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A new species of the genus *Cottus* (Scorpaeniformes, Cottidae) from the Baltic Sea Basin and its phylogenetic placement

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Abstract. The genus *Cottus* contains more than 60 species that are common in freshwater bodies of northern Eurasia and North America. Despite the abundance of species, this genus has been insufficiently studied in Eastern European rivers and lakes. The new taxon *Cottus cyclophthalmus* sp. nov. was found in the Neman/Nemunus and Venta river systems (Baltic Sea Basin). *Cottus cyclophthalmus* is sister and most morphologically similar to *Cottus gobio*. The new species has the following diagnostic characters: round, protruding (tubular) eyes near front of head; dermal papillae on top and sides of head, naked body, absence of bony prickles, full trunk canal with 32–36 pores. The description of *Cottus cyclophthalmus* and a new finding of *Cottus microstomus* improved understanding of the Eastern European biodiversity.

Keywords. Mitochondrial DNA, morphology, multivariate analysis, Neman, phylogenetic relationships, sculpins.

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Introduction

Freshwater sculpins of the genus *Cottus* Linnaeus, 1758 distributed in freshwaters of Europe belong to two species groups: the Western European species group “*Cottus gobio*” and the Eastern European group “*Cottus koshewnikowi*” (Sideleva *et al.* 2019).

Molecular genetic study of the mtDNA sequences (control region and cytochrome b) revealed the existence of three phylogenetic lineages: a W lineage occurs to the west of the Baltic Sea, a SE lineage to the south and southeast of it, an E lineage is found to the east and northeast of the Baltic Sea (Kontula &

Väinölä 2001). In our opinion, the W lineage corresponds to the “*Cottus gobio*” species group, the E lineage corresponds to the “*Cottus koshewnikowi*” species group, the SE lineage from South Estonia is *Cottus* sp. These results support the presence of two species groups (“*Cottus gobio*” and “*Cottus koshewnikowi*”) identified using morphological characters (length of the trunk canal, number and distribution of spines on the body) (Witkowski 1979).

The “*Cottus gobio*” species group consists of 12 species: *Cottus gobio* Linnaeus, 1758, *C. aturi* Freyhof, Kottelat & Nolte, 2005, *C. haemusi* Marinov & Dikov, 1986, *C. hispaniolensis* Băcescu & Băcescu-Meşter, 1964, *C. metae* Freyhof, Kottelat & Nolte 2005, *C. microstomus* Heckel, 1837, *C. perifretum* Freyhof, Kottelat & Nolte, 2005, *C. petiti* Băcescu & Băcescu-Meşter, 1964, *C. rhenanus* Freyhof, Kottelat & Nolte, 2005, *C. rondeleti* Freyhof, Kottelat & Nolte, 2005, *C. sabaudicus* Sideleva, 2009, and *C. transsilvaniae* Freyhof, Kottelat & Nolte, 2005 (Freyhof *et al.* 2005; Sideleva 2009; Bravničar *et al.* 2020). All species of the “*Cottus gobio*” group have common morphological features that distinguish them from the species group “*Cottus koshewnikowi*” (absence or small number of bony prickles on the body and a full trunk canal).

The species group “*Cottus koshewnikowi*” is represented by two species: *C. koshewnikowi* Gratzianov, 1907 and *C. gratzianowi* Sideleva, Naseka & Zhidkov, 2015 (Sideleva *et al.* 2015a, 2015b). This group of species has such morphological features as the presence of bony prickles on the body and a short (incomplete) trunk canal.

The sculpins of each species group are distributed in different parts of Europe (Freyhof *et al.* 2005; Sideleva *et al.* 2015a). The representatives of the “*Cottus gobio*” group are found from the Pyrenees in the west to the Dniester River in the east. The species of the “*Cottus koshewnikowi*” group are distributed in Eastern Europe from Finland and the Western Dvina (Daugava) River basin in the west to the Urals in the east.

The Dniester River (Black Sea Basin) can be considered a conventional faunistic boundary between the species groups “*Cottus gobio*” and “*Cottus koshewnikowi*”. However, to the north of the source of the Dniester River, there are large rivers and river systems whose faunas include sculpins with an unclear taxonomic status. These rivers include the Neman/Nemunas and Venta with their tributaries and the Krasnaya River. The mentioned river systems belong to the Baltic Sea Basin.

The fact that these rivers are inhabited by sculpins is known from the publications of Zograf (1907), Zhukov (1958), Alekseev & Probatov (1969), and Tylik & Shibaev (2008). The sculpins have been identified as *C. gobio*. In 2019, specimens of sculpins were caught in the Nemunas/Neman and Venta river systems (including the Krasnaya River) and morphological and molecular genetic studies were carried out. These studies revealed the presence in these rivers of the species *C. microstomus* and of a new form different from the known species of the genus *Cottus*.

Material and methods

Study area and sampling

For this study, sculpins were collected in August 2019 in three tributaries of the Neman River: Neris, Žeimena, and Siesartis (Lithuania). In addition, specimens were caught in the Krasnaya River connected to the Neman by an artificial canal (Kaliningrad Region, Russia). In the Venta River system, samples were collected in its small tributary Šerkšnė River (Lithuania) (Fig. 1). The coordinates of each sampling site are presented in the description of the new species.

The sculpins were caught using a hand net and a battery-powered electric fishing gear (HANS GRASSL GmbH, model IG200/2). The fish were anesthetized with clove oil and then fixed in 96% ethanol. For

morphological and DNA studies, 134 specimens were used. All fish specimens, their tissues and DNA are kept in the ichthyological collection of the Zoological Institute of the Russian Academy of Sciences (ZIN) in St. Petersburg.

For comparative morphological studies, we used sculpins (*C. microstomus*, *C. koshevníkowi*, and *C. gobio*) from the collections of the Zoological Institute of the Russian Academy of Sciences (ZIN) and the Finnish Museum of Natural History (MZP):

Cottus microstomus from the Vistula River system: ZIN 56393, n = 10, San River at Średnia Wieś (49°25'55.6" N, 22°19'65.7" E), 10 Sept. 2012; ZIN 56330, n = 8, Mierzawa R., tributary of the Nida River, at Pawłowice, at Pińczów (50°30'32.2" N, 20°27'58.2" E), 29 Nov. 2013; ZIN 56394, n = 7, Czarny Dunajec at Nowy Targ (49°29'08.5" N, 20°00'53.5" E), 1 Oct. 2013;



Fig. 1. The map of sampling sites showing the distribution of *Cottus cyclophthalmus* sp. nov. The numbers indicate sampling sites in various rivers: 1. Krasnaya River. 2. Neris River. 3. Žeimena River. 4. Siesartis River. 5. Šerkšnė River. The star marks the type locality of the new species; the circles mark sampling sites of non-type specimens; the triangle marks the locality where specimen of *Cottus microstomus* sp. nov. was caught.

C. koshevníkowi from the Volga River system: ZIN 55582, n = 60, Oka River at Kaluga city (54°30' N, 36°14' E), July 2012;

C. gobio from the Weser River system: UK 1889, n = 18, Fulda River, Hessen, Germany, 1960.

In total, 237 specimens were included in this study. Topotypes for three species (*C. gobio*, *C. koshevníkowi*, and *C. metae*) have been studied morphologically and genetically. Selecting data from the GenBank NCBI for other species, we took into account the proximity of the collection site to the type habitat. Therefore, we did not take all the data available.

For molecular genetic analysis of mitochondrial DNA, the tissue samples of three species were used: *C. koshevníkowi* (10 specimens), *C. poecilopus* Heckel 1837 (4 specimens), and a new species *C. cyclophthalmus* (31 specimens from all studied localities). For other species of Cottoidei, data from GenBank NCBI (<https://www.ncbi.nlm.nih.gov/genbank/>) were used (the list of species in Supplementary file 2). Thus, the sequences of the mtDNA control region were taken for five valid species belonging to the “*Cottus gobio*” group.

The control region is widely used for species identification and differentiation between closely related species of cottoid fishes of the genus *Cottus* (Kontula & Väinölä 2001; Šlechtová *et al.* 2004; Yokoyama *et al.* 2008; Bravničar *et al.* 2021).

