European Journal of Taxonomy 861: 1–20
https://doi.org/10.5852/ejt.2023.861.2061

ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2023 · Bogutskaya N.G. *et al.*

Research article

urn:lsid:zoobank.org:pub:CDFF090D-045A-4C21-A48C-208A91343646

Novel data support validity of *Phoxinus chrysoprasius* (Pallas, 1814) (Actinopterygii, Leuciscidae)

Nina G. BOGUTSKAYA [1,*], Oleg A. DIRIPASKO [2] & Anja PALANDAČIĆ [3]

[1, 3] Naturhistorisches Museum Wien, Vienna, Austria.
[2] Institute of Fisheries and Marine Ecology, Berdyansk, Ukraine.
[3] University of Ljubljana, Biotechnical Faculty, Department of Biology, Jamnikarjeva 101, SI-1000, Ljubljana, Slovenia.

[*] Corresponding author: nina.bogutskaya@nhm-wien.ac.at
[2] Email: olegdiripasko@ukr.net
[3] Email: anja.palandacic@nhm-wien.ac.at

[1] urn:lsid:zoobank.org:author:F6957EF1-366E-484E-BF40-211E17A93A1B
[2] urn:lsid:zoobank.org:author:B6B4CAE9-13F2-456C-BCA8-E1D5FC7C0D98
[3] urn:lsid:zoobank.org:author:7E4E4D47-A7EA-4907-8DCB-1145240126AF

Abstract. The common minnow species *Cyprinus chrysoprasius*, previously synonymised to *Phoxinus phoxinus*, was originally described from the Crimean Peninsula (Black Sea – Sea of Azov basin). A genetic analysis of the mitochondrial cytochrome oxydase 1 in the context of a phylogenetic study of European *Phoxinus* showed that it represents a distinct genetic clade and potentially a valid species. In the present study, we approach the issue following a broader, both genetic and morphological, study in order to check and support the validity of native Crimean *Phoxinus* under the earliest available name of the species: *P. chrysoprasius*. Our data demonstrate a reliable genetic distance of this minnow from geographically neighbouring clades and species, and a certain morphological distinctiveness. In order to determine the taxonomic concept of *P. chrysoprasius*, as a species involved in a genetically well-differentiated, but phenotypically poorly structured complex of east-European *Phoxinus*, a neotype for the species, based on topotypical material, is herein described and designated. The original type locality of the species is also clarified.

Keywords. Common minnow, Crimea, mitochondrial clade, morphology, neotypification.

Bogutskaya N.G., Diripasko O.A. & Palandačić A. 2023. Novel data support validity of *Phoxinus chrysoprasius* (Pallas, 1814) (Actinopterygii, Leuciscidae). *European Journal of Taxonomy* 861: 1–20. https://doi.org/10.5852/ejt.2023.861.2061

Introduction

In the 20th century, *Phoxinus phoxinus* (Linnaeus, 1758) was commonly considered as a senior synonym of all available species-group names applied to common minnows widely distributed throughout the Palaearctic region, in the basins of the Atlantic, North and Baltic seas, and the Arctic and northern

Pacific oceans (see for a review Kottelat 1997: 73). In the last two decades, the European *Phoxinus* Rafinesque, 1820 have been intensively researched and studies revealed high levels of molecular and morphological diversity, and a complex taxonomy (Palandačić *et al.* 2015, 2017, 2020; Vucić *et al*. 2018; Bogutskaya *et al*. 2019; Corral-Lou *et al.* 2019; De Santis *et al.* 2020). Some genetically and morphologically diagnosable clades were given the rank of species, either new (Bogutskaya *et al*. 2019; Denys *et al.* 2020) or re-validated (Bogutskaya & Naseka 2004; Palandačić *et al.* 2017; Bogutskaya *et al*. 2019), e.g., *P. marsilii* Heckel, 1836 and *P. colchicus* Berg, 1910. However, a number of clades requiring integrated research to help clarify their phylogeny and taxonomic identities, remained. Furthermore, some nomenclatural issues such as the applicability of older available names, have become of paramount importance in this field. While studies on these issues have mainly analysed the material of *Phoxinus* from the western part of its range (Kottelat 2007; Palandačić *et al.* 2015, 2017, 2020; Vucić *et al*. 2018; Bogutskaya *et al*. 2019; Corral-Lou *et al.* 2019; De Santis *et al.* 2020; Denys *et al.* 2020), the areas to the east and north-east of the Danube Basin have been less thoroughly studied.

Inland from the northern and north-eastern coasts of the Black Sea and the Caspian Sea, species of *Phoxinus* occur in river drainages of (from west to east) Dniester, Dnieper, Salhir (Salgir, Salhyr), Don, Kuban, Volga and Ural, as well as small river drainages along the Black Sea coast of Russia and Georgia. The pattern of distribution in these areas (Fig. 1) seems to be determined by habitat preference, as *Phoxinus* are known here to inhabit only small streams and shallow rivulets with a pronounced current, cold water and hard substrate, although common minnows have also been recorded from reservoirs along mountain rivers (Beling 1914; Movchan & Smirnov 1981; Plotnikov 2001; Emtyl’ & Ivanenko 2002; Luzhnyak 2003; Movchan *et al*. 2003; Chernyshev 2010; Karpova & Boltachev 2011; Movchan 2011;

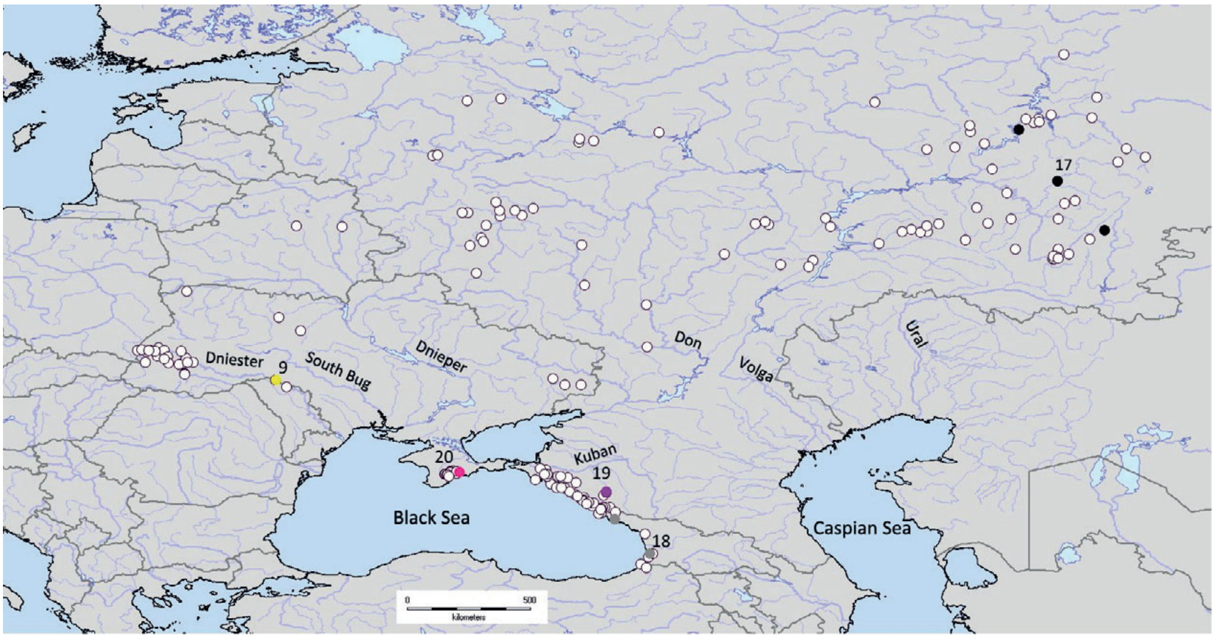

Fig. 1. Map of the localities of *Phoxinus* Agassiz, 1835 in river drainages of the northern and north-eastern coasts of the Black Sea and the Caspian Sea based on numerous published sources (references available in Supp. file 1) and public museum collections (Museum of Zoology, National Museum of Natural History, Kyiv, Ukraine; Natural History Museum, Vienna, Austria; Zoological Research Museum Alexander Koenig, Bonn, Germany). Coloured circles and numbers correspond to genetically examined individuals of clades specified in Palandačić *et al.* (2017, 2020): yellow circle = *P. marsilii*, Clade 9; black circles = ‘Baltic *Phoxinus*’, Clade 17; grey circles = *P. colchicus*, Clade 18; purple circle = ‘Kuban *Phoxinus*’, Clade 19; pink circle = ‘Crimean *Phoxinus*’, Clade 20.

Moshu & Trombitskiy 2013; Artaev *et al*. 2021). From genetic data, Dniester *Phoxinus* was identified as Clade 9 (*P. marsilii*), Crimean *Phoxinus* as Clade 20 (*Cyprinus chrysoprasius* Pallas, 1814 as an earliest available name), Kuban *Phoxinus* as Clade 19, north-eastern Black Sea *Phoxinus* as Clade 18 (*P. colchicus*), and Volga and Ural drainages (upper reaches) as Clade 17 (still missing an available species name). A geographically nearby Clade 14 from the non-Danubian Black Sea rivers in Bulgaria and Turkey are assigned, at the present level of knowledge, to *P. strandjae* Drensky, 1926 (Palandačić *et al.* 2015, 2017, 2020).

The goal of the present study was to clarify the nomenclature and taxonomic status of the species name *Cyprinus chrysoprasius* as applied to Crimean *Phoxinus* in a geographical context using barcoding and morphological data for the neighbouring clades and species.

