted using the equipment at the Laboratory of Ichthyology of ZIN. Sequencing was performed by Beagle LLC, also located in St Petersburg.

The nucleotide sequences were manually edited and aligned using Geneious Prime 2021.1.1 (Kearse et al., 2012). Unique haplotypes were identified using DnaSP v6.12.03 (Rozas et al., 2017). Pairwise p-distances among different forms, species, and genera were calculated using MEGA X software (Kumar et al., 2018).

Both of the mtDNA markers (CR and *cytb*) were concatenated to construct the phylogenetic tree. The phylogenetic analysis was performed using Bayesian inference in Mr. Bayes 3.1.2 (Huelsenbeck & Ronquist, 2001) and the Maximum Likelihood (ML) method in IQ-TREE 1.6.12 (Nguyen et al., 2015). The optimal model of nucleotide substitution was determined using the algorithm implemented in IQ-TREE 1.6.12, based on the Bayesian Information Criterion (BIC). The best-fit models selected for each marker were HKY+F+G4 for *CR* and TN+F+G4 for *cytb*. The statistical reliability of the ML tree was assessed using the bootstrap method with 1,000 iterations. The Markov-chain Monte Carlo (MCMC) process was set for four chains to run simultaneously for 107 generations, with sampling trees at every 1,000 generations. The first 25% of the trees were discarded in the computation of the majority-rule consensus tree. Posterior probabilities were calculated by generating a 50% majority rule consensus tree from the remaining trees. The phylogenetic trees resulting from the ML and BI analyses were visualised using FigTree 1.4.4 software.

The inner group on the phylogenetic tree also included 15 species of cottoid fish of the subfamily Myoxocephalinae (see below). Nucleotide sequences for the following species were obtained from GenBank NCBI: Argyrocottus zanderi Herzenstein, 1892 [MT890585, MT890586]; Megalocottus platycephalus (Pallas, 1814) [MK936041, MK936042]; Microcottus sellaris (Gilbert, 1896) [MT909823]; Myoxocephalus aenaeus (Mitchill, 1814) [AY338277, AY338278]; M. brandtii (Steindachner, 1867) [JF290401, JF290403]; M. jaok (Cuvier, 1829) [MN162694, MN162695]; M. ochotensis Schmidt, 1929 [JF290409, JF290410]; M. octodecemspinosus (Mitchill, 1814) [AY338279]; M.polyacanthocephalus (Pallas, 1814) [MK621914, MK621915]; M. scorpius (Linnaeus, 1758) [MK321578, MT410889]; M. stelleri Tilesius, 1811 [AY338282, AB188174]; Porocottus allisi (Jordan et Starks, 1904) [MT906795, MT906796]; P. camtschaticus (Schmidt, 1916) [KM057908]; P. japonicus Schmidt, 1935 [MH172296]; P. minutus (Pallas, 1814) [MH172289]; and Triglopsis thompsonii [AY833369]. The species Taurulus bubalis (Euphrasen, 1786) [MT410868] was utilised as an outgroup for the phylogenetic analysis.

## Results

Morphological features of the Baltic, Ladoga and Onega forms. The comparative analysis of the external morphological features of T. quadricornis individuals (similar in size) revealed differences in the state of some characters. In the three studied forms, a sequential decrease of bony outgrowths on the head was observed (Figs 1, 2). The largest outgrowths, consisting of four protuberances with a needle-like structure, are found in the Baltic fourhorn sculpin (Figs 1A, 2A). In contrast, the Ladoga specimens exhibit weakly prominent outgrowths in the form of small tubercles (Figs 1B, 2B), while specimens from Lake Onega show an absence of outgrowths altogether (Figs 1C, 2C). Additionally, the preoperculum shows a gradual reduction in armament, characterised by a decrease in the size of the spines and a change in their orientation. In the specimens from the Baltic



Fig. 1. Photographs of type specimens: A, *Cottus quadricornis* Linnaeus, 1758, syntype, NRM 2491, Baltic Sea; B, *Myoxocephalus quadricornis lonnbergi* Berg, 1916, holotype, ZIN 20623, Lake Ladoga; C, *Myoxocephalus quadricornis onegensis* Berg et Popov, 1932, holotype, ZIN 9026a, Lake Onega. Scale bars: 1 cm.