Lateral line, axial skeleton and 3D scan of the lateral, dorsal and ventral views

The sensory canals and pores of the lateral line were studied by injecting methylene blue into the canals with a syringe. The canals were colored blue and photographed (Sideleva 1982).

An X-ray unit (PRDU, manufactured by Eltekhmed) and a Soredex Digora PCT scanner were used to obtain a digital image of the axial skeleton of the sculpins.

A 3D scan of type specimens and the new species *Cottus cyclophthalmus* sp. nov. was performed at St. Petersburg State University. A RangeVision Spectrum scanner was used. The resulting model was imported into 3D modeling software Autodesk Meshmixer (ver. 3.3.15) which was used to capture an image of the fish in three projections (lateral, dorsal and ventral views).

Statistical analyses

To study morphometric characters, such multivariate statistical methods as principal component analysis (PCA) and discriminant function analysis (DFA) were used. The scheme of measurements (developed by Taliev 1955 and Sideleva *et al.* 2015b) included 26 distances. This scheme is designed specifically for cottids.

For these data, a principal component analysis (PCA) based on the correlation matrix was performed (absolute measurements were used). A reduced set of orthogonal vectors was generated from the original variables. The obtained principal components are considered to be new uncorrelated characters. Most of the original morphometric characters have high and positive factor loadings on the first principal component (PC1). This means that PC1 has the greatest contribution to the total variability and mainly determines the size differences between individuals (Somers 1986). A scatterplot in the space of the second and third components was created to describe the differences between samples not related to size variability. Each sample forms its own cluster (morphospace) on the diagram. The degree of cluster overlap indicates their morphological differentiation.

Morphometric features that were analyzed using PCA were also used for a discriminant analysis. However, relative measurements (% SL) were used for this analysis. The quality of discrimination was

assessed based on the Wilks' Lambda and F-test statistics. Wilks' Lambda values close to 0 indicate a strong discrimination. The level of differences between species was determined based on the values of the squared Mahalanobis distance. The contribution of each character to the discriminating power of the model was estimated by the Partial Lambda value. The lower this indicator, the higher the contribution of the variable. Canonical analysis was used to compute orthogonal discriminant functions. The result of this analysis is visualized using a scatterplot of canonical values in the space of the first and second discriminant axes.

Both statistical analyzes were performed using the STATISTICA 10 software (StatSoft).

DNA extraction, PCR amplification and sequencing

DNA was isolated from fin-clip tissue samples (100–200 mg) fixed in 96% ethanol using QIAamp DNA Mini Kit (Qiagen, Germany). The complete *CR* was amplified using primers L16638 (AACTCCTACCCCTAACTCCCAAAGC) and H1122 (GGAGTGCGGAGACTTGCAT) (Kocher *et al.* 1989), resulting in 1000 bp amplicons that included fragments of flanking tRNA genes.

Amplification was undertaken in a BioRad C1000 Touch in a 15 μ L reaction volume containing 1 \times buffer, 1.5 μ M MgCl₂, 10 μ M of each primer, 0.2 μ M of each dNTP, 1 μ L of template DNA solution, and 1U of HS Taq polymerase (Evrogen, Moscow). The conditions for PCR were as follows: 3 min of initial denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 20 s, primer annealing at 59.2°C for 60 s, DNA elongation at 72°C for 60 s, and final elongation at 72°C for 10 min. The sequencing of the amplified fragments was performed in a 3500 Genetic Analyzer (Applied Biosystems) using the primers mentioned above.

Isolation and amplification of DNA was carried out using the equipment of the Laboratory of Ichthyology in Zoological Institute RAS (St. Petersburg). Sequencing was performed in Papanin Institute of Biology of Inland Waters RAS (Borok).

Alignment, sequence diversity statistics, and phylogenetic reconstructions

The inner group included 50 species of cottoid fish: seven are European species (*Cottus poecilopus*, *C. gobio*, *C. metae*, *C. perifretum*, *C. rhenanus*, *C. koshewnikowi*, and *C. cyclophthalmus* sp. nov.); nine Asian species [*C. szanaga* Dybowski, 1869, *C. kolymensis* Sideleva & Goto, 2012, *C. sibiricus* Warpachowski, 1889, *C. volki* Taranetz, 1933, *C. czerskii* Berg, 1913, *C. amblystomopsis* Schmidt, 1904, *C. hangiongensis* Mori, 1930, *C. koreanus* Fujii, Choi & Yabe, 2005, and *Mesocottus haitej* (Dybowski, 1869)]; four species from the Japanese Islands (*C. kazika* (Jordan & Starks, 1904), *C. nazawae* Snyder, 1911, *C. pollux* Günther, 1873, and *C. reinii* Hilgendorf, 1879); seven American species (*C. carolinae* (Gill, 1861), *C. specus* Adams & Burr, 2013, *C. cognatus* Richardson, 1836, *C. bairdii* Girard, 1850, *C. marginatus* (Bean, 1881), *C. aleuticus* Gilbert, 1896, and *Leptocottus armatus* Girard, 1854); 23 species of Baikalian “species flocks” (see Supplement 2). One of the most primitive representatives of Cottoidei, *Trachidermus fasciatus* Heckel, 1837, was used as an outgroup. This species is traditionally used in phylogenetic analyzes of freshwater cottoid fishes (Yokoyama *et al.* 2008; Goto *et al.* 2015).

Sequences were edited by eye and aligned using Geneious Prime 2021.1.1 (<https://www.geneious.com>). Identification of unique haplotypes was performed using DnaSP ver. 6.12.03 (Rozas *et al.* 2017). Excluding the outgroup (*Trachidermus fasciatus*), the final alignment (865 base pairs) included 114 sequences.

The phylogenetic trees were reconstructed using Bayesian analysis in Mr Bayes 3.1.2 (Huelsenbeck & Ronquist 2001) and the Maximum Likelihood (ML) method in IQ-TREE 1.6.12 (Nguyen *et al.* 2016). The choice of the best model for nucleotide substitutions was carried out using algorithm implemented

in IQ-TREE (HKY+I+G5+F with parameters I = 0.28 and G = 0.51 was selected as best model). The Bayesian inference of phylogeny was done by using the selected model (nst = 2, rates = invgamma). The MCMC process was set for four chains to run simultaneously for 10^7 generations, with sampling trees at every 1000 generations. The first 25% of trees were discarded in the computation of the majority-rule consensus tree. Posterior probabilities were calculated by generating a 50% majority rule consensus tree with the remaining trees. The statistical reliability of the ML tree was assessed by the bootstrap method (2000 pseudoreplications). The phylogenetic tree was visualized using FigTree 1.4.4 software (<http://tree.bio.ed.ac.uk/software/figtree>).

To assess the genetic diversity of *C. cyclophthalmus*, the average number of nucleotide substitutions, haplotype diversity (Hd), and nucleotide diversity (π) were estimated. The calculations were performed using the algorithm implemented in DnaSP ver. 6.12.03 (Rozas *et al.* 2017). Gaps were treated as the fifth state.

Pairwise p-distances between different species and between haplotypes of the same species were calculated using MEGA X (Kumar *et al.* 2018). The bPTP web server was used for species delimitation (Zhang *et al.* 2013). The bPTP analysis was performed using 10^5 MCMC generations (thinning interval = 100, burn-in = 0.1).

Results

Class Osteichthyes Huxley, 1880
Order Scorpaeniformes Garman, 1899
Family Cottidae Bonaparte, 1831
Genus *Cottus* Linnaeus, 1758

Cottus cyclophthalmus sp. nov.

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Figs 2–3; Table 1

Cottus gobio – Zograf 1907: 17.

Cottus gobio – Zhukov 1958: 156.

Cottus gobio – Alekseev & Probatov 1969: 7.

Diagnosis

Cottus cyclophthalmus sp. nov. has round, protruding (tubular) eyes near front of head; dermal papillae present on top and sides of head, body naked, bony prickles absent; full trunk canal with 32–36 pores.