Material and methods

Genetic analysis

For the genetic analysis, 112 previously published mitochondrial cytochrome oxidase I (CO1) sequences (GenBank; Supp. file 2) were downloaded, aligned and trimmed to 651 bp. Subsequently, an unrooted minimum-spanning (haplotype) network was built using the median-joining algorithm implemented in Network 5.1 (www.fluxus-engineering.com) with default settings. The pairwise differences were calculated in MEGA6 using the Tajima-Nei model (Tajima & Nei 1984), which was estimated as the most fitting evolutionary model using ModelFinder (Kalyaanamoorthy *et al.* 2017) applying the BIC criterion.

Morphological analyses

Morphological analysis was based on historical and recent museum samples deposited in the Fish Collection of the Natural History Museum in Vienna, Austria (NMW), and the Alexander Koenig Zoological Research Museum in Bonn, Germany (ZFMK). Recent samples from Bulgaria (2003–2017) deposited in NMW were collected under permits of the Ministry of Agriculture, Food and Forestry of Bulgaria to Tihomir Stefanov at the National Museum of Natural History, Sofia (Bulgaria), and those from Ukraine, deposited in ZFMK, under the permit issued on 9 June 2003, by the State Department of Fisheries of the Ministry of Agrarian Policy of Ukraine (Krymazcherrybvod) to the Institute of Fisheries and Marine Ecology in Berdyansk, Ukraine (AzYugNIRO).

Fin ray counts follow Kottelat & Freyhof (2007). Terminology for the pattern of breast scale patches follows Kottelat (2007: 147, fig. 1) with subsequent detailing as described in Bogutskaya *et al.* (2019: 383, fig. 4, table 1). Methods, terminology, and abbreviations for the pattern of scalation on the breast and anterior belly, vertebrae and scale counts, and morphometric characters follow Bogutskaya *et al.* (2019: 380, fig. 2, tables 2–3) and are explained in that publication, as well as in Table 1 and in Supp. files 3–4. Standard length (SL) was measured from the tip of the upper jaw to the posterior margins of hypurals. Head length (HL), postorbital distance and interorbital width include the skin fold. Cranium roof length measured from the anterior margin of the supraethmoid to the base of the supraoccipital crest. Two characters (vertebral formula and type of scalation pattern on the breast and anterior belly) were numerically coded and included into statistical analysis. General terminology describing breeding tuberculation follows Chen & Arratia (1996).

To test if the examined samples (clades and potentially valid species identified from molecular analyses) were separated from each other in the morphospace, forward stepwise discriminant function analysis (DFA) was employed. Evaluation of the contribution of individual characters to the overall discrimination was based upon Wilks' Lambda values. The statistical analysis was performed using Microsoft Excel, Statistica 12 (StatSoft) and PAST ver. 4.09 (Hammer 1999–2022).

Table 1 (continued on the next page). Measurements, counts (variable in examined sample) and scalation type on breast and anterior belly (defined in Bogutskaya *et al*. 2019: table 1) of *P. chrysoprasius* (Pallas, 1814) (ZFMK 93640–59, 93921–30): ♀ (n = 13) and ♂ (n = 16, including the neotype).

	♀				♂				
Characters	**min**	**max**	**mean**	**S.D.**	**neotype**	**min**	**max**	**mean**	**S.D.**
Measurement									
SL (mm)	61.6	87.2			67.1	49.5	71.0		
Body depth at dorsal-fin origin (% SL)	23.3	25.7	24.6	0.8	22.7	20.9	24.3	23.0	1.1
Minimum depth of caudal peduncle (% SL)	9.3	11.3	10.2	0.6	10.5	9.9	11.9	10.9	0.5
Minimum depth of caudal peduncle (% length of caudal peduncle)	37.2	44.0	41.6	2.0	40.9	35.0	45.8	41.6	2.4
Maximum caudal peduncle depth (% SL)	11.9	13.5	12.6	0.5	13.1	12.1	14.1	13.3	0.5
Body width at dorsal-fin origin (% SL)	13.5	15.4	14.6	0.6	11.7	11.7	14.6	13.4	0.7
Caudal peduncle width (% SL)	9.3	10.8	10.0	0.5	8.9	8.8	10.4	9.8	0.5
Predorsal length (% SL)	54.2	56.8	55.6	0.7	52.4	47.3	56.9	53.1	1.9
Postdorsal length (% SL)	33.5	37.7	35.9	1.4	37.2	32.5	38.8	36.3	1.4
Prepelvic length (% SL)	46.9	51.0	48.6	1.3	44.2	40.7	48.1	45.6	1.8
Preanal length (% SL)	64.7	68.4	66.1	1.0	62.8	56.2	65.6	63.0	2.1
Pectoral – pelvic-fin origin length (% SL)	22.3	25.9	24.4	1.1	20.5	20.0	24.6	22.1	1.2
Pelvic – anal-fin origin length (% SL)	17.7	19.7	18.7	0.6	18.9	15.9	19.8	18.2	1.1
Caudal peduncle length (% SL)	22.4	26.2	24.5	1.2	25.7	23.7	28.5	26.3	1.3
Dorsal-fin base length (% SL)	11.2	13.2	12.3	0.7	12.8	11.5	13.7	12.7	0.5
Dorsal-fin depth (% SL)	16.4	19.5	18.5	1.0	20.7	19.0	24.1	21.0	1.2
Anal-fin base length (% SL)	10.2	12.6	11.6	0.8	11.5	10.5	13.1	11.7	0.6
Anal-fin depth (% SL)	15.4	18.8	17.6	0.8	19.4	17.2	20.6	19.5	1.0
Pectoral-fin length (% SL)	17.0	19.1	18.2	0.5	19.2	16.8	22.5	19.5	1.3
Pelvic-fin length (% SL)	14.3	16.2	15.2	0.5	16.4	16.2	18.5	17.6	0.8
Head length (% SL)	24.9	27.0	25.8	0.6	25.0	23.3	27.9	25.9	1.0
Head length (% body depth)	97.8	112.8	105.0	4.3	110.1	101.4	118.7	112.5	5.4
Head depth at nape (% SL)	15.3	17.9	17.1	0.8	17.8	16.0	18.6	17.5	0.9
Head depth at nape (% HL)	61.4	69.3	66.4	2.2	71.1	61.9	73.8	67.7	3.4
Head depth through eye (% HL)	48.8	56.4	51.6	1.9	51.0	46.1	55.8	51.7	3.2
Maximum head width (% SL)	13.1	16.3	14.8	0.9	13.5	12.6	14.9	13.9	0.7
Maximum head width (% HL)	51.5	61.8	57.3	2.7	53.9	49.6	59.4	53.7	2.3
Snout length (% SL)	7.6	9.1	8.4	0.4	7.8	7.6	9.0	8.3	0.4
Snout length (% HL)	30.3	35.2	32.4	1.5	31.0	30.0	34.9	32.1	1.4
Eye horizontal diameter (% SL)	5.5	6.2	5.7	0.2	5.5	5.2	6.3	5.7	0.3
Eye horizontal diameter (% HL)	20.7	24.5	21.9	1.1	21.8	19.9	23.7	22.1	1.0
Eye horizontal diameter (% interorbital width)	55.9	62.2	59.6	2.0	58.7	56.2	72.5	64.0	4.4
Postorbital distance (% HL)	46.7	53.1	49.8	1.8	50.4	46.5	53.3	50.9	1.5
Interorbital width (% SL)	8.8	10.4	9.6	0.5	9.3	8.2	9.8	8.9	0.5
Interorbital width (% HL)	34.7	41.1	37.2	1.6	37.2	30.9	38.7	34.6	2.3
Length of upper jaw (% HL)	30.4	34.8	32.5	1.3	29.9	28.2	32.7	29.9	1.1
Length of upper jaw (% SL)	7.8	9.9	8.5	0.6	7.5	6.8	8.6	7.7	0.4
Length of lower jaw (% SL)	9.2	10.2	9.7	0.3	8.7	8.2	12.0	9.3	0.9
Length of lower jaw (% HL)	34.9	39.4	37.6	1.2	34.9	32.7	43.0	36.0	2.4
Length of lower jaw (% interorbital width)	90.0	113.4	101.4	6.1	93.9	85.8	139.3	104.5	11.9
Length of lower jaw (% depth of operculum)	100.2	124.5	111.0	7.4	105.4	101.3	122.1	106.3	5.1
Length of lower jaw (% cranium roof length)	51.3	59.9	57.1	2.8	51.8	47.4	66.9	53.1	4.1
Cranium roof length (% SL)	15.9	18.4	17.0	0.7	16.9	15.6	19.8	17.5	0.9

Table 1. (continued).