Sea, all four preopercular spines are well developed. Among these, the upper spine is the longest, while the others gradually decrease in length from the upper to the lower spine (Fig. 2D). All spines are oriented almost perpendicular to the margin of the preoperculum. In the Ladoga form, the upper preopercular spine is longer than the others, but it is noticeably shorter than that of the Baltic fish (Fig. 2E). In the fourhorn sculpin from Lake Onega, the shape of the preopercular bone changes, becoming narrow and elongated. The upper spine is long, yet narrow and sharp, making it more delicate than those found in the Baltic and Ladoga forms (Fig. 2F). The two lower spines are significantly reduced and oriented forward. In the neurocranium, a gradual increase in the size of the orbit was observed (Figs 2A–2C). This increase is correlated with the measurements of the eye diametre (Fig. 3A). The Baltic form has small eyes, with an average eye diametre of 5.7% *SL* (range: 5.2-6.4%); the Ladoga form has an average of 6.7% *SL* (range: 6.0-7.0%); the specimens from Lake Onega have the largest eyes among the studied forms, measuring 8.1% *SL* (range: 7.2-10.0%) (Table 1).

According to Berg (1949), all known forms of T. quadricornis have bony dermal plates on the dorsal side of the trunk. Our observations showed that the number of these plates decreases in similarly sized individuals, following the same

| Table 1. Morphometric d | lata for <i>Triglopsis</i> | quadricornis | (Linnaeus, | 1758) from | the Baltic Sea, | Lake Ladoga a | nd |
|-------------------------|----------------------------|--------------|------------|------------|-----------------|---------------|----|
| Lake Onega.             |                            |              |            |            |                 |               |    |