Etymology

The name of the new species is derived from the Latin word for ‘round-eyed’ and is associated with the round and convex shape of eyes.

Type material

Holotype

RUSSIA • ♂, SL 83.3 mm, TL 99.0 mm; Krasnaya River, near Tokarevka village, Kaliningrad Region; 54°24′59.4″ N 22°23′50.4″ E; 31 Aug. 2019; ZIN 56687.

Paratypes

RUSSIA • 8 specimens, SL 81.0–48.7 mm; same collection data as for holotype; ZIN 56688.

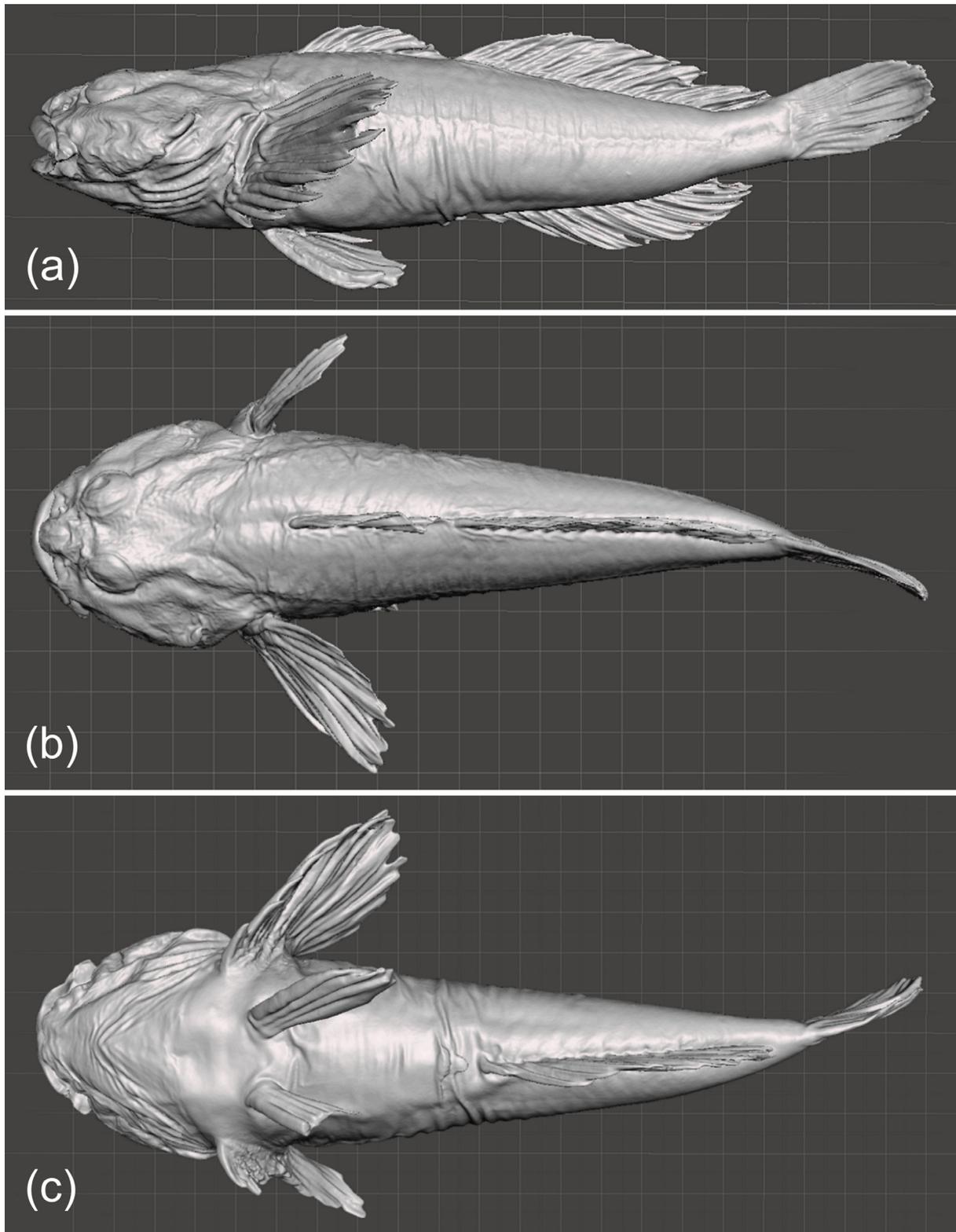


Fig. 2. *Cottus cyclophthalmus* sp. nov., holotype, ♂ (ZIN 56687), SL 83.3 mm, TL 99.0 mm, Krasnaya River, near Tokarevka village, 54°24'59.4" N 22°23'50.4" E. 3D scan images. **a.** Lateral view. **b.** Dorsal view. **c.** Ventral view.

Non-type specimens

LITHUANIA • 26 specimens, SL 56.9–72.1 mm; Žeimena River, tributary of the Neris River, Nemunas/Neman River Basin; 54°58'01.4" N, 25°44'11.1" E; Sep. 2019; ZIN 56689 • 24 specimens, SL 51.4–74.1 mm; Neris River, tributary of the Nemunas/Neman River; 54°50'06.4" N, 25°22'31.5" E; Sep. 2019; ZIN 56690 • 22 specimens, SL 56.1–69.7 mm; Siesartis River, Nemunas/Neman River Basin; 55°17'23.8" N, 24°53'02.7" E; Sep. 2019; ZIN 56691 • 16 specimens, SL 48.8–79.2 mm; Šerkšnė River, tributary of the Venta River; 56°19'35.2" N, 22°12'47.7" E; Sep. 2019; ZIN 56692.

Description

Body shortened, its average maximum depth at origin of first dorsal fin four times SL. Body massive, preanal distance more than half SL. Caudal peduncle short, its length 14–17% SL (14.9% in holotype), average height of caudal peduncle half its length (Table 1). Trunk naked, bony prickles (modified scales) absent.

Head large with smooth dorsal profile from head to back, its length more than 30% SL in type specimens (33% in holotype). Dermal papillae numerous on top and sides of head, sometimes in form of circles.

Postorbital length large, always more than half head length (53% HL in holotype). Snout short, more than half postorbital length (59.4% in holotype). Anterior nostrils small, tubular, highly pigmented; posterior nostrils in form of short tubes. Mouth small, terminal; upper jaw does not reach vertical line of anterior edge of eye. Teeth on jaws and vomer small, numerous, of same shape and size. Upper lip thick, fleshy, twice thicker than lower lip.

Eye round and protruding, near to front of head, average eye diameter 7.3% SL (7.4% SL or 22.4% HL in holotype). Interorbital space narrow, on average 1.5 times less than eye diameter. Preoperculum with three spines; upper spine sharp, directed backwards and slightly curved inward. Second and third spines small, in form of tubercles hidden under the skin. Interbranchial length large, on average 1.5 times less than length of gill slit (16.6% in holotype).