	♀				♂				
Characters	**min**	**max**	**mean**	**S.D.**	**neotype**	**min**	**max**	**mean**	**S.D.**
Cranium width between margins of pterotics (% cranium roof length)	62.6	70.2	67.4	1.9	63.0	58.3	66.7	64.2	2.4
Cranium width between margins of sphenotics (% cranium roof length)	56.0	61.6	58.5	1.4	49.0	49.0	58.6	54.1	2.4
Ratios									
Interorbital width/eye horizontal diameter	1.6	1.8	1.7	0.1	1.70	1.4	1.8	1.6	0.1
Snout length/eye horizontal diameter	1.3	1.6	1.5	0.1	1.42	1.3	1.6	1.5	0.1
Head depth at nape/eye horizontal diameter	2.7	3.2	3.0	0.2	3.26	2.7	3.3	3.1	0.2
Head length/caudal peduncle depth	2.3	2.8	2.5	0.1	2.38	2.2	2.6	2.4	0.1
Length of caudal peduncle/caudal peduncle depth	2.3	2.7	2.4	0.1	2.44	2.2	2.9	2.4	0.1
Length of lower jaw/caudal peduncle depth	0.9	1.1	1.0	0.1	0.83	0.7	1.1	0.9	0.1
Pectoral fin length/pectoral–pelvic-fin origin distance	0.7	0.8	0.7	0.0	0.94	0.7	1.1	0.9	0.1
Predorsal length/head length	2.1	2.3	2.2	0.1	2.09	1.9	2.2	2.1	0.1
Counts									
Number of branched pectoral-fin rays	15	17	15.6	0.7	15	15	16	15.8	0.4
Total number of scales in lateral series	84	98	90.3	5.3	86	83	97	88.5	3.4
Total number of lateral-line (pored) scales	57	91	78.1	8.6	55	55	85	73.5	8.3
Number of lateral-line scales in first complete section of lateral line	11	83	41.2	23.7	30	14	85	52.0	22.4
Number of predorsal vertebrae	14	16	14.6	0.7	15	14	15	14.6	0.7
Number of abdominal vertebrae	22	23	22.5	0.5	22	22	23	22.4	0.5
Number of caudal vertebrae	17	19	18.0	0.6	18	17	19	18.0	0.6
Total vertebrae	39	42	40.5	0.9	40	39	42	40.5	0.7
Number of anal-fin pterygiophores in front of first haemal spine	4	6	5.0	0.7	5	4	6	5.0	0.7
Type of scale pattern on breast and anterior belly	3	6	4.2	1.2	3	3	6	4.1	1.1

Results

Examined material

Species identification was based upon taxonomic and molecular clade assignments as in Palandačić *et al.* (2017, 2020) and Bogutskaya *et al.* (2019). In the following list '*' indicates samples that contain specimens (specs) examined genetically.

Phoxinus chrysoprasius, Clade 20

UKRAINE • 17 specs, 49.5–87.2 mm SL; at Krasna Sloboda village, upper reaches of Kuchuk-Karasu, Salhir drainage; 44°58′26″ N, 34°44′19″ E; 16 Jun. 2003; N. Bogutskaya, O. Diripasko, A. Naseka and J. Freyhof leg.; ZFMK 93640–59* • 12 specs, 54.3–73.7 mm SL; River Salhir upstream of Simferopol Reserve, at Pereval'ne; 44°51′12″ N, 34°18′47″ E; 17 Jun. 2003; N. Bogutskaya, O. Diripasko, A. Naseka and J. Freyhof leg.; ZFMK 93921–30.

P. marsilii, Clade 9

AUSTRIA • lectotype, 64.1 mm SL; Wien River, Danube drainage; 1836; collector unknown; NMW 51225* • 5 paralectotypes, 46.8–74.1 mm SL; same collection data as for lectotype; NMW 98672* • 21 specs, 44.9–77.2 mm SL; Wien River at Purkersdorf and Hütteldorf; 48°12′30″ N, 16°12′27″ E; 10 Sep. 2014; D. Ramler leg.; NMW 98664*.

P. strandjae, Clade 14

BULGARIA • Subclade 14a (Bulgaria): 12 specs, 43.1–50.1 mm SL; Luda Kamchiya R., downstream of Ichera, Kamchiya drainage; 42°46′43.5″ N, 26°29′24″ E; 26 Jul. 2010; T. Stefanov leg.; NMW 100449 • 51 specs, 25.0–63.3 mm SL; Medvenska R., at Dabovitsa, Kamchiya drainage; 42°48′47″ N, 26°37′37″ E; 26 Jul. 2010; T. Stefanov leg.; NMW 100450 • 40 specs, 35.1–55.7 mm SL; Izvorska R. at Indzhe Voyvoda;, 42°09′50″ N, 27°05′52″ E; 26 Jul. 2010; T. Stefanov leg.; NMW 100452 • 42 specs, 33.5–64.3 mm SL; Orlyashka R. at Vizitsa, Kitenska [Karaagach] R; 42°08′19″ N, 27°36′53″ E; 26 Sep. 2014; T. Stefanov leg.; NMW 100453• 42 specs, 41.4–59.3 mm SL; Mladezhka R., Veleka [Kocadere] drainage; 42°05′31″ N, 27°23′47″ E; 18 Oct. 2017; T. Stefanov leg.; NMW 99092.

TURKEY • Subclade 14b 24 specs, 40.0–58.4 mm SL; Kurtköy stream at Kurtköy, Lake Sapanca basin, Sakarya; 40°42′46″ N, 30°10′30″ E, 8 Jun. 2006; ZFMK 93593–624* • 16 specs, 42.6–56.3 mm SL; Çanakkale, Gönen R. west of Akçakoyun, Biga Peninsular; 39°49′36″ N; 27°08′36″ E, 30 Oct 2007; ZFMK 94196–215.

P. csikii, Clade 5a

BULGARIA • neotype, 84.4 mm SL; Rožaje, Ibar, Zapadna Morava-Velika Morava system, Danube, Montenegro, ca 42°50′39″ N, 20°09′59″ E; 1917; NMW 51266* • 5 specs, (neotype), 78.9–91.1 mm SL; same data as neotype; NMW 98673* • 30 specs, 46.2–68.1 mm SL; Nishava R. at Tuden, Velika Morava system, Danube, Bulgaria, 43°0′13″ N 23°0′26″ E; T. Stefanov, 2 Nov. 2017; NMW 99088 • 33 specs, 45.1–67.3 mm SL; Beli Vit R. at Ribaritsa, Danube; 42°50′16″ N, 24°22′52″ E; T. Stefanov, 6 Nov. 2017; NMW 99089 • 55 specs, 50.9–78.4 mm SL; Palakaria R. at Yarlovo, Iskar system, Danube; 42°28′58″ N, 23°16′12″ E; 20 Oct. 2017; T. Stefanov; NMW 99090.

P. colchicus, Clade 18

GEORGIA • Ilia State University, Institute of Zoology, Tbilisi, no catalogue numbers • 2 ♂♂, SL 32.9–43.6 mm, 1 ♀, SL 76.5 mm; Kintrishi River; 41°48′13″ N, 41°46′42″ E; Jul. 2018; B. Japoshvili leg. • 4 specs, SL 30.6–34.8 mm, 1 ♀, SL 59.3 mm; Natanebi River, Georgia, 41°55′35″ N, 41°58′2″ E; Aug. 2016; B. Japoshvili leg.

Phoxinus sp. Kuban’, Clade 19

UKRAINE • 6 specs, 42.2–48.7 mm SL; Adagum River at Krymsk, Kuban’ drainage; 44°54′03″ N, 37°58′31″ E; 23 Jul. 2001; N. Bogutskaya, A. Naseka and J. Freyhof leg.; ZFMK 79044–49.

Genetic analysis

A single haplotype was detected in all five examined Crimean specimens (Supp. file 2), previously recognised as genetic clade 20 from the Salhir drainage (Palandačić *et al.* 2017, 2020). From the CO1 mitochondrial gene sequences, this haplotype forms its own haplogroup in the network (Fig. 2) and is 33–97 mutational steps distant from mitochondrial clades analysed in this study, supporting its distinctiveness. Pairwise distances between *P. chrysoprasius* and the other clades or species range between 4% and 7% (Supp. file 5).

Statistical morphological analysis

Samples were first compared using only non-morphometric characters to exclude the influence of sexual dimorphism and be able to include the *P. marsilii* samples for which we did not have a complete set of morphometric data because of variable preservation condition of the specimens. The number of specimens per sample with SL > 45 mm that are sub-adult and adult (314 specimens in total), and a list of characters (11 counts and 2 coded characters represented by 97 character states) are reported in Supp. file 4. DFA (Fig. 3) did not reveal a clear differentiation of the groups. We found that only 63.7% of the specimens were classified correctly. However, the Crimean specimens were differentiated at a relatively high level with 26 out of 29 specimens correctly classified. Characters that contributed most to the discrimination between the groups were the relative length of the first uninterrupted section of the lateral line, the total number of vertebrae and the numbers of abdominal and caudal vertebrae.

DFA was then performed using all characters (meristic, coded and morphometric) (Fig. 4) in the samples geographically closest to the Black Sea (Bulgarian *P. strandjae*, Turkish *P. strandjae*, *P. colchicus* and Crimean) for females and males separately, as the species exhibits considerable sexual dimorphism as