| Maaaaaa                                    | Baltic Se   | ea (n = 8)        | Lake Lado   | ga (n = 16)    | Lake Onega (n = 22) |               |  |
|--|-------------|-------------------|-------------|----------------|---------------------|---------------|--|
| Measurements                               | min–max     | nin-max mean ± SD |             | mean ± SD      | min–max             | mean ± SD     |  |
| <i>SL</i> , mm                             | 127.3-189.0 | 156.9             | 108.7-151.2 | 127.8          | 77.0-116.6          | 89.5          |  |
| %SL  |             |                   |             |                |                     |               |  |
| Predorsal length                           | 36.8-40.1   | $38.1\pm1.2$      | 35.1-40.3   | $38.0\pm1.3$   | 34.7-43.4           | $37.2\pm1.6$  |  |
| Postdorsal length                          | 15.3–17.6   | $16.7\pm0.8$      | 14.9–18.6   | $17.0\pm1.1$   | 15.2 - 20.2         | $16.9\pm1.2$  |  |
| Preanal length                             | 54.6-59.0   | $57.5\pm1.4$      | 52.5-60.1   | $56.3 \pm 1.8$ | 49.8-67.6           | $54.8\pm3.5$  |  |
| Postanal length                            | 41.5-46.1   | $43.3\pm1.3$      | 38.2-46.4   | $43.8\pm1.9$   | 44.0-49.7           | $46.5\pm1.5$  |  |
| Maximum body depth                         | 17.8-23.2   | $19.7\pm1.8$      | 16.0-19.3   | $17.9 \pm 1.3$ | 15.9-21.0           | $19.2\pm1.4$  |  |
| Length of caudal peduncle                  | 15.6–17.9   | $16.5\pm0.8$      | 13.7–19.9   | $16.8\pm1.4$   | 14.3-19.7           | $16.7\pm1.2$  |  |
| Depth of caudal peduncle                   | 4.1-5.4     | $4.9\pm0.5$       | 4.0-5.3     | $4.6\pm0.3$    | 4.7 - 6.2           | $5.2\pm0.4$   |  |
| Length of 1st dorsal-fin base              | 17.2-23.2   | $19.5\pm2.2$      | 14.4-20.2   | $17.8\pm1.5$   | 14.1-18.9           | $16.7\pm1.3$  |  |
| Length of 2 <sup>n</sup> d dorsal-fin base | 21.2-24.4   | $23.2\pm0.9$      | 18.8–25.1   | $22.8\pm1.8$   | 21.9-29.2           | $25.5\pm1.9$  |  |
| Length of anal-fin base                    | 22.3-25.2   | $23.9 \pm 1.0$    | 21.4-26.5   | $23.6\pm1.5$   | 23.7 - 28.7         | $26.5\pm1.2$  |  |
| Length of longest 1st dorsal-fin ray       | 8.4–13.8    | $10.4\pm1.6$      | 8.6-13.2    | $10.5\pm1.3$   | 7.9–13.9            | $10.2\pm1.6$  |  |
| Length of longest 2nd dorsal-fin ray       | 15.9–20.5   | $17.2\pm1.5$      | 15.2-29.0   | $18.4\pm4.4$   | 13.7-24.1           | $18.3\pm3.7$  |  |
| Length of longest anal-fin ray             | 11.8–13.9   | $13.1\pm0.7$      | 8.5-14.3    | $11.6\pm1.4$   | 9.4-13.8            | $11.7\pm1.3$  |  |
| Pectoral fin length                        | 26.0-31.2   | $28.0\pm1.6$      | 25.4-29.8   | $27.6\pm1.5$   | 26.4 - 33.5         | $29.8\pm2.1$  |  |
| Pelvic fin length                          | 16.6-22.5   | $18.5\pm1.8$      | 11.0-22.3   | $16.4\pm2.7$   | 10.1-17.1           | $13.9\pm1.9$  |  |
| Distance between 1st and 2nd dorsal fins   | 5.1-10.1    | $7.4\pm2.1$       | 5.0-10.8    | $7.2\pm1.7$    | 4.4-9.6             | $7.1 \pm 1.5$ |  |
| Head length (HL)                           | 35.7-37.6   | $36.5\pm0.7$      | 33.0-36.8   | $34.3\pm1.1$   | 33.4 - 40.4         | $35.1\pm1.4$  |  |
| Postorbital length                         | 19.6-21.4   | $20.6\pm0.7$      | 16.5–19.5   | $17.9\pm0.9$   | 16.8-20.6           | $18.0\pm0.8$  |  |
| Head depth                                 | 17.0-20.4   | $18.4\pm1.2$      | 15.6-18.4   | $16.9\pm0.9$   | 14.5 - 18.7         | $16.8\pm1.3$  |  |
| Head width                                 | 28.2-35.9   | $31.6\pm2.8$      | 25.6-35.3   | $30.7\pm3.2$   | 26.9 - 34.9         | $31.1\pm2.4$  |  |
| Eye horizontal diameter                    | 5.2 - 6.4   | $5.7\pm0.4$       | 6.0-7.0     | $6.7\pm0.3$    | 7.2-10.0            | $8.1\pm0.6$   |  |
| Snout length                               | 9.5–11.6    | $10.4\pm0.7$      | 8.8-11.6    | $10.1\pm0.8$   | 8.8-10.6            | $9.7\pm0.5$   |  |
| Interorbital width                         | 5.1-6.2     | $5.5\pm0.4$       | 4.5-5.4     | $5.0\pm0.3$    | 2.9 - 4.5           | $3.6\pm0.4$   |  |
| Upper jaw length                           | 16.5-18.8   | $17.6\pm0.8$      | 15.7–17.9   | $16.9\pm0.7$   | 15.4-18.3           | $16.9\pm0.6$  |  |
| Length of gill slit                        | 20.8-23.9   | $22.1\pm1.1$      | 19.3-23.0   | $20.8\pm1.0$   | 17.8 - 24.0         | $20.4\pm1.2$  |  |
| Length of upper spine on preoperculum      | 4.6-6.2     | $5.6\pm0.5$       | 3.3–5.6     | $4.6\pm0.5$    | 3.9–5.5             | $4.5\pm0.7$   |  |