Two dorsal fins follow each other without gap. First dorsal fin low, length of its longest rays 1.5 times as long as rays of second dorsal fin. Narrow light border along edge of first dorsal fin. Second dorsal fin

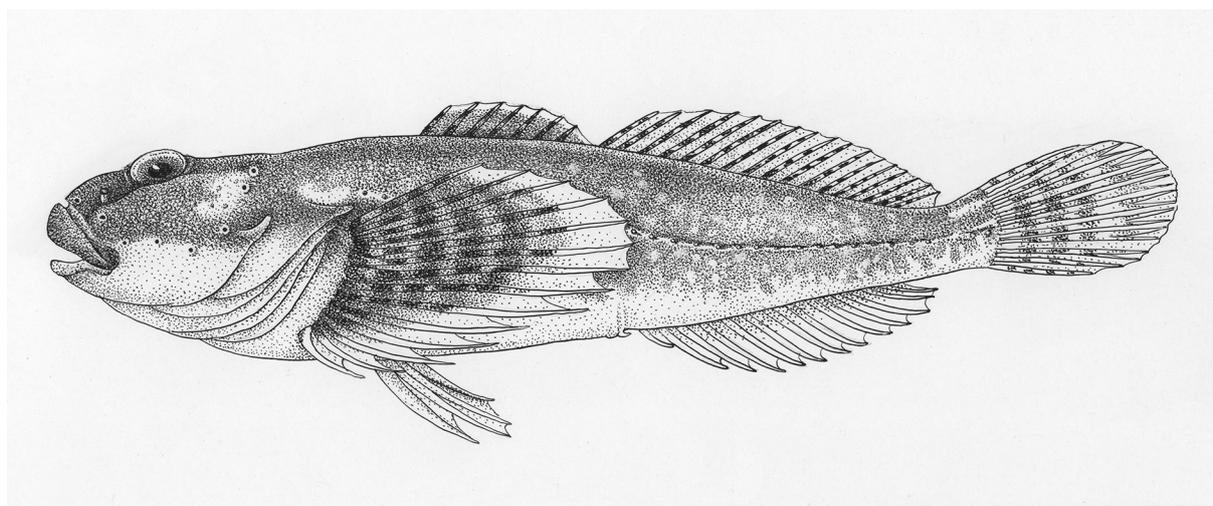


Fig. 3. The zoological picture of the holotype of *Cottus cyclophthalmus* sp. nov. (ZIN 56687), SL 83.3 mm, lateral view.

Table 1. Proportional measurements as percentages of standard length of holotype and paratypes of *Cottus cyclophthalmus* sp. nov. from Krasnaya River.

	Holotype			Paratypes (n=8)					
Standard length (SL), mm	83.3	81.0	68.5	66.4	63.3	54.4	53.3	50.4	48.7
	In % SL								
Predorsal length	35.5	37.3	36.8	37.2	37.0	36.0	34.9	34.9	36.8
Postdorsal length	9.7	8.9	11.5	11.3	11.1	11.0	12.2	11.1	10.3
Preanal length	55.0	58.4	51.7	54.2	55.9	55.5	54.4	52.0	56.7
Postanal length	47.3	42.6	48.5	47.7	48.2	48.9	47.3	48.2	46.0
Maximum body depth	24.2	24.8	21.3	22.9	22.9	23.2	21.2	22.0	22.2
Length of caudal peduncle	14.9	14.3	17.2	16.6	16.3	14.5	15.2	15.3	14.0
Depth of caudal peduncle	8.5	7.9	7.0	7.4	7.1	7.4	7.3	6.9	7.0
Length of first dorsal fin base	14.8	13.6	13.9	15.1	12.5	13.2	12.8	14.1	12.1
Length of second dorsal fin base	36.9	36.7	34.6	34.9	35.4	35.5	36.2	36.9	37.2
Length of anal fin base	30.1	27.7	29.2	28.6	28.9	28.9	29.6	27.6	27.9
Length of longest first dorsal fin spines	8.3	8.9	8.8	9.3	8.8	7.4	9.2	9.7	8.6
Length of longest second dorsal fin rays	12.5	13.3	13.3	13.9	12.2	13.2	13.3	13.7	12.5
Length of longest anal fin rays	14.5	15.8	13.4	15.2	14.2	13.2	12.4	14.3	12.7
Pectoral fin length	28.6	27.3	25.8	27.7	26.1	26.5	25.7	26.6	27.3
Pelvic fin length	20.3	20.2	20.4	21.2	19.6	20.8	19.7	18.3	19.9
Head length	33.0	35.7	32.6	33.6	33.0	32.4	31.3	32.1	34.7
Postorbital length	17.5	18.8	17.7	17.2	17.4	16.5	15.6	16.5	17.7
Head depth	21.4	22.1	19.4	20.5	20.5	20.2	18.9	18.3	21.6
Head width	30.6	34.6	32.3	31.2	32.2	30.9	30.8	28.6	32.0
Eye horizontal diameter	7.4	7.2	7.0	6.9	7.0	7.5	7.3	7.9	7.2
Snout length	10.4	11.0	10.2	10.1	10.3	9.9	9.8	9.7	10.7
Interorbital width	4.9	6.0	4.4	5.1	5.2	5.3	4.7	4.8	6.0
Upper jaw length	13.2	14.6	11.5	12.3	12.5	11.4	10.5	11.3	12.7
Interbranchial width	11.4	12.8	9.9	12.0	11.5	12.1	9.9	9.1	8.6
Length of gill slit	19.0	20.4	16.8	18.4	18.5	16.4	16.5	17.7	18.5

long, its base 2.5 times as long as base of first dorsal fin. Origin of anal fin at short distance (3% SL) from anus, on vertical line of second ray of second dorsal fin; length of longest rays in anal fin 1.2 times that in second dorsal fin. Pectoral fin short, reaching vertical of first ray of second dorsal fin. Pelvic fin long (20% SL), not reaching anus.

Axial skeleton: total number of vertebrae counts 31–32: 10–11 abdominal and 21–22 caudal (11 + 21 = 32 in holotype). The first vertebra with fully developed neural spine. The posteriormost abdominal vertebrae (from 8th–9th to 10th–11th) are carrying two or three pairs of pleural ribs (three in holotype).

The first proximal pterygiophore of the dorsal series is placed between first and second vertebrae. It supports first dorsal fin spine which is in supernumerary position (morphotype A according to Yabe

1985). One interdorsal pterygiophore is placed between first and second dorsal fin. The last proximal pterygiophore of dorsal series supports one or two rays (one in holotype). The last pterygiophore of anal fin supports one or two fin rays (two in holotype).

The caudal skeleton is composed of single hypural-parhypural complex bone and three epurals. The complex bone has deep notch posteromedially and supports principal caudal-fin rays. Medial principal rays of caudal fin are branched.

Number of rays in fins: first dorsal fin with six to eight spines (seven in holotype); second dorsal fin with 16 to 19 rays (17 in holotype); anal fin with 12 to 15 rays (13 in holotype); pectoral fin with 12 to 15 rays (14 in holotype); four rays in pelvic fin; caudal fin with 12 principal rays (eight branched and four unbranched).

Lateral line of *Cottus cyclophthalmus* is typical of the genus *Cottus*. All sensory canals (with exception of preopercular-mandibular canal) are interconnected and form a unified system.

Three small pores are in supraorbital canal, they located in anterior part of canal, up to coronal commissure. It connects left and right supraorbital canals to each other. One small pore is in center of coronal commissure. The infraorbital sensory canal opens outward with nine pores, of which second and third pores are large slit-like. The temporal canal and occipital commissure each have three pores. The preopercular-mandibular sensory canal opens with 10 pores. The canals of left and right sides are interconnected, and on chin they open with common oval pore. The trunk canal is full (reaches the base of the caudal fin), located closer to dorsal part of body and opens with 31–34 small pores.

Coloration: the upper part of the body is dark to the medial line. Below, there are numerous small spots formed by clusters of melanophores. The upper part of the head, including the upper lip, is dark, the radii branchiostegii are light. The ventral side of head and body are light. The dorsal fins variegated and have dark transverse stripes. A narrow light border is at the edge of the first dorsal fin. Weakly expressed dark spots and stripes are present at origin of pectoral and caudal fins. The external parts of the pectoral, anal and pelvic fins are light, not pigmented.

Distribution

Cottus cyclophthalmus sp. nov. is distributed in the rivers Krasnaya, Neris, Žeimena and Sesartis (Neman/Nemunus River system) and Šerkšnė River (Venta River system), Baltic Sea Basin (Fig. 1).

Variation of morphometric features and numbers of rays in *Cottus cyclophthalmus* sp. nov.

For a comparative analysis of morphometric characters of type and non-type specimens of *Cottus cyclophthalmus* sp. nov. from the rivers Krasnaya, Neris, Šerkšnė, Siesartis and Žeimena, PCA was used (Fig. 4). The variability of 26 external features was analyzed. (Table 2). The principal component analysis revealed a variability of initial data. This data is represented in a scatter plot with uncorrelated second and third principal components. Each component reflects a proportion of a variability of variance-covariance matrix of features. The conducted analysis showed that the first principal component describes 84.6% of total variability of measurements in five studied samples of sculpins. The first component is characterized by close positive values of factor loadings (from 0.758 to 0.982, or 0.918 on average). All other 25 components describe 15.4% of total variability. Figure 4 shows morphospaces of studied samples in the space of second and third components. The morphospaces of samples of *C. cyclophthalmus* overlap significantly. There are no discrete geographic groupings. This indicates that the five studied samples are not differentiated by morphometric characters and belong to the same species *C. cyclophthalmus*.