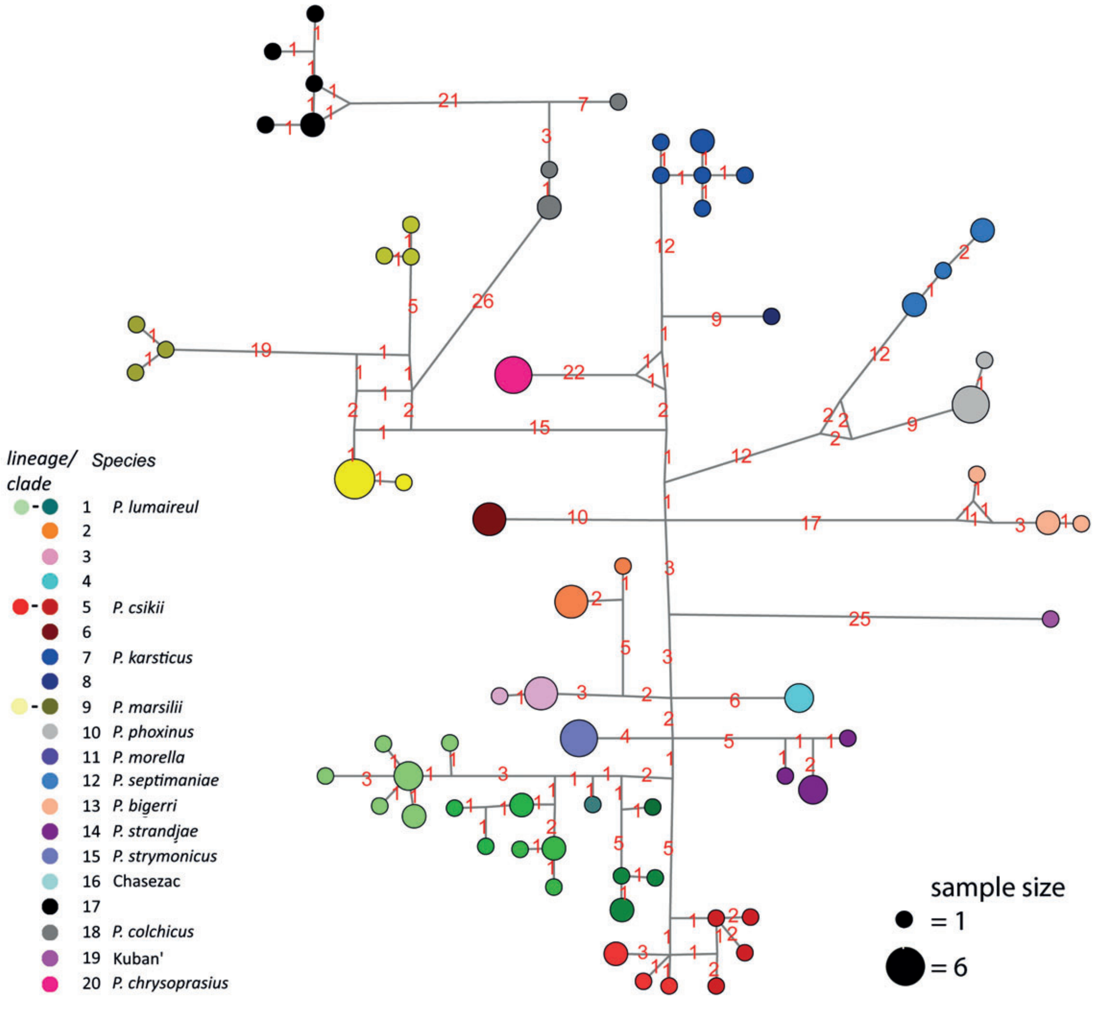

Fig. 2. A haplotype network based on cytochrome oxidase I (CO1) fragment using 112 previously published GenBank sequences and representing 20 genetic clades numbered as in Palandačić *et al.* (2017, 2020), of which 12 are considered valid species including *P. chrysoprasius* (Pallas, 1814) and Kuban' *Phoxinus* (an available species name not known). The lines carry the number of mutational steps (shown in red).

described below. All specimens were classified successfully (100%), and both males and females of *P. chrysoprasius* were clearly distant from the respective subsamples of *P. colchicus* and *P. strandjae* (Fig. 4A). Total number of lateral-line scales contributed most to the discrimination between samples, followed by the caudal-peduncle length (% SL), caudal-peduncle depth (both % of caudal-peduncle length and % SL), prepelvic length (% SL) and total number of scales in the lateral series.

As some of the result might be influenced by differences in the number of females and males (only two males in Turkish *P. strandjae* and absent in *P. colchicus*), we undertook an analysis for females only (Fig. 4B). All female specimens were 100% classified. The subsample of Crimean females was almost equidistant from *P. colchicus* and Bulgarian *P. strandjae* females (squared Mahalanobis distance 568.445 and 609.09, respectively), but less so (419.83) from the Turkish *P. strandjae* subsample.

In combination, CO1 and the morphological data of the present study strongly support the validity of the native Crimean *Phoxinus* under the earliest available name of the species: *P. chrysoprasius*.

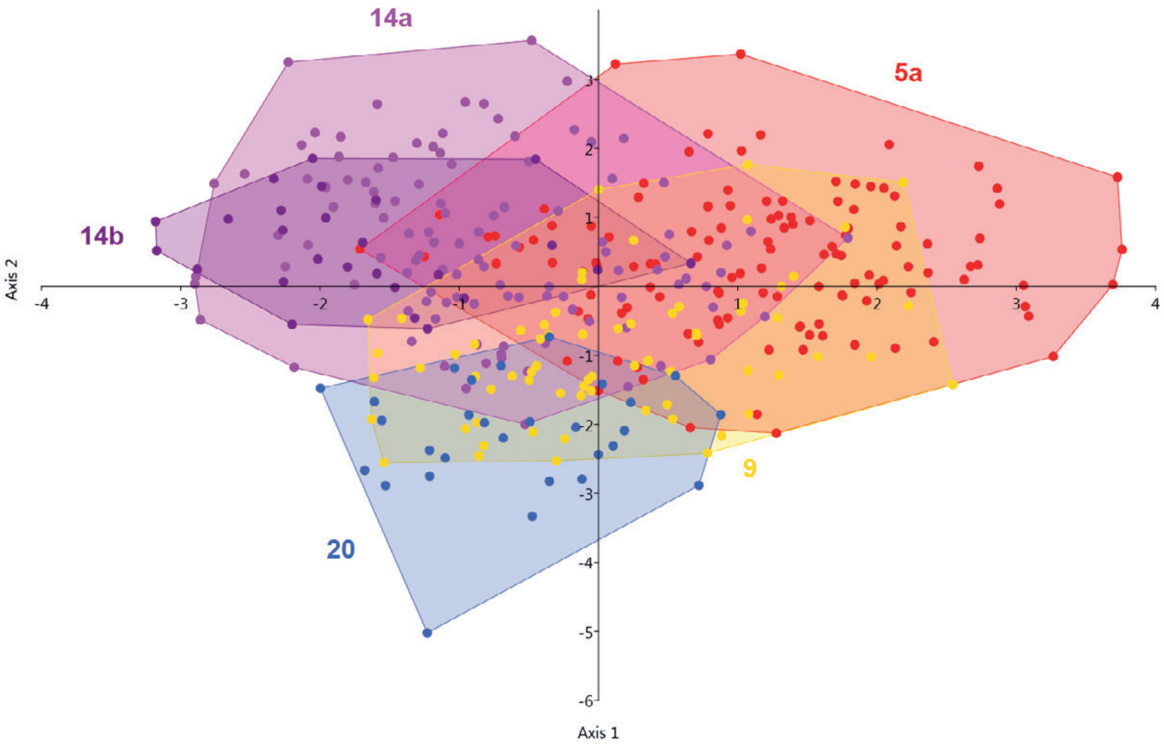

Fig. 3. DFA based on 11 counts and 2 coded characters as in Supp. file 4. Clade 5 = 5a, Danubian tributaries in Bulgaria (Nishava, Beli Vit, and Palakaria samples and *P. csikii* Hankó, 1922 from type locality). Clade 14 = non-Danubian rivers of the Black Sea coast in Bulgaria (14a, Izvorska, Veleka, Karaagach and Kamchiya) and Turkey (14b, Gönen and Sapanca). Clade 9 = *P. marsilii* Heckel, 1836. Clade 20 = Crimea, Salhir. DFA statistics values: Wilks' Lambda 0.16694, approx. F (45.1345) = 14.709, p < 0.0000 (perfect discrimination).