pattern as the other aforementioned characters. The highest number of bony plates, ranging from 39 to 55 with an average of 47, is observed in the individuals from the Baltic Sea (Fig. 4). In contrast, *T. quadricornis* from Lake Ladoga exhibits significant variation in the number of plates, ranging from 20 to 41, with an average of 30, which is lower than that of the Baltic form. The fewest bony plates were found in the Onega form, where 11% of the examined individuals lacking any bony

plates altogether. Among those individuals that do possess these plates, the quantity ranges from 1 to 15, with an average of 5 (Fig. 4).

In addition to morphological (osteological) changes, the freshwater forms show a gradual decrease in the body size. The average standard length of sexually mature individuals in our samples is as follows: 156.9 mm for the fourhorn sculpin from the Baltic Sea, 127.8 mm for the Ladoga form, and 89.5 mm for the specimens from Lake



Fig. 2. Tomograms of the cranium and preoperculum of *Triglopsis quadricornis* (Linnaeus, 1758) from: Baltic Sea, ZIN 26934 (A, D); Lake Ladoga, ZIN 56996 (B, E); and Lake Onega, ZIN 57053 (C, F).

Onega. A process of gradual miniaturisation occurred during the colonisation of freshwater lakes by marine fish, as previously shown by Kullander (1999).

From meristic characters, the number of rays in four fins (the first and second dorsal fins, anal fin, and pectoral fin) was examined (Table 2, Fig. 5). The numbers of rays in all fins does not differ between the marine and freshwater forms. The only minor difference observed is in the modal number of rays in the second dorsal fin. All specimens of the Baltic and Ladoga fourhorn sculpin exhibit either 13 or 14 rays in this fin, with nearly equal proportions. In Lake Onega, specimens with 14 rays are predominant (82%), while only one specimen has 13 rays in the second dorsal fin (Table 2). Small differences were observed in the variation of the number of rays, ranging from two to four states of the character. In general, meristic characters of the marine and freshwater forms have considerably more similarities than differences.

Data on the variability of 27 morphometric characters are presented in Table 1. A comparative analysis of the data revealed that the majority

**Table 2.** Counts of fin rays in *Triglopsis quadricornis* (Linnaeus, 1758) from the Baltic Sea, Lake Ladoga and LakeOnega. The modal values are indicated in bold font.

| T a colitar |    | DI |   |    |   | DII |    |    | Α  |    |    | Р  |    |    |    |    |    |
|-------------|----|----|---|----|---|-----|----|----|----|----|----|----|----|----|----|----|----|
| Locality    | n  | 6  | 7 | 8  | 9 | 10  | 12 | 13 | 14 | 15 | 12 | 13 | 14 | 15 | 15 | 16 | 17 |
| Baltic Sea  | 14 | 1  | 4 | 6  | 3 |     |    | 8  | 6  |    |    | 2  | 9  | 3  | 4  | 10 |    |
| Lake Ladoga | 22 |    | 4 | 14 | 3 | 1   | 3  | 9  | 10 |    | 1  | 4  | 16 | 1  | 6  | 13 | 3  |
| Lake Onega  | 22 | 1  | 1 | 14 | 6 |     |    | 1  | 18 | 3  |    | 10 | 12 |    | 5  | 17 |    |



**Fig. 3.** Scatter plots showing the relationships between eye horizontal diametre (**A**), length of anal-fin base (**B**), interorbital width (**C**), length of upper spine on preoperculum (**D**), and *SL* in *Triglopsis quadricornis* (Linnaeus, 1758) from the Baltic Sea, Lake Ladoga and Lake Onega.