Data on variation in number of rays in dorsal, anal, and pectoral fins in five samples of *Cottus cyclophthalmus* sp. nov. are presented in Table 3. A comparison of presented data shows that sculpins

from different localities have a similar degree of variation in the number of rays in fins. The differences in number of rays in each fin ranged from 2 to 4 values. In the first dorsal fin in the type specimens from Krasnaya River, the number of rays varied only within two ranges (six to seven rays). In non-type individuals from tributaries of the Nemunas/Neman and Venta rivers, the number of rays varied from six to eight. In all samples (except for sculpins from Šerkšnė River), fish with seven rays in the first dorsal fin dominated (76–94% of all specimens). The number of rays in the second dorsal fin ranged from 16 to 19. Type specimens from Krasnaya River had the smallest (16–17) number of rays in the second dorsal fin. The difference between the samples lies in modal values, with the majority of studied fish (69.5%) having 17–18 rays in the second dorsal fin. In the anal fin, the number of rays varied from 12 to 16. Type specimens of *Cottus cyclophthalmus* had 13–14 rays in anal fin. The same number of rays dominated in 94% of the studied fish. In the pectoral fin, the number of rays varies from 12 to 15. Fish with a modal number of 14 rays dominated in all samples.

Thus, in all five samples of *C. cyclophthalmus* sp. nov., the number of rays in two dorsal, the anal and pectoral fins had a similar level of variation. The differences in number of rays in sculpins from different

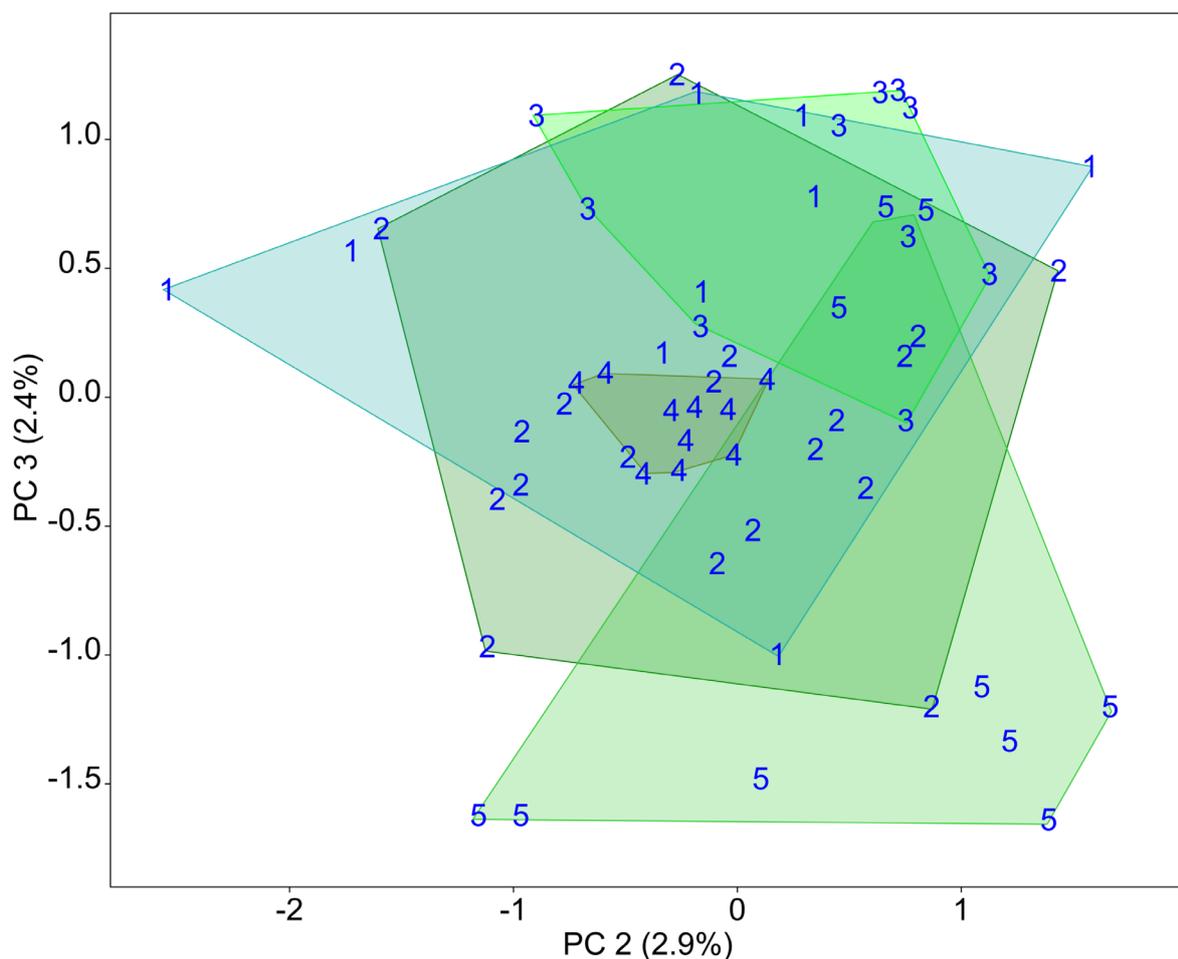


Fig. 4. The result of statistical analysis of morphometric characters of type and non-type specimens of *Cottus cyclophthalmus* sp. nov. from rivers Krasnaya, Neris, Šerkšnė, Siesartis, and Žeimena (method of principal components was used). The numbers correspond to the places where the sculpins were caught, as indicated on the map (Fig. 1).

Table 2 (continued on next page). Proportional measurements of type and non-type specimens of *Cottus cyclophthalmus* sp. nov. from Pregolya, Neman and Venta River systems (Russia, Lithuania). Note: numerator = mean (M) \pm standard deviation (SD); denominator = range.