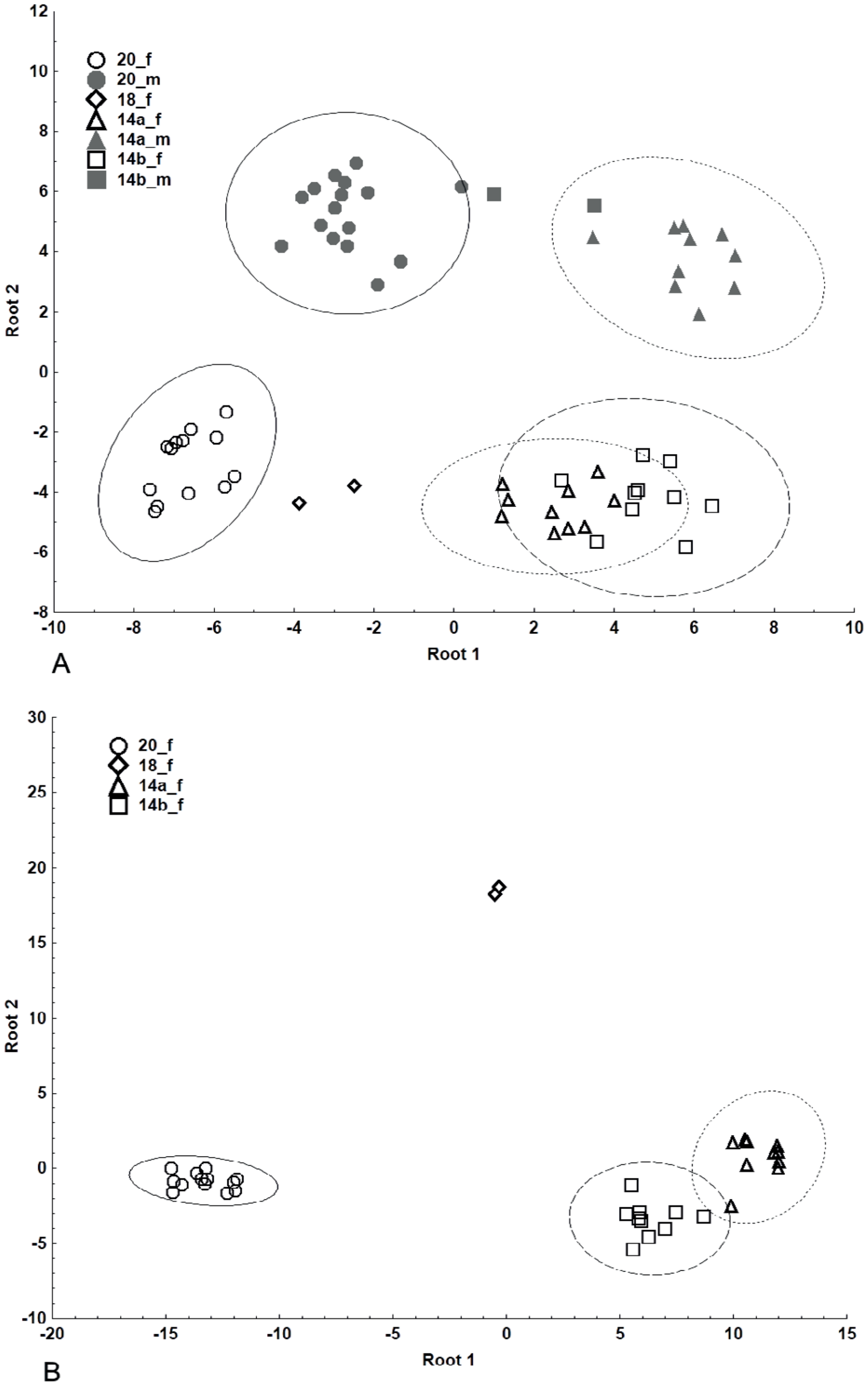

Fig. 4. DFA based on 11 counts, two coded characters, and 55 relative measurements, for males and females separately (**A**) and for females only (**B**). Numbers of samples or clades as in Fig. 3. Abbreviations: f = females; m = males. DFA statistics values: **A**. Wilks' Lambda 0.00000, approx. *F* (156.189) = 11.390, p < 0.0000. **B**. Wilks' Lambda 0.00002, approx. *F* (48.48) = 36.279, p < 0.0000 (perfect discrimination).

Taxonomy

Class Actinopterygii Klein, 1885
Order Cypriniformes Bleeker, 1859
Family Leuciscidae Bonaparte, 1835
Genus *Phoxinus* Agassiz, 1835

Phoxinus chrysoprasius (Pallas, 1814)
Figs 5–6

Cyprinus chrysoprasius Pallas, 1814: 318 (mountain rivulets of the Crimea Peninsula).

Cyprinus phoxinus – Gablitz 1785: 182 (Salhir with tributaries Kuchuk-Karasu and Biyuk-Karasu).
Cyprinus chrysoprasius – Rathke 1836: 345 (Crimea). — Nordmann 1840: 483 (Crimea).
Phoxinus laevis – Kessler 1859: 539 (River Salhir at Simferopol'); 1877: 257 (partim: Crimea). — Dybowski 1862: 105 (partim: Crimea).
Phoxinus phoxinus – Berg 1912: 246 (partim: Crimea).

Morphological diagnosis

Among the species and clades examined of *Phoxinus* in this study, in the geographically close areas or drainages (*P. colchicus*, *P. csikii* and *P. strandjae*), *P. chrysoprasius* is distinguished by having a less scaled breast and anterior belly (type 3–6, commonly 3 or 4, vs type 3 to 12, commonly 5 to 11), the longest lateral line, with 55–91, commonly 71–90, pored scales, averaging 75.6 (vs 12–80, commonly 31–70, averaging 44.7–61.8), and the longest first uninterrupted section of the lateral line, 11–85 pored scales, commonly 21–60, averaging 47.1 (vs 2–71, commonly 11–50, averaging 15.2–36.0) pored scales. *Phoxinus chrysoprasius* is closest to the geographically distant Upper Danubian *P. marsilii* which is also characterised by a relatively less scaled breast and anterior belly (type 3–7, commonly 3 or 4) and a long lateral line (45–90, commonly 71–80, pored scales), but is distinguishable from the latter by a smaller difference between numbers of abdominal and caudal vertebrae, 3–6, averaging 4.8 (vs 1–4, averaging 3.0), a deeper caudal peduncle: caudal-peduncle depth 2.2–2.9, averaging 2.4, × in its length (vs 2.8–3.5, averaging 3.2), and no breeding tubercles on the breast scales in males (vs present).

Additionally, *P. chrysoprasius* is distinguished from other species or clades of *Phoxinus* (*P. krkae* Bogutskaya *et al*., 2019, *P. lumaireul* (Schinz, 1840), *P. septimaniae* Kottelat, 2007) examined earlier (Bogutskaya *et al*. 2019) by a combination of characters, none of which is unique, as follows: a deep caudal peduncle, minimum depth of caudal peduncle 2.2−2.9 × in caudal peduncle length; upper lip projecting beyond lower lip; rostral fold absent; mouth clearly subterminal, tip of mouth at level markedly below lowest point of eye; scales relatively small, total number of scales in lateral series 83−98; lateral line long, with 55−91 pored scales; lateral-line interruptions occurring in few places, commonly only on posterior body and caudal peduncle; scales on belly not extending over middle of the distance between pelvic- and pectoral-fin origins; patches of breast scales disconnected (commonly widely disconnected); total vertebrae commonly 40−41 (most frequent vertebral formulae 22 + 18 and 23 + 18); 4−6, commonly 5, anal-fin proximal pterygiophores anterior of first haemal spine of first caudal vertebra.

Molecular diagnosis (CO1 barcoding)

The single CO1 haplotype of *P. chrysoprasius* is presented in Supp. file 2. It is 33 mutational steps distant from the genetically closest mitochondrial clade (Clade 8: unnamed *Phoxinus* from Lake Ohrid) and 55 mutational steps distant from the geographically closest mitochondrial clade (Clade 19: unnamed *Phoxinus* from River Kuban').

Etymology

The etymology is not explained in the original description. The species epithet, the adjective "*chrysoprasius*", apparently refers to a characteristic feature of the colouration being a combination of "*chrys*" (Greek for "gold or yellow") and "*prase*" (from the Greek for "leek", referring to some greenish colouring also present).

Type series

Specimens belonging to the type series of the species, *Cyprinus chrysoprasius* Pallas, 1814: 318, cannot be located at present and were apparently not preserved by Pallas (at least we are unaware of any published indications of their existence). The original description (Pallas 1814: 318) does not unambiguously distinguish the species.

Material examined

Neotype (Fig. 5A)

UKRAINE • ♂, 67.1 mm SL; upper reaches of the river Kuchuk-Karasu at Krasna Sloboda village, a tributary of Biyuk-Karasu, a tributary of Salhir; 44°58′26″ N, 34°44′19" E; 16 Jun. 2003; collectors N. Bogutskaya, O. Diripasko, A. Naseka and J. Freyhof leg.; ZFMK 93640–59.

Remarks

It was examined morphologically and genetically (CO1) and designated herein as neotype. The entire set of data of the neotype in provided in Supp. file 2 (rhodopsin and CO1 data), and in Table 1 and

Fig. 5. A. Neotype of *Phoxinus chrysoprasius* (Pallas, 1814), ♂ (ZFMK 93640-59). **B**. ♀ (ZFMK 93640-59), 87.2 mm SL, same locality and date as the neotype.

Supp. files 3–4 (counts, scalation and morphometrics). The neotype has a moderately long lateral line and scalation pattern on the breast and anterior belly of type 3 (scale patches widely disconnected); scales in total lateral series, 86; scales in total lateral line, 55; scales in first uninterrupted section of lateral line, 39; predorsal abdominal vertebrae, 15; total vertebrae, 40; abdominal vertebrae, 22; and anal-fin pterygiophores anterior of first haemal spine, 5.

Comments on neotypification

Since the 1920s, Crimean freshwater drainages have been altered considerably by constructions of diverse network of irrigation channels and reservoirs, including the Severo-Krymsky (North Crimean) irrigation canal, which connected Crimea with the Dnieper drainage. As a result, 19 non-native taxa appeared in the Crimean inland waters (Karpova & Boltachev 2011; Karpova 2017). Therefore, we cannot exclude anthropogenic translocations or invasions, although not yet recorded, of non-native *Phoxinus* on the peninsula, as has occurred with some clades of *Phoxinus* in other regions of Europe (reviewed in Palandačić *et al.* 2020). Neotypification provides nomenclatural support for the name *Phoxinus chrysoprasius* to be irrefutably applied to the native common minnow of the Salhir River in the Crimea Peninsula in the modern concept based on molecular data.

Accordingly, a specimen, examined morphologically and genetically (CO1), is designated herein as neotype for *P. chrysoprasius* under the conditions stipulated in art. 75.3 of the International Code of Zoological Nomenclature (ICZN 1999). This act satisfies the provisions of art. 75 of the Code by clarifying its taxonomic identity in the modern concept (art. 75.3.1); nominating the above-mentioned combination of its phenotypic characters and CO1 haplotype as diagnostic features (art. 75.3.2); providing data and description sufficient to ensure recognition of the designated specimen (art. 75.3.3); giving reasons and references for believing that original type material is lost (art. 75.3.4); selecting a neotype consistent with the original description of the species (art. 75.3.5); choosing a neotype from the originally cited type locality, the Crimea Peninsula (art. 75.3.6); and recording that the neotype is the property of a recognised scientific institution, the Zoological Research Museum Alexander Koenig in Bonn (art. 75.3.7).

Fig. 6. *Phoxinus chrysoprasius* (Pallas, 1814), living specimen, spawning male, Angara River, Salhir drainage, 44°51′12″ N, 34°18′47″ E; 16 Jun. 2003.

Description

The general appearance of Crimean *Phoxinus* is shown in Figs 5–6; meristic characters, types of scalation pattern on the breast and anterior belly and relative measurements of 29 adult specimens of both sexes (SL 49.5–87.2 mm), including the male neotype of *P. chrysoprasius* (designated above), are given in Supp. files 3–4.