of relative measurements (85%) exhibit similar or very close values. However, four features showed a consistent and noticeable change in their values. Notably, of these characters, the length of the anal fin base is significant (Fig. 3B): the Baltic and Ladoga forms have shorter anal fin bases compared to the Onega form (mean lengths of 23.9% and 23.6% *SL vs.* 26.5% *SL*). This difference is statistically significant (p < 0.05). Additionally, a gradual increase is observed in relative eye diametre (Fig. 3A): 5.7% *SL* in *T. quadricornis* from the Baltic, 6.7% *SL* in the Ladoga individuals, and 8.1% *SL* in the form from Lake Onega. A gradual decrease in size was recorded for the interorbital space (Fig. 3C) and the length of the upper preopercular spine (Fig. 3D). The interorbital space



**Fig. 4.** Number of bony plates in upper dorsal row in three forms of *Triglopsis quadricornis* (Linnaeus, 1758) from the Baltic Sea, Lake Ladoga and Lake Onega. Vertical bars indicate ranges from minimum to maximum values. The average values are connected by a red line.

equal to the eye diametre (5.5% vs. 5.7% SL) was found in the Baltic form; the interorbital space slightly smaller than the eye diametre (5.0% vs. 6.7% SL) was observed in the Ladoga form, and the interorbital space considerably smaller than the eye diametre (3.6% vs. 8.1% SL) was found in the fish from Lake Onega. The upper spine on the preoperculum is the longest in the Baltic fourhorn sculpin, with a mean length of 5.6% SL; in both freshwater lake forms, its length measures 4.5-4.6% SL. In addition to the four characters listed above, a consistent decrease in values was also observed for the length of the first dorsal-fin base, pelvic fin length, and preanal length (Table 1). The range of variation observed in these features across all samples is substantial, which limits the ability to draw definitive conclusions.

Among the morphological characters examined, sexual dimorphism was observed for some of them. It was found that males of the fourhorn sculpin from Lake Onega have higher second dorsal fins ( $21.5 \pm 2.1\%$  vs.  $15.6 \pm 2.0\%$  SL) and anal fins ( $12.9 \pm 0.6\%$  vs.  $10.7 \pm 0.7\%$  SL), as well as longer pectoral fins ( $31.3 \pm 1.5\%$  vs.  $28.6 \pm 1.8\%$  SL) and pelvic fins ( $15.2 \pm 1.1\%$  vs.  $12.8 \pm 1.5\%$  SL) compared to females. For subsequent multivariate analysis, these characters were excluded due to significant intrasample variability resulting from sexual dimorphism.

Principal component analysis (PCA) was used for the comparative study of morphometric characters in the marine and two lake forms of T. quadricornis. The results of the PCA are illustrated in a scatter diagram that plots the second and third principal components (Fig. 6). Each component represents the proportion of variability in the correlation matrix of the characters. The first principal component accounts for 92.6% of the total variability in morphometric characters among the three studied forms of T. quadricornis from the Baltic Sea, Lake Ladoga and Lake Onega. This indicates that the differences among specimens are primarily influenced by their individual body sizes. The first principal component is characterised by high positive factor loadings, ranging from 0.726 to 0.996, with a mean of 0.960. The second and third principal components account for 4.5% of the total variability (PC2: 3.37%, PC3: 1.17%). The two features with the highest loadings on PC2 and PC3 are the eye diametre (0.375 and 0.395, respectively) and the distance between the first and second dorsal fins (0.643 and -0.178, respectively). The polygons presented in Fig. 6 represent the morphospaces of the studied samples along the PC2 and PC3 axes. The morphospaces of the three samples of T. quadricornis significantly overlap, indicating that there are no clear differences in body shape between the marine and lake forms of the fourhorn sculpin.

Despite the miniaturisation of the Ladoga and, especially, the Onega forms of *T. quadricornis*, the morphometric characters of the freshwater specimens are similar to those of the individuals from the Baltic Sea. The absence of well-defined morphometric differentiation further supports the conclusion that all studied forms belong to a single nominal subspecies, *T. quadricornis quadricornis*.



Fig. 5. X-ray images of the axial skeleton of type specimens: (A) *Cottus quadricornis* Linnaeus, 1758, syntype, NRM 2491, Baltic Sea; (B) *Myoxocephalus quadricornis lonnbergi* Berg, 1916, holotype, ZIN 20623, Lake Ladoga; (C) *Myoxocephalus quadricornis onegensis* Berg et Popov, 1932, holotype, ZIN 9026a, Lake Onega.