	Krasnaya R. (n=9)	Neris R. (n=19)	Zeimena R. (n=10)	Siesartis R. (n=10)	Šerkšnė R. (n=10)
	Type specimens		Non-type specimens		
SL, mm	63.3 48.7–83.3	61.6 51.4–74.1	65.6 56.9–72.1	62.3 56.1–69.7	61.4 48.8–79.2
In % SL†					
Predorsal length	36.3 \pm 0.9 34.9–37.3	36.3 \pm 1.3 34.8–39.7	34.9 \pm 1.2 32.4–36.5	35.4 \pm 0.5 34.7–36.2	34.9 \pm 1.2 32.4–36.5
Postdorsal length	10.8 \pm 1.0 8.9–12.2	10.3 \pm 0.8 9.0–12.5	9.9 \pm 1.2 8.2–11.9	10.0 \pm 0.3 9.6–10.3	9.9 \pm 1.2 8.2–11.9
Preal length	54.9 \pm 2.1 51.7–58.4	53.6 \pm 1.5 51.0–56.2	55.0 \pm 2.0 51.9–57.2	54.2 \pm 0.4 53.5–54.9	55.0 \pm 2.0 51.9–57.2
Postanal length	47.2 \pm 1.9 42.6–48.9	48.2 \pm 1.6 45.1–50.5	46.8 \pm 1.6 43.6–48.6	47.7 \pm 0.7 46.2–48.8	46.8 \pm 1.6 43.6–48.6
Maximum body depth	22.7 \pm 1.2 21.2–24.8	21.9 \pm 1.2 20.0–23.8	20.5 \pm 0.9 19.3–21.7	21.5 \pm 0.5 20.7–22.3	20.5 \pm 0.9 19.3–21.7
Length of caudal peduncle	15.4 \pm 1.1 14.0–17.2	15.1 \pm 1.1 13.0–16.9	14.7 \pm 1.4 12.7–16.6	14.9 \pm 0.2 14.5–15.3	14.7 \pm 1.4 12.7–16.6
Depth of caudal peduncle	7.4 \pm 0.5 6.9–8.5	7.4 \pm 0.5 6.8–8.7	7.0 \pm 0.4 6.4–7.9	7.0 \pm 0.2 6.6–7.4	7.0 \pm 0.4 6.4–7.9
Length of first dorsal fin base	13.6 \pm 1.0 12.1–15.1	13.8 \pm 0.7 12.3–15.4	13.5 \pm 0.8 12.1–14.7	13.6 \pm 0.2 13.3–13.9	13.5 \pm 0.8 12.1–14.7
Length of second dorsal fin base	36.0 \pm 0.9 34.6–37.2	36.8 \pm 1.5 32.3–39.1	36.5 \pm 1.7 33.0–38.5	38.1 \pm 0.4 37.2–38.7	36.5 \pm 1.7 33.0–38.5
Length anal fin base	28.7 \pm 0.9 27.6–30.1	28.7 \pm 1.4 26.3–31.2	28.3 \pm 1.4 26.6–31.0	29.9 \pm 0.4 29.1–30.4	28.3 \pm 1.4 26.6–31.0
Length of longest first dorsal fin spines	8.8 \pm 0.7 7.4–9.7	8.8 \pm 0.9 7.2–10.3	8.0 \pm 0.9 6.6–9.3	9.1 \pm 0.3 8.7–9.8	8.0 \pm 0.9 6.6–9.3
Length of longest second dorsal fin ray	13.1 \pm 0.6 12.2–13.9	13.6 \pm 0.7 12.6–15.2	13.3 \pm 0.9 11.4–14.5	13.4 \pm 0.3 12.9–14.1	13.3 \pm 0.9 11.4–14.5
Length of longest A ray	14.0 \pm 1.1 12.4–15.8	13.8 \pm 0.8 12.8–15.6	13.5 \pm 0.6 12.4–14.5	14.1 \pm 0.6 13.2–15.0	13.5 \pm 0.6 12.4–14.5
Pectoral fin length	26.8 \pm 1.0 25.7–28.6	28.6 \pm 1.5 25.4–32.3	25.9 \pm 1.4 23.8–28.9	26.2 \pm 0.8 25.1–27.9	25.9 \pm 1.4 23.8–28.9
Pelvic fin length	20.0 \pm 0.8 18.3–21.2	20.2 \pm 1.3 17.9–23.2	19.0 \pm 0.8 18.0–20.6	19.7 \pm 0.5 18.8–20.4	19.0 \pm 0.8 18.0–20.6
Head length	33.2 \pm 1.3 31.3–35.7	32.6 \pm 1.0 30.6–34.4	32.0 \pm 1.2 30.0–33.7	32.3 \pm 0.9 31.2–33.9	32.0 \pm 1.2 30.0–33.7
Postorbital length	17.2 \pm 0.9 15.6–18.8	17.1 \pm 0.6 16.0–18.1	16.1 \pm 1.0 14.7–17.7	16.9 \pm 0.7 16.1–18.3	16.1 \pm 1.0 14.7–17.7
Head depth	20.3 \pm 1.3 18.3–22.1	19.5 \pm 1.1 17.1–21.3	19.2 \pm 1.0 17.7–20.6	19.5 \pm 0.6 18.6–20.4	19.2 \pm 1.0 17.7–20.6
Head width	31.5 \pm 1.6 28.6–34.6	29.3 \pm 2.2 24.9–33.3	29.3 \pm 1.3 26.5–30.9	29.0 \pm 0.7 27.8–30.1	29.3 \pm 1.3 26.5–30.9

Table 2 (continued). Proportional measurements of type and non-type specimens of *Cottus cyclophthalmus* sp. nov. from Pregolya, Neman and Venta River systems (Russia, Lithuania). Note: numerator = mean (M) ± standard deviation (SD); denominator = range.

	Krasnaya R. (n=9)	Neris R. (n=19)	Zeimena R. (n=10)	Siesartis R. (n=10)	Šerkšně R. (n=10)
	Type specimens		Non-type specimens		
Eye horizontal diameter	7.3±0.3 6.9–7.9	7.2±0.4 6.5–8.0	7.6±0.3 7.1–8.2	6.9±0.3 6.5–7.3	7.6±0.3 7.1–8.2
Snout length	10.2±0.4 9.7–11.0	9.1±0.5 7.6–9.8	9.4±0.9 8.0–10.9	9.0±0.3 8.6–9.5	9.4±0.9 8.0–10.9
Interorbital width	5.2±0.6 4.4–6.0	4.9±0.4 4.1–5.8	4.0±0.6 3.1–4.8	4.8±0.2 4.5–5.1	4.0±0.6 3.1–4.8
Upper jaw length	12.2±1.2 10.5–14.6	11.8±0.8 10.6–13.8	10.8±0.5 10.0–11.6	11.7±0.3 11.4–12.5	10.8±0.5 10.0–11.6
Interbranchial width	10.8±1.5 8.6–12.8	10.2±0.8 8.5–11.8	9.7±1.1 8.3–11.9	11.1±1.0 8.9–12.5	9.7±1.1 8.3–11.9
Length of gill slit	18.0±1.3 16.4–20.4	17.7±0.8 16.1–19.1	16.6±1.0 14.7–18.0	16.3±0.6 15.6–17.3	16.6±1.0 14.7–18.0

localities were in modal values. This level of variability corresponds to differences between individual populations of *C. cyclophthalmus*.

Variation in mitochondrial DNA sequences

To identify intraspecific genetic diversity of *C. cyclophthalmus* sp. nov., we studied nucleotide sequences (858 bp) of mtDNA control region. Table 4 contains the data on haplotypes found in samples of *C. cyclophthalmus* from four rivers (Krasnaya, Žeimena, Siesartis, and Šerkšně). Nine haplotypes were identified (CCY1–CCY9). The most common haplotype was CCY6 (52% of specimens of the new species). It was found in sculpins from each of the studied rivers. The sample of a new species from the Šerkšně River was characterized by the greatest haplotype diversity (6 unique haplotypes). The number of detected polymorphic sites (S) was seven. The nucleotide diversity of mtDNA control region among individuals of *C. cyclophthalmus* had a low value ($\pi = 0.00139 \pm 0.00024$). The haplotype diversity (Hd) was high (0.718 ± 0.080). The average number of nucleotide differences was 1.196.

All data indicate a low level of genetic differentiation between the studied specimens. This is the evidence that all individuals belong to species *C. cyclophthalmus*.

Discussion

Morphological differentiation between *Cottus cyclophthalmus* sp. nov. and other species of genus *Cottus*

The new species *C. cyclophthalmus* sp. nov. is characterized by morphological features, the states of which are similar to that of *C. gobio*. The characters that are common to *C. cyclophthalmus* and *C. gobio* are as follows: a shortened body with a similar postanal distance (47.2% SL in type specimens vs 48.7); short caudal peduncle (15.4 vs 16.1); similar height of the caudal peduncle (7.4 vs 7.1); short ventral fin not reaching the anus (20.0 vs 19.0); narrow interorbital space (5.2 vs 4.6), as well as the absence of bony spines on the body and a complete trunk canal reaching the rays of the caudal fin.

The presence of dermal papillae (of a different shape) on surface of head is found in Eastern European sculpin *C. koshewnikowi* (Sideleva *et al.* 2015a).

Cottus cyclophthalmus sp. nov. differs from all European species of the genus *Cottus* by the following complex of morphological characters: round bulging eyes, short snout, dermal papillae on top and sides of head, thick upper lip, and light coloration of external part of pectoral fin.