Longest examined specimen (female) 87.2 mm SL (Fig. 5B). Body moderately stout, with deep caudal peduncle (minimum depth of caudal peduncle 2.2−2.9 × caudal peduncle length), dorsal and ventral profile roughly symmetrical with dorsal profile sometimes slightly more convex, and small hump at nape present in some larger specimens. Snout markedly stout and rounded. Mouth subterminal, tip of mouth at level to slightly to markedly below lowest point of eye, mouth cleft short. Upper lip projecting beyond lower lip, rostral fold absent.

Dorsal fin with 3 unbranched and 7½ branched rays. Distal dorsal-fin margin slightly concave, dorsal-fin origin markedly behind pelvic-fin insertion. Anal fin with 3 unbranched and 7½ branched rays, distal anal-fin margin is about straight or slightly convex.

Lateral line well developed though incomplete and interrupted in, commonly, posterior section only. First uninterrupted section of lateral line variable (11–85 scales), interruptions occurring in a few to many places in posterior region of body and caudal peduncle, lateral line containing 55–91 scales in total, commonly reaching beyond anal base. Total lateral series (including scales at caudal-fin base) 83–98 scales.

Scales on belly occasionally extending forward to beyond middle of distance between pelvic-fin base and pectoral-fin origin, but usually less. Patches of breast scales widely or narrowly separated (types 3 and 4) in 19 out of 29 examined specimens; few scales in between patches (type 5) and patches connected by 1–3 scales (type 6) found in 4 and 6 specimens, respectively.

Total vertebrae 39–42, commonly 40–41; abdominal vertebrae 22−23; predorsal vertebrae 14–15 (16 in one specimen); most common vertebral formulae 22 + 18 and 23 + 18; anal-fin pterygiophores anterior of first haemal spine not numerous, 4−6.

COLOURATION. In formaldehyde-preserved specimens (Fig. 5), basic colour pattern consisting of 10–16 blackish mid-lateral bars or blotches, sometimes fused, not extending below horizontal of lower half of eye, pale lower half of flank and belly, and dark marking on back; black spot at middle of caudal-fin base. Dark bars or blotches are not apparent when the fish are alive, when the overall colouration is golden bronze or brown with a wide mid-lateral stripe of varying golden intensity overlaid on blackish stripe (Fig. 6). Golden colouration first recorded as early as the 18th century by Gablitz (1785) who wrote about the "gold" stripe on the sides of Crimean minnow, while three decades later, Pallas (1814) named the species referring to its "golden" colour. No extensive areas of pink, orange or red observed in adults collected in June 2003, immediately after spawning period, apart from a slight pink tinge to the bases of the pelvic and anal fins. Also, iridescent blue patches observed in larger adult males (in June) on the snout, gill covers and behind the head.

SEXUAL DIMORPHISM. Not observed with regard to the breast scalation type or meristic characters, though statistically significant for 10 morphometric characters, and prominent for % SL of pectoral-fin length (greater in males, reaching or almost reaching pelvic-fin origin), pelvic-fin length (greater in males, reaching or almost reaching anal-fin origin), anal-fin depth (greater in males), maximum body depth (greater in females), caudal-peduncle width (greater in females), head width (greater in females), and the % cranium roof length of cranium width between margins of pterotics (greater in females). DFA analysis clearly differentiated males from females in morphospace (DFA statistics values: Wilks'

Lambda 0.00006, approx. *F* (40.18) = 59.386, p < 0.0000, perfect discrimination). Pectoral-fin in males broad and markedly rounded (Fig. 5A) with thickened rays vs narrow pointed fin without thickened rays in females (Fig. 5B). Genital papilla in males well developed, located just behind the anus and often protruding beyond the latter vs absence of genital papilla or a poorly developed papilla located at some distance from the anus in females. Mature males bear breeding tuberculation on head (size of the tubercles varies significantly; we roughly grouped them as 'large', 'medium', 'small' and 'very small'). Breeding tubercles arranged in a regular manner and in larger sized (> 55 mm SL) males (Fig. 5A). Paired rows (number of tubercles on both sides of the head): in front of the naris (1 or 2, medium to large), between nares (2, large), in front of and below the orbit (0–4, commonly 0 or 1; commonly small if present) and along dorsal rim of the orbit (3–6, medium to large). Unpaired rows: a group of tubercles in interorbital space between rows along the dorsal rim of the orbit (3–7, small to large) and at the nape (7–28, small to medium). Tubercles along the temporal sensory canal and just behind the head along the lateral line, with variable number (0–13, very small to medium if present); the most numerous tubercles in this area – 6 on the left and 13 on the right – were found in the largest male of 71 mm SL. Tubercles were apparent neither on pectoral fin nor on breast scales.

Distribution

Type locality

The type locality from the original description (Pallas 1814: 318) reads "In rivulis saxosis montosae orae Chersonesi taurica" ("at rocky banks of mountainous rivulets of the Tauric Chersonese [Crimea Peninsula]").

Note on type locality

The type locality of *P. chrysoprasius* is commonly misinterpreted because of incorrect translation of the original Latin text. For example, in Eschmeyer's Catalog of Fishes, the type locality is given as "hill streams on Mount Chersones, Crimea" (Fricke *et al.* 2021) but there is no Mount Chersones in the Crimea. The word "chersonese" (ancient Greek "χερσόνησος") translates as "a peninsula", and "Chersonesi taurica" literally means "the peninsula of Tavria". The toponym Tauric Chersonese, applied to the whole of the Crimean Peninsula, should not be confused with the name Chersonesos, an ancient Greek colony, which aptly describes the site (a peninsula) on which the colony was established.

Peter Simon Pallas, a native German, worked in Russia from 1767 on and performed extensive natural history studies in various regions of the Russian Empire. During the period 1793–1794, he conducted expeditionary research in its southern regions, as a result of which he published, in German, a two-volume book "*Bemerkungen auf einer Reise in die südlichen Statthalterschaften des Russischen Reichs in den Jahren 1793 and 1794*" (Observations made during a trip to the southern governorships of the Russian state in 1793–1794) (Pallas 1801). The second volume contains a description of the journey to the Crimea. Even from the first paragraphs of this description, it is clear that Pallas was well acquainted with the history of the peninsula, using the word "Crimea" (derived from the Tatar toponym Qirim or Qyrym), and at the same time gives its ancient name, the Tauric Chersonesus, for example, "Der erste Wohnplatz der eigentlichen Krym oder des Alters so genannten Taurischen Chersones, ist Perekop, oder Or-Kapi, wie es die Tataren nennen" (The first dwelling place of the actual Krym or of the age of the, so-called Taurian Chersonese, is Perekop, or Or-Kapi as the Tatars call it) (Pallas 1801: 4). While staying in the vicinity of Sevastopol, Pallas paid attention to the description of the ancient town of Chersones, and it is clear that he distinguished between the name of the ancient Greek colony and the name of the peninsula.

Comments on distribution

The Crimean minnow was first reported by Gablitz (1785: 182) in the upper reaches of the Salhir River and its tributaries Kuchuk-Karasu and Biyuk-Karasu (= Malaya Karasevka and Bol'shaya Karasevka) under the name *Cyprinus phoxinus*. Pallas visited Salhir during his trips and described its fishes (Pallas 1801: 20) but later Pallas (1814) did not specify the names of the rivers where he observed the species described as *C. chrysoprasius*, perhaps because he took for granted the occurrence of the species in other rivers of Crimea with a similar biotope. Rathke (1836: 345) and Nordmann (1840: 483) also used the name *Cyprinus chrysoprasius* in their studies of the fauna of Crimea, and also provided no indication of the rivers where the species occurred, though Nordmann (1840: 483) mentioned that it was "abundant in all rivers of Crimea, especially in the Salhir". In later publications on the freshwater fish fauna of the Russian Empire, researchers indicated the distribution of *C. chrysoprasius* Pallas, 1814 (synonymised with *Phoxinus laevis* Agassiz, 1832 or *Phoxinus phoxinus* (Linnaeus, 1758)) as 'Crimea': Kessler (1859: 539–540) described specimens of the Salhir drainage in the Simferopol region; Dybowski (1862: 105) reported it "in the Crimean mountain rivers"; and Kessler (1877: 257) and Berg (1912: 260), as "occurring in the Crimea". Tseeb (1929) was apparently the first to limit the range of '*Phoxinus phoxinus*' on the Crimea Peninsula to the rivers of the Salhir drainage (Sea of Azov basin), emphasising that it did not occur in the rivers of the Crimea flowing to the Black Sea. Berg (1949: 590) noticed this significant clarification, and corrected the range of Crimean *Phoxinus* to "in the Crimea, but only in the Salhir drainage".

The historical and present distribution of the common minnow in Crimea has been thoroughly revised and analysed in recent publications (Miroshnichenko 2003; Karpova & Boltachev 2011, 2012; Karpova 2017) clearly indicating that it only occurs in the Salhir River drainage.

Discussion

The results of the present study are in congruence with previous findings (Palandačić *et al.* 2020) and confirm the mitochondrial genetic clade 20 (Salhir) as well distinguished. In addition, our morphological analysis of the same sample confirms its distinctiveness from species or clades in neighbouring areas or drainages.

Clade 20 was previously recognised as a distinctive mitochondrial lineage, possibly corresponding to *P. chrysoprasius* based on an analysis of the barcoding fragment of CO1 (Palandačić *et al.* 2020). In that earlier study, while the clade itself was well supported, the relationships between geographically adjacent clades remained unresolved and the deep nodes of the tree not statistically supported. In addition, the data on nuclear genes were inconclusive or missing. In the present study, based on the haplotype network, clade 20 was well distinguished (Fig. 2), and the genetic distances between the sequences ranged between 4% and 7% (Supp. file 5). The CO1 "barcoding gap" ought not serve as a single basis for species delimitation and remains a rough approximation at best (Ferguson 2002; Padial *et al.* 2010; Goldstein & DeSalle 2010). Nevertheless, a CO1 divergence of 4–7% provides a solid argument favouring resurrection of *P. chrysoprasius* as a valid species, especially as it exceeds the widely used value of 2%, considered a universal threshold in freshwater fishes between taxonomically distinct species (e.g., Hebert *et al.* 2003a, 2003b; Geiger *et al.* 2014; Rossini *et al*. 2016).