Genetic differentiation of the Baltic, Ladoga and Onega forms. To assess the genetic differentiation among the three forms of T. quadricornis, the sequences of two mitochondrial markers were studied: a polymorphic control region of mtDNA (CR, 849 bp) and the more conservative cvtochrome b gene (cytb, 1129 bp). The CR sequences of T. quadricornis from the Baltic Sea and lakes had a single site with indel (pos. 740). The phylogenetic tree reconstructed using the combined sequences (Fig. 7) shows that all representatives of the genus Triglopsis belong to a well-supported single clade. This finding supports the monophyly of the genus Triglopsis, and its distinct separation from representatives of the genus Myoxocephalus. The average p-distance between these two genera was calculated to be  $9.4 \pm 0.6\%$ .

The haplotypes of *T. quadricornis* from the Baltic Sea, Lake Ladoga and Lake Onega are closely

clustered on the phylogenetic tree. They are distinctly separated from the Arctic clade (p-distance  $0.60 \pm 0.14\%$ ), which includes haplotypes of Triglopsis sp. from the White Sea, and from the haplotypes of Triglopsis thompsonii Girard, 1851, a freshwater species from the American Great Lakes (p-distance  $0.81 \pm 0.18\%$ ). Genetic differentiation among the forms of T. quadricornis from the Baltic Sea, Lake Ladoga and Lake Onega is lower than that observed between species (Table 3). The Baltic and Ladoga forms are the most closely related, exhibiting the smallest genetic distance between them (0.05  $\pm$  0.03%). Triglopsis quadricornis from Lake Onega is more genetically distinct from the Baltic form, with a p-distance of  $0.38 \pm 0.12\%$ . Furthermore, three haplotypes of the Onega fourhorn sculpin formed a fairly well-supported subclade on the phylogenetic tree. This finding further confirms the greater genetic isolation of



**Fig. 6.** Result of PCA carried out using morphometric characters for *Triglopsis quadricornis* (Linnaeus, 1758) from the Baltic Sea, Lake Ladoga and Lake Onega. *PC2* and *PC3* – second and third principal components.

the fish from Lake Onega compared to the other two studied forms.

The data obtained show different levels of genetic distances among: (1) *T. quadricornis* from the Baltic Sea and other species of the genus *Triglopsis* (0.60% and 0.81%), and (2) individuals from the Baltic Sea, Lake Ladoga and Lake Onega (ranging from 0.05% to 0.38%). These distance values

indicate a close genetic affinity between the marine and freshwater forms of *T. quadricornis*. The level of divergence among the Baltic, Ladoga and Onega forms is 1.5 to 16 times lower than that observed between species within the genus *Triglopsis*. Based on the genetic findings, the forms from Lake Ladoga and Lake Onega belong to the nominative subspecies *T. quadricornis quadricornis*.

**Table 3.** Average p-distances (%) with standard error estimatescalculated using combined dataset (CR + cytb, 1978 bp).

| Species (Locality)                | [1]             | [2]       | [3] |
|-----------------------------------|-----------------|-----------|-----|
| T. quadricornis (Baltic Sea) [1]  | X               |           |     |
| T. quadricornis (Lake Ladoga) [2] | $0.05 \pm 0.03$ | X         |     |
| T. quadricornis (Lake Onega) [3]  | 0.38±0.12       | 0.36±0.11 | x   |

#### Conclusion

The process of colonisation of Lake Ladoga and Lake Onega by the fourhorn sculpin from the Baltic Sea was accompanied by significant morphological transformations associated with a reduction in skeletal mass. This is accomplished through the thinning of skull bones and the partial or complete reduction of post-



**Fig. 7.** Bayesian phylogenetic tree of the subfamily Myoxocephalinae reconstructed using *CR* and *cytb* (mtDNA) sequences. The topologies of the BI and ML trees were generally concordant. For each node, the values of the posterior probability (left) and the bootstrap support (right) are indicated.