The discriminant function analysis (DFA) based on morphometric data revealed significant morphological differences in body shape between three species: *C. cyclophthalmus*, *C. gobio*, and *C. koshewnikowi* (Fig. 5). Statistical analysis (Wilks' Lambda = 0.00852, approx. $F(50, 136) = 26.745$, $p < 0.0000$) indicated a significant discrimination of the three species. The new species from the Nemunas/Neman and Venta river systems was the most different from the Western European species *C. gobio* (squared Mahalanobis distance between these species was 93.82). Mahalanobis distance between samples of *C. cyclophthalmus* and Eastern European *C. koshewnikowi* was slightly lower (55.02). Partial Lambdas demonstrating individual contribution of variables to discriminatory power of model are shown in Table 5. The most important characters (Partial Lambda < 0.8) for discriminating samples of species were horizontal diameter of eye and length of base of first dorsal fin. Thus, *C. cyclophthalmus* is well differentiated from *C. gobio* and *C. koshewnikowi* by the complex of morphometric characters.

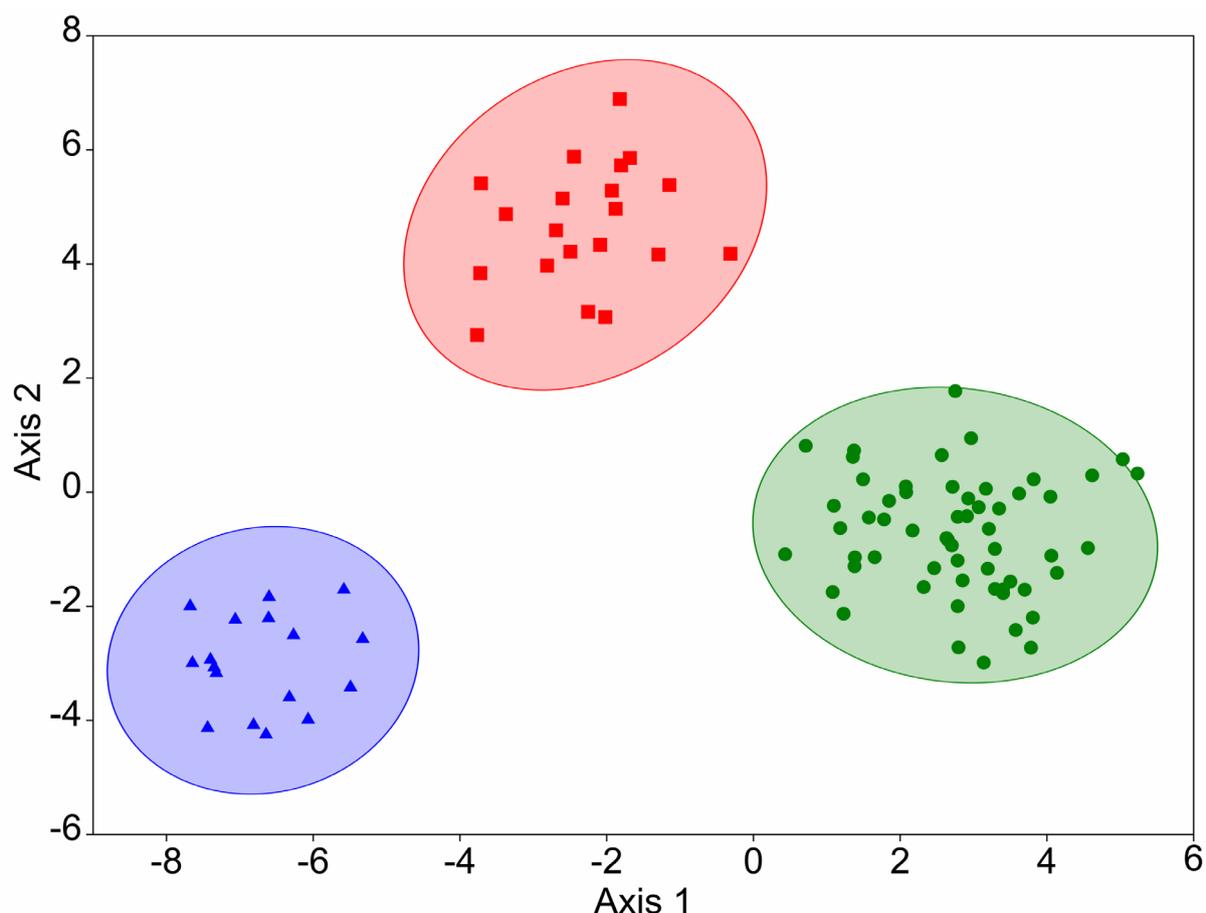


Fig. 5. The result of the DFA carried out on morphometric characters to discriminate *Cottus cyclophthalmus* sp. nov. (green), *Cottus gobio* Linnaeus, 1758 (blue), and *Cottus koshewnikowi* Gratzianov, 1907 (red).

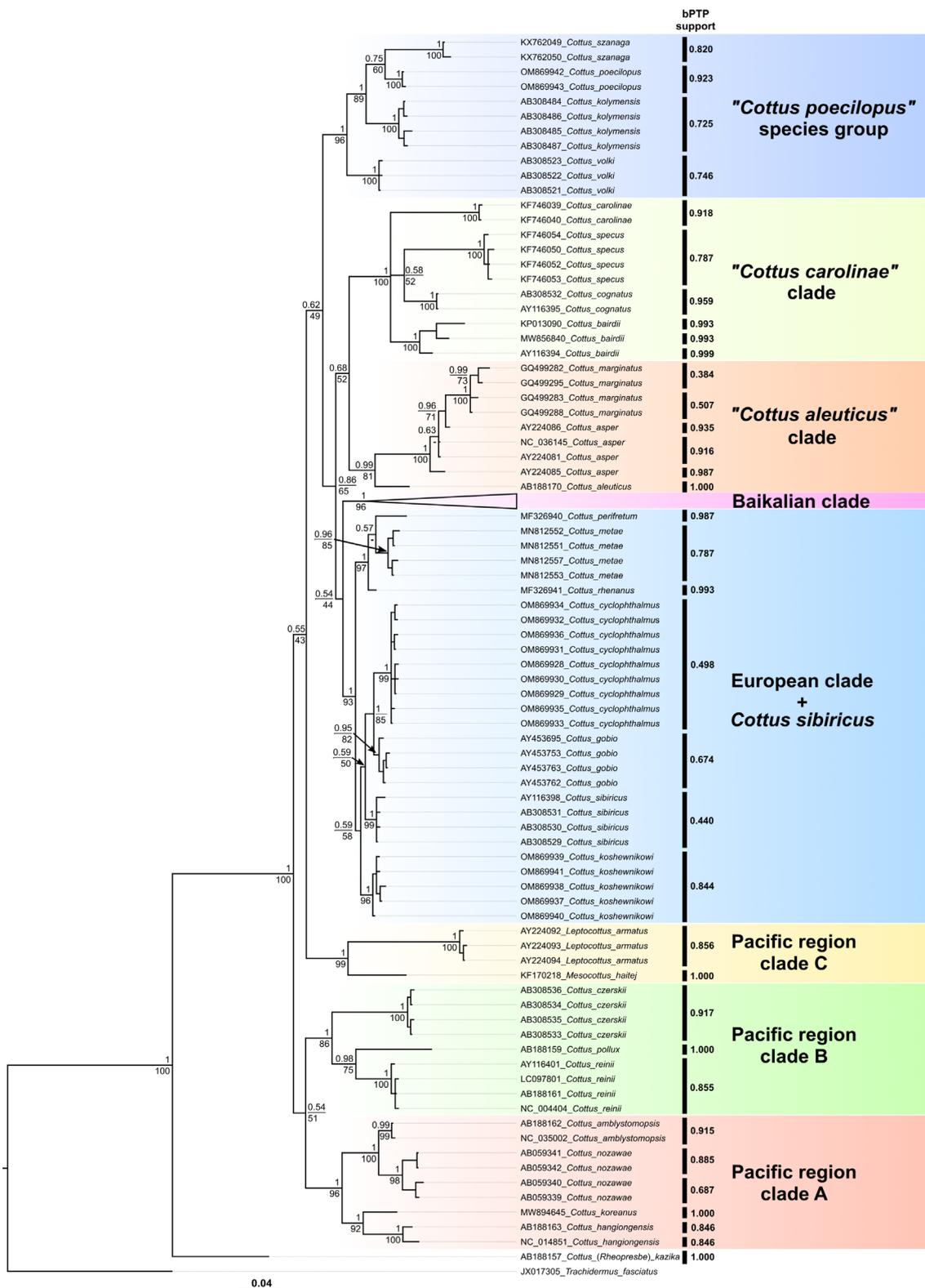


Fig. 6. Bayesian phylogenetic tree of the freshwater species of Cottidae reconstructed using mtDNA control region sequences. Bayesian posterior probabilities (on the top side) and bootstrap values from a ML analysis (on the down side) are shown. Results of species delimitation with bPTP support values are presented.