As to phenotypic diagnosability, not a single character used in the morphological analysis clearly distinguished Crimean *P. chrysoprasius* from the geographically neighbouring clades or species. This is unsurprising as many species of *Phoxinus* are highly similar when traditional complexes of characters (colouration, scalation, length of lateral line) are used (Bogutskaya *et al*. 2019; present study: Supp. file 4). Nevertheless, these characters in combination with morphometric characters provide a better discrimination signal, especially when males and females are compared separately. Thus, our data show that the Crimean clade possesses a morphological specificity, distinguishing it from all other examined samples, particularly with the presence of the longest lateral line (absolute and relative numbers of total lateral-line scales), and the longest first uninterrupted section of the lateral line. As data on live colouration in different species of European *Phoxinus* from different localities are rare, they do not enable a diagnostic value of the 'golden stripe' and overall bronze-brownish tint in live Crimean *Phoxinus* (most morphological data come from preserved specimens which lack the gold tint). Our preliminary data on the breeding tuberculation pattern in various samples (Bogutskaya *et al.* 2019; this study) confirmed its possible taxonomic value but, owing to the quality of preservation and different seasons of collection, our data did not allow us to examine the pattern of tuberculation in equal detail. Therefore, we do not discuss further this pattern here.

A particularly interesting result of the present study was that the geographically closest forms *P. chrysoprasius*, *P. colchicus*, and Kuban' *Phoxinus* (Fig. 1) were genetically the most distant. Pairwise distances based on CO1 between *P. chrysoprasius* and *P. colchicus*, and between *P. colchicus* and the Kuban' sample were 7% (Supp. file 5). The specimens of *P. colchicus* genetically examined in the present study came from Natanebi River, the type locality of *P. colchicus*, originally described by Berg (1910: 169) from Bachvis Tzchali River (Bakhvistsqali) in the upper reaches of Natanebi River in Ozurgety District, Georgia (Black Sea basin, around 42°52′ N, 42°19.5′ E). *Phoxinus* from Kuban' drainage in Russia (Sea of Azov basin) was commonly considered conspecific with *P. colchicus* following Berg (1949: 590). However, Emtyl' and Ivanenko (2002: 90, fig. 59) refer to an earlier study of Emtyl' and co-authors in 1994 that demonstrated morphological differences between Kuban' *Phoxinus* and *P. colchicus* (we failed to find that earlier publication) and used the name '*Phoxinus phoxinus kubanicum* sp. nov.' (where it ought to be *kubanicus* as *Phoxinus* is masculine). The name is accompanied by a comparative description but not available because it does not comply with the criteria for the conditions stipulated in art. 16.4 of the International Code of Zoological Nomenclature (ICZN 1999) for species-group names proposed after 1999, as it is not accompanied by an explicit fixation of a holotype or syntypes for the nominal taxon (art. 16.4.1.) and a statement of deposit in a collection (art. 16.4.2.). The name *Phoxinus kubanicus* is later used at least once (e.g., Otrishko & Emtyl' 2012), but again in a manner that does not make the name available. Unfortunately, we did not have enough material of *P. colchicus* and none from the Kuban' drainage to implement a proper morphological comparison, and the taxonomic status of the two species requires further study.

Meanwhile, the genetic data reported in the present study (and in previous studies) support the occurrence of three distinctive species with a remarkable 5–7 % genetic difference among them.

Acknowledgements

This research was undertaken through research programmes of the Austrian Science Foundation (FWF), Lise Meitner Programme Project M2183–B25. Our sincere thanks go to Ernst Mikschi and all other members of the NMW Fish Collection, to Bela Japoshvili (Institute of Zoology, Tbilisi, Georgia), and to Fabian Herder and Serkan Wesel (ZFMK), for their valuable help in studying the collections under their care as well as to Alexander Naseka (Slovenia), Jorg Freyhof (Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany), and Tihomir Stefanov (Natural History Museum, Sofia, Bulgaria) for the organisation of the field trips and collecting. Alexander Naseka is also much appreciated for providing the photographs. We are also immensely grateful to Iain Wilson (University of Natural Resources and Life Sciences, Vienna, Austria) for linguistic revision and proofreading of earlier versions of the manuscript, to Felipe Otttoni, Section Editor, Pedro Braganca, the reviewer, and the anonymous reviewer for their comments that greatly improved the manuscript.

References

Artaev O., Pashkov A., Vekhov D., Saprykin M., Shapovalov M., Levina M. & Levin B. 2021. Fish occurrence in the Kuban' River Basin (Russia). *Biodiversity Data Journal* 9: e76701. https://doi.org/10.3897/BDJ.9.e76701

Beling D.E. 1914. Essays on the ichthyofauna of the Dnieper 1. Ichthyofauna of the Dnieper basin near Kiev. *Trudy Dneprovskoy Biologicheskoy Stantsii* [*Proceedings of the Dnieper Biological Station*] 1: 53–110. [In Russian.]

Berg L.S. 1910. Rapport sur une mission zoologique au Caucase en 1909. *Zapiski Zoologicheskago Muzeya Imperatorskoi Akademii Nauk* [*Proceedings of the Zoological Museum of the Imperial Academy of Sciences*] 15: 153–170. [In Russian.]

Berg L.S. 1912. *Faune de la Russie et des Pays limitrophes fondés principalements sur les Collections du Musée zoologique de l'Académie impériale des Sciences de St.-Pétersbourg. Poissons (Marsipobranchii et Pisces). Vol. III. Ostariophysi. Part 1.* L'Académie impériale des Sciences, St.-Pétersbourg. [In Russian.]

Berg L.S. 1949. *Freshwater fishes of the U.S.S.R. and Adjacent Countries. 4th ed.*, *vol. 2.* Guide to the Fauna of the U.S.S.R., Freshwater fishes of the U.S.S.R. and adjacent countries 29: 467–925, Akademiya Nauk SSSR, Moskva. [In Russian.]

Bogutskaya N.G. & Naseka A.M. 2004. *Catalogue of Agnathans and Fishes of Fresh and Brackish Waters of Russia with Comments on Nomenclature and Taxonomy*. KMK-Publishers, Moscow. [In Russian.]

Bogutskaya N.G., Jelić D., Vucić M., Jelić M., Diripasko O.A., Stefanov T. & Klobučar G. 2019. Description of a new species of *Phoxinus* from the upper Krka River (Adriatic Basin) in Croatia (Actinopterygii: Leuciscidae), first discovered as a molecular clade. *Journal of Fish Biology* 96 (2): 378–393. https://doi.org/10.1111/jfb.14210

Chen X.-Y. & Arratia G. 1996. Breeding tubercles of *Phoxinus* (Teleostei: Cyprinidae): morphology, distribution, and phylogenetic implications. *Journal of Morphology* 228: 127–144.

Chernyshev A.A. 2010. Ichthyofauna of the Kursk region: knowledge, problems of protection and rational use. Scientific notes. *Electronic Scientific Journal of Kursk State University 1* (13): 15–36. [In Russian.]

Corral-Lou A., Perea S., Aparicio E. & Doadrio I. 2019. Phylogeography and species delineation of the genus *Phoxinus* Rafinesque, 1820 (Actinopterygii: Leuciscidae) in the Iberian Peninsula. *Journal of Zoological Systematics and Evolutionary Research* 57 (4): 926–941. https://doi.org/10.1111/jzs.12320

De Santis V., Delmastro G.B., Vanetti I.J., Britton R. & Zaccara S. 2020. Species composition of introduced and natural minnow populations of the *Phoxinus* cryptic complex in the westernmost part of the Po River Basin (north Italy). *Biological Invasions* 23 (3): 657–668. https://doi.org/10.1007/s10530-020-02406-2

Denys G.P.J., Dettaï A., Persat H., Daszkiewicz P., Hautecoeur M. & Keith P. 2020. Revision of *Phoxinus* in France with the description of two new species (Teleostei, Leuciscidae). *Cybium* 44 (3): 205–237. https://doi.org/10.26028/cybium/2020-443-003

Dybowski B.N. 1862. Versuch einer Monographie der Cyprinoiden Livlands, nebst einer synoptischen Aufzählung der europäischen Arten dieser Familie. *Archiv für die Naturkunde Liv-*, *Ehst- und Kurlands. Zweite Serie. Biologische Naturkunde*, *Dorpat* 3: 133–362. [In Russian.]

Emtyl' M.Kh. & Ivanenko A.M. 2002. *Fishes of the South-west of Russia*. Kubanskiy Gosudarstvennyy Universitet, Krasnodar. [In Russian.]

Ferguson J.W.H. 2002. On the use of genetic divergence for identifying species. *Biological Journal of the Linnean Society* 75 (4): 509–516. https://doi.org/10.1046/j.1095-8312.2002.00042.x

Fricke R., Eschmeyer W.N. & Van der Laan R. (eds). 2021. *Eschmeyer's Catalog of Fishes: genera*, *species*, *references.* Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [accessed 30 Sep. 2021].

Gablitz K.I. 1785. *Physical description of the Tauride region*, *according to its location*, *and for all three kingdoms of nature*. I. Veitbrekht, Saint-Petersburg. [In Russian.]