cranial dermal bony elements. The massive bony outgrowths characteristic of the skull in the Baltic form are diminished to small tubercles or may be entirely absent in the freshwater forms. The number of bony plates on the trunk is reduced. Alongside these osteological changes, body proportions are altered: the lake forms have smaller body sizes and larger eye diametres. Morphological structures undergo these transformations in a sequential manner. The largest sizes are characteristic of the fish from the Baltic, medium sizes are observed in the Ladoga form, and the smallest sizes are found in the Onega form. Due to the

Zoosystematica Rossica, Vol. 33, No. 2, pp. 293–307

gradual nature of these changes, it is not possible to assign diagnostic features that would distinguish the lake forms from the marine individuals.

Genetic differences between the haplotypes (cytb + CR) of the fish from the Baltic Sea, Lake Ladoga and Lake Onega are minimal. The average p-distances are 0.05% and 0.38%, respectively. These values of genetic divergence are characteristic of intraspecific forms of cottoid fishes.

The data obtained clearly indicate that both marine and freshwater forms belong to the nominative subspecies *T. q. quadricornis*. However, the freshwater forms exhibit varying degrees of differentiation from the original Baltic form. The fourhorn sculpin from Lake Ladoga is most similar to the fish from the Baltic Sea, while the Onega form has a more advanced state of characters and is more isolated from the Baltic form. Certain morphological characters, such as the absence of bony outgrowths on the head, larger eye size, and a reduced number or complete absence of bony plates on the body, exceed the level of intraspecific differences. A comparative analysis of the morphological characters of the "extreme" forms (Baltic vs. Onega) reveals differences at the species level, which could result in the erroneous description of new taxa. However, between the "extremes" there is the intermediate freshwater Ladoga form. The study of this fish has shown that changes in morphological characters are sequential and gradual, with no discrete differences. Molecular genetic data also show a very low level of differentiation among the lake forms of T. quadricornis. The genetic differences are comparable to those found between distinct populations of the same species. This suggests that taxonomic diversification has not yet occurred across different lake systems.

*Triglopsis quadricornis* is an excellent example of the significant morphological differences that can arise between marine and freshwater forms of the same species (Kullander, 1999). In these instances, relying solely on morphological differences is inadequate for drawing taxonomic conclusions; instead, an integrative approach is essential.

#### Acknowledgments

The authors would like to express their gratitude to Bo Delling (Swedish Museum of Natural History, Stockholm) for providing the photographs and X-ray images of the type specimen of *Cottus quadricornis*; Viktoria Nikiforova (ZIN RAS, St Petersburg) for her assistance with the tomography of sculpins; and Andrei Detsko and Alexandr Khristenko (Petrozavodsk) for their support in the collection of sculpins from Lake Onega. This study was supported by the Russian Science Foundation grant No. 23-24-00406.

# References

Berg L.S. 1916. Ryby presnykh vod Rossiyskoy Imperii [The fishes of fresh waters of the Russian Empire]. Moscow: Publishing House of the Department of Agriculture. 563 p. (In Russian).