Table 3. Frequency of occurrence of fin-rays counts in *Cottus cyclophthalmus* sp. nov. Note: † = values for the holotype.

Locality (number of specimens)	First dorsal fin			Second dorsal fin				Anal fin				Pectoral fin			
	6	7	8	16	17	18	19	12	13	14	15	12	13	14	15
Krasnaya R. (n=9)	1	8†		5	4†				4†	5			1	6†	2
Neris R. (n=21)		19	2	1	10	9	1	4	10	5	2	1	3	9	8
Žeimena R. (n=26)	2	20	4	1	10	14	1	2	13	11		1	1	18	6
Siesartis R. (n=21)	1	16	4	1	7	10	3	1	5	12	3		1	17	3
Šerkšnė R. (n=15)		5	10		3	8	4	1	6	6	2		2	10	3

Phylogenetic placement of *Cottus cyclophthalmus* sp. nov. in freshwater Cottoidei

An analysis of phylogenetic relationships of freshwater Cottidae was performed based on the mtDNA control region sequences using Bayesian and ML methods. Both analyzes generated trees with similar topology. The Bayesian tree is shown in Fig. 6.

Among the species of the ingroup, the most isolated, basal position in the phylogenetic tree was occupied by *Cottus (Rheopresbe) kazika*, a catadromous species inhabiting the rivers of Honshu Island (Japan) (Goto *et al.* 2015). According to the mtDNA control region data, the average p-distance between this species and all other presented lineages was 14.5% (from 10.8 to 18.8%). The average p-distances between the eight major clades ranged from 5.0 to 12.4% (mean value 8.7%). The genetic differences between species varied greatly (0.1–15.6%), and the average interspecific p-distance was 7.3%. The clade of endemic Baikal cottoid fishes was well supported on the phylogenetic tree. In our dataset, it was represented by 23 species from three families (Cottidae, Abyssocottidae, and Comephoridae). All the studied lineages of European representatives of the “*Cottus gobio*” and “*Cottus koshewnikowi*” species groups (including the new species *C. cyclophthalmus*) and the Siberian species *Cottus sibiricus* from the Ob and Yenisei drainages formed single clade. This result does not confirm the morphological differentiation of the two identified species groups. The morphological similarity of *C. gobio* and *C. sibiricus* has previously been known. L. Berg (1949: 1148) wrote about the Siberian sculpin: “It is close to *C. gobio*, which it replaces in Siberia”. Nevertheless, these species are well differentiated by osteological features. The ranges of *C. sibiricus* and *C. gobio* do not overlap. *Cottus koshewnikowi* differs from *C. gobio* by a number of morphological characters: presence of prickles, short trunk canal with smaller number of pores (22–27 vs 30–36). The p-distances between these three pairs of species are rather low (1.1% between *C. gobio* and *C. sibiricus*, 1.3% between *C. gobio* and *C. koshewnikowi*, and 0.8% between *C. koshewnikowi* and *C. sibiricus*).

All of these data suggest complex phylogenetic relationships between these species that require further study. The average interspecific p-distance within this clade was 1.8% (from 0.8 to 2.9%), which could indicate recent divergence between lineages.

The new species *C. cyclophthalmus* sp. nov. and *C. gobio* were found to be most closely related taxa. The genetic distance between them was 1.3%. According to the results of species delimitation using the bPTP web server (Fig. 6), the lineage of *C. cyclophthalmus* has sufficient support (0.498, with a threshold value of 0.331). This result supports its species status.

The first record of the sculpin *Cottus microstomus* in the Neman/Nemunas River basin

Studying fish from the tributary of the Neman River (Siesartis River), one specimen (ZIN 56723, SL 78.4 mm) was found that did not belong to the new species *C. cyclophthalmus* sp. nov.

Table 4. The geographic distribution of detected mt *CR* haplotypes of *Cottus cyclophthalmus* sp. nov., *C. koshewnikowi* Gratzianov, 1907, and *C. poecilopus* Heckel, 1837.

Species	mt CR haplotype	GenBank Accession No.	Sampling sites	Number of specimens
<i>C. cyclophthalmus</i> sp. nov.	CCY1	OM869928	Krasnaya R.	2
	CCY2	OM869929	Krasnaya R., Siesartis R., Šerkšnė R.	4
	CCY3	OM869930	Krasnaya R., Žeimena R.	2
	CCY4	OM869931	Šerkšnė R.	1
	CCY5	OM869932	Šerkšnė R.	1
	CCY6	OM869933	Krasnaya R., Neris R., Siesartis R., Žeimena R., Šerkšnė R.	16
	CCY7	OM869934	Šerkšnė R.	1
	CCY8	OM869935	Neris R., Žeimena R.	3
	CCY9	OM869936	Šerkšnė R.	1
<i>C. koshewnikowi</i>	CKO1	OM869937	Moskva R.	2
	CKO2	OM869938	Moskva R.	2
	CKO3	OM869939	Sominka R.	2
	CKO4	OM869940	Volchya R.	1
	CKO5	OM869941	Burnaya R.	3
<i>C. poecilopus</i>	CPO1	OM869942	Luga R.	3
	CPO2	OM869943	Sista R.	1

The diagnostic features of the found specimen are the following: low depth of caudal peduncle; head smoothly passes into body, leathery wrinkles on upper surface of head; eye oval, not bulging; in male, lobular genital papilla; dorsal, pectoral and caudal fins with dark transverse stripes; bony prickles on trunk absent; trunk canal full (Fig. 7). The number of rays in fins: first dorsal fin with 8 rays, second dorsal fin with 18 rays, anal fin with 14 rays, pectoral fin with 15 rays, and 12 principal caudal-fin rays.

According to these characters, the specimen was identified as *C. microstomus*. This species is known from the Vistula and Dniester rivers. The type locality of *C. microstomus* is near the city of Krakow (Poland). There have been no data on the distribution of this species outside the Vistula and Dniester river systems. In the system of the Nemunas/Neman River, representatives of this species have previously not



Fig. 7. *Cottus microstomus* Heckel, 1837 (ZIN 56723), SL 78.4 mm, Siesartis River, Neman/Nemunas River basin (Lithuania).

been recorded. *Cottus microstomus* is rare in the Neman/Nemunas river system. Among 134 individuals caught, only one specimen of *C. microstomus* was found. The first finding of this species testifies to its wider range and expands the list of fish species of Lithuania.

Conclusion

The new species *C. cyclophthalmus* sp. nov. differs from other species of the genus *Cottus* by the set of morphological characters. Taxonomically important features are the shape and location of the eyes, as well as the presence of well-defined dermal papillae above and on the sides of the head. According to other morphological features, this species belongs to the “*Cottus gobio*” species group.

The shape of the body, the absence of prickles, and the full trunk sensory canal significantly distinguish *C. cyclophthalmus* from a closely related *C. koshewnikowi*, which has an adjacent range. Based on morphological and molecular genetic data *C. cyclophthalmus* is a distinct taxon. Its position within the genus *Cottus* has been clearly defined.

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Supplementary files

Supp. file 1. Nucleotide alignment of *CR* sequences used for phylogenetic tree reconstruction; data acquired in the present study and from the GenBank NCBI (trimmed to the length of 865 bp). <https://doi.org/10.5852/ejt.2022.834.1897.7535>

Supp. file 2. Data from GenBank NCBI used in this study. <https://doi.org/10.5852/ejt.2022.834.1897.7537>