Geiger M.F., Herder F., Monaghan M.T., Almada V., Barbieri R., Bariche M., Berrebi P., Bohlen J., Casal-Lopez M., Delmastro G.B., Denys G.P.J., Dettai A., Doadrio I., Kalogianni E., Kärst H., Kottelat M., Kovačić M., Laporte M., Lorenzoni M., Marčić Z., Özuluğ M., Perdices A., Perea S., Persat H., Porcelotti S., Puzzi C., Robalo J., Šanda R., Schneider M., Slechtová V., Stoumboudi M., Walter S. & Freyhof J. 2014. Spatial heterogeneity in the Mediterranean Biodiversity Hotspot affects barcoding accuracy of its freshwater fishes. *Molecular Ecology Resources* 14 (6): 1210–1221. https://doi.org/10.1111/1755-0998.12257

Goldstein P.Z. & DeSalle R. 2010. Integrating DNA barcode data and taxonomic practice: Determination, discovery, and description. *BioEssays* 33 (2): 135–147. https://doi.org/10.1002/bies.201000036

Hammer Ø. 1999–2022 *PAST. Paleontological Statistics*. Ver. 4.09. Reference Manual. Available from https://www.nhm.uio.no/english/research/resources/past/ [accessed 30 Sep. 2021].

Hebert P.D.N., Cywinska A., Ball S.L. & de Waard J.R. 2003a. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B*, *Biological Sciences* 270: 313–21. https://doi.org/10.1098/rspb.2002.2218

Hebert P.D.N., Ratnasingham S. & de Waard J.R. 2003b. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* 270 (suppl. 1): 96–99. https://doi.org/10.1098/rsbl.2003.0025

ICZN 1999. *International Code of Zoological Nomenclature*, *Fourth Edition: Adopted by the International Union of Biological Sciences*. The International Trust for Zoological Nomenclature, London.

Kalyaanamoorthy S., Minh B., Wong T., von Haeseler A. & Jermiin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. https:// doi.org/10.1038/nmeth.4285

Karpova E.P. 2017. *Transformation of Fish Communities in Water Bodies of the Crimean Peninsula under the Influence of Anthropogenic Factors*. Candidate of Sciences thesis, Institute of Marine Biological Research, Sevastopol'. [In Russian.]

Karpova E.P. & Boltachev A.R. 2011. Features of formation and current state of the ichthyofauna of inland waters of the Crimea. *Proceedings of the Zoological Museum (National Museum of Natural History*, *Kyiv*, *Ukraine)* 42: 75–91. [In Russian].

Karpova E.P. & Boltachev A.R. 2012. *Fishes of Inland Waters of the Crimean Peninsula*. BiznessInform, Simferopol'. [In Russian].

Kessler K.F. 1859. Auszüge aus dem Berichte über die nordwestlichen Küsten des schwarzen Meeres und durch die westliche Krym unternommene Reise. *Bulletin de la Société impériale des Naturalistes de Moscou* 32 (pt 1, No. 2): 520–546.

Kessler K.F. 1877. *Fishes Occurring and Found in the Aralo-Caspian-Pontic Ichthyological Province*. Proceedings of Aral-Caspian Expedition 4, Obshchestvo Estestvoispytateley, Saint-Petersburg. [In Russian.]

Kottelat M. 1997. European freshwater fishes. *Biologia (Bratislava)* 52 (suppl. 5): 1–271. https://doi.org/10.5281/zenodo.1311773

Kottelat M. 2007. Three new species of *Phoxinus* from Greece and southern France (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters* 18 (2): 145–162.

Kottelat M. & Freyhof J. 2007. *Handbook of European Freshwater Fishes*. Kottelat, Cornol, Switzerland and Freyhof, Berlin.

Luzhnyak V.A. 2003. Ichthyofauna of rivers and estuaries of the Black Sea coast of Russia. *Voprosy Ikhtiologii* 43 (4): 457–463. [In Russian.]

Miroshnichenko A.I. 2003. Fishes of inland waters of the Crimea. *In*: Tarasenko V.S. (ed.) *Sustainable Crimea. Water resources*: 142–145. Tavrida, Simferopol’. [In Russian.]

Moshu A. & Trombitskiy I. 2013. *Peștii din Nistrul Mijlociu și de Jos (ghid pentru păzitorii râului)*. Renasterea Rurala & Vitaliti, Chișinău. [In Moldovan].

Movchan Yu.V. 2011. *Fishes of Ukraine (Keys and References)*. Zolotye Vorota, Kyiv. [In Ukrainian].

Movchan Yu.V. & Smirnov A.I. 1981. *Fauna of Ukraine*. *Vol. 8. Fishes. Issue 2*, *Part 1*. Naukova dumka, Kyiv. [In Ukrainian].

Movchan Yu.V., Manilo L.G., Smirnov A.I. & Shcherbukha A.Ya. 2003. Catalogue of the collections of the of the Museum of Zoology of the NNHM of NAS of Ukraine. *Cyclostomata and Fishes*. NNHM, Kyiv. [In Russian.]

Nordmann A. von 1840. Prodrome de l'ichthyologie pontique. *In:* Démidoff A. de (ed.) *Voyage dans la Russie méridionale et la Crimée* 3: 353–635, 748–755. E. Bourdin et Ce., Paris.

Otrishko M.P. & Emtyl’ M.Kh. 2012. The current state of the ichthyofauna of the Adagum River. *Privolzhskiy Nauchnyy Vestnik* 8 (24): 21–24. [In Russian.]

Padial J.M., Miralles A., De la Riva I. & Vence M. 2010. The integrative future of taxonomy. *Frontiers in Zoology* 7 (1): 16. https://doi.org/10.1186/1742-9994-7-16

Palandačić A., Bravničar J., Zupančič P., Šanda R. & Snoj A. 2015. Molecular data suggest a multispecies complex of *Phoxinus* (Cyprinidae) in the Western Balkan Peninsula. *Molecular Phylogenetics and Evolution* 92: 118–123. https://doi.org/10.1016/j.ympev.2015.05.024

Palandačić A., Naseka A., Ramler D. & Ahnelt H. 2017. Contrasting morphology with molecular data: an approach to revision of species complexes based on the example of European (Cyprinidae). *BMC Evolutionary Biology* 17: 184. https://doi.org/10.1186/s12862-017-1032-x

Palandačić A., Kruckenhauser L., Ahnelt H. & Mikschi E. 2020. European minnows through time: museum collections aid genetic assessment of species introductions in freshwater fishes (Cyprinidae: *Phoxinus* species complex). *Heredity* 124 (3): 410–422. https://doi.org/10.1038/s41437-019-0292-1

Pallas P.S. 1801. *Bemerkungen auf einer Reise in die südlichen Statthalterschaften des Russischen Reichs in den Jahren 1793 und 1794. Zweyter Band. Mit Colorirten Kupfern*. Gottfried Martini, Leipzig. https://doi.org/10.3931/e-rara-16624

Pallas P.S. 1814. *Zoographia Rosso-Asiatica*, *sistens omnium animalium in extenso Imperio Rossico et adjacentibus maribus observatorum recensionem*, *domicilia*, *mores et descriptiones anatomen atque icones plurimorum*, 3. Academia Scientiarum, Petropolis, Saint-Petersburg. [In Russian.]

Plotnikov G.K. 2001. *Ichthyofauna of Various Aquatic Ecosystems of the Northwestern Caucasus*. Kubanskiy Gosudarstvennyy Universitet, Krasnodar. [In Russian.]

Rathke H. 1836. Zur Fauna der Krym. Mémoires présentés à l'Académie impériale des Sciences de St.-Petersbourg par Divers Savants et lus dans ses Assemblées. *Mémoires des Savants étrangers* 3: 291–454, 771–774.

Rossini B.C., Oliveira C.A.M., de Melo F.A.G., Bertaco V.D., de Astarloa J.M.D., Rosso J.J., Foresti F. & Oliveira C. 2016. Highlighting Astyanax species diversity through DNA barcoding. *PLoS ONE* 11: e0167203. https://doi.org/10.1371/journal.pone.0167203

Tajima F. & Nei M. 1984. Estimation of evolutionary distance between nucleotide sequences. *Molecular Biology and Evolution* 1: 269–285. https://doi.org/10.1093/oxfordjournals.molbev.a040317

Tseeb Ya.Ya. 1929. Preliminary results of the study of the ichthyofauna of small Crimean rivers. *Trudy Krymskogo NII*, *Simferopol'* 2 (2): 112–123. [In Russian.]

Vucić M., Jelić D., Žutinić P., Grandjean F. & Jelić M. 2018. Distribution of Eurasian minnows (*Phoxinus*: Cypriniformes) in the Western Balkans. *Knowledge and Management of Aquatic Ecosystem* 419: 11. https://doi.org/10.1051/kmae/2017051

Manuscript received: 6 June 2022
Manuscript accepted: 3 November 2022
Published on: 6 March 2023
Topic editor: Tony Robillard
Section editor: Felipe Polivanov Ottoni
Desk editor: Marianne Salaün

Supplementary material

Supp. file 1. List of reference on the distribution of *Phoxinus* Agassiz, 1835 in the northern Black Sea and Sea of Azov basins. https://doi.org/10.5852/ejt.2023.861.2061.8563

Supp. file 2. List of material used in genetic analysis and primary CO1 data for *P. chrysoprasius* (Pallas, 1814) and samples sequenced in this study. https://doi.org/10.5852/ejt.2023.861.2061.8565

Supp. file 3. Primary morphological data of examined *Phoxinus chrysoprasius* (Pallas, 1814). https://doi.org/10.5852/ejt.2023.861.2061.8573

Supp. file 4. Counts and type of scale pattern on breast and anterior belly in *P. chrysoprasius* (Pallas, 1814). https://doi.org/10.5852/ejt.2023.861.2061.8575

Supp. file 5. Estimates of net evolutionary divergence between groups of sequences. https://doi.org/10.5852/ejt.2023.861.2061.8577