- Berg L.S. 1923. *Ryby presnykh vod Rossii* [Freshwater fishes of Russia]. Second edition. Moscow: State Publishing House. 535 p. (In Russian).
- Berg L.S. 1949. Ryby presnykh vod SSSR i sopredel'nykh stran [Freshwater fishes of the USSR and adjacent countries], 3. Moscow – Leningrad: Academy of Sciences of USSR. 452 p. (In Russian).
- Berg L.S. & Popov A.M. 1932. A review of the forms of Myoxocephalus quadricornis (L.). *Doklady Akademii Nauk SSSR*, 1932: 152–160.
- Fricke R., Eschmeyer W.N. & Van der Laan R. 2024. Eschmeyer's catalog of fishes: genera, species, references [online]. San Francisco: California Academy of Sciences. http://researcharchive.calacademy. org/research/ichthyology/catalog/fishcatmain.asp [viewed 10 July 2024].
- **Gratzianow V.I.** 1907. The experience of reviewing the fish of the Russian Empire in systematic and geographical aspects. *Trudy Otdela ikhtiologii Russkogo obshchestva akklimatizatsii zhivotnykh i rasteniy*, 4: 1–567. (In Russian).
- Huelsenbeck J.P. & Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8): 754–755. https://doi.org/10.1093/ bioinformatics/17.8.754
- Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P. & Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199
- Kocher T.D., Thomas W.K., Meyer A., Edwards S.V., Pääbo S., Villablanca F.X. & Wilson A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, 86(16): 6196–6200. https://doi. org/10.1073/pnas.86.16.6196
- Kontula T. & Väinölä R. 2003. Relationships of Palearctic and Nearctic 'glacial relict' Myoxocephalus sculpins from mitochondrial DNA data. *Molecular Ecology*, 12: 3179–3184. http://doi. org/10.1046/j.1365-294X.2003.01963.x
- Kottelat M. 1997. European freshwater fishes. *Biologia (Bratislava)*, **52**(suppl. 5): 1–271.
- Kudersky L.A. & Lotarev V.A. 1964. Finding of the Onega fourhorn sculpin (Myoxocephalus quadricornis onegensis Berg et Popov) in a small lake of the Onego-Segozersky Isthmus. *Rybnoe Khozyaystvo Karelii*, 8: 210–214. (In Russian).
- Kullander S.O. 1999. Fish species how and why. Reviews in Fish Biology and Fisheries, 9: 325–352. https://doi.org/10.1023/A:1008959313491

- Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, **35**(6): 1547–1549. https://doi. org/10.1093/molbev/msy096
- Lilljeborg W. 1891. *Sveriges och Norges fauna* [The fauna of Sweden and Norway], **1**. Uppsala: Schulz. i-xxi + 782 p. (In Swedish).
- Lönnberg E. 1919. Några ord om Vänerns hornsimpa [A few words about fourhorn sculpin in Vänern Lake]. *Fauna och Flora (Uppsala)* [Fauna and Flora (Uppsala)], 14(5): 163–172. (In Swedish).
- Neelov A.V. 1979. Seysmosensornaya sistema i klassifikatsiya kerchakovykh ryb (Cottidae: Myoxocephalinae, Artediellinae) [The seismosensory system and classification of cottoid fishes (Cottidae: Myoxocephalinae, Artediellinae)]. Leningrad: Nauka. 208 p. (In Russian).
- Nguyen L.T., Schmidt H.A., Von Haeseler A. & Minh B.Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximumlikelihood phylogenies. *Molecular Biology and Evolution*, **32**(1): 268–274. https://doi.org/10.1093/ molbev/msu300
- Palumbi S.R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's

tale. Journal of experimental marine Biology and Ecology, **203**: 75–92. https://doi.org/10.1016/0022-0981(96)02571-3

- Rozas J., Ferrer-Mata A., Sánchez-DelBarrio J.C., Guirao-Rico S., Librado P., Ramos-Onsins S.E. & Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, 34(12): 3299– 3302. https://doi.org/10.1093/molbev/msx248
- Segerstråle S. 1956. The distribution of glacial relicts in Finland and adjacent Russian areas. *Societas scientiarum Fennica*, 15(18): 1–35.
- Sideleva V.G., Prirodina V.P., Reshetnikov Y.S. & Zhidkov Z.V. 2015. Redescription of Cottus koshewnikowi (Cottidae) and its morphological variability in tributaries of the Upper Volga. *Journal of Ichthyology*, 55(1): 30–39. https://doi.org/10.1134/ S0032945215010191
- Sideleva V. & Zhidkov Z. 2022. Evaluation of differences between the Baltic Triglopsis quadricornis and White Sea Triglopsis sp. using morphological and genetic data. *Diversity*, 14(11): 983. https://doi. org/10.3390/d14110983
- Tilesius W.G. 1811. Piscium Camtschaticorum descriptiones et icones. Mémoires de l'Académie impériale des sciences de St.-Pétersbourg, 3: 225–285.

Received 22 July 2024 / Accepted 29 November 2024. Editorial responsibility: D.A. Gapon