

The incidence of the anomaly P syndrome in water frogs (*Anura*, *Ranidae*, *Pelophylax*) from the Middle Volga River (Russia)

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Abstract

The anomaly P is a widespread morphological anomaly, which occurs in some groups of amphibians, caused by the trematode parasite *Strigea robusta* (Digenea: Strigeidae). This anomaly has been previously recorded in water frogs of the genus *Pelophylax* and toads of the genera *Bufo* and *Bufo*. The anomaly P includes symmetrical polydactyly cases as a mild attenuated form of the complex syndrome, which in severe cases includes strong deformations of hindlimbs and forelimbs. *Strigea robusta* has a complex 3-host life cycle using planorbid mollusks as the first intermediate hosts, amphibian larvae as the second intermediate hosts, and anadit birds as the definitive hosts. Herein, we described new records of the anomaly P syndrome in water frogs of the genus *Pelophylax* from the northeastern parts of their ranges. Symmetrical polydactyly (as a mild form of the anomaly P syndrome) was found in 30 individuals of three species of water frogs from seven localities: in 25 individuals of *P. lessonae* from four waterbodies, in four individuals of *P. ridibundus* from three waterbodies, and one individual of *P. esculentus*. In Gusevo pond, three individuals of *P. lessonae* with severe cases of the syndrome were found. This is the first record of the anomaly P in reliably identified hybridogenetic edible frogs (*P. esculentus*) that have been identified in nature. Additionally, we provided new data about the occurrence of the anomaly P and the prevalence of the trematode *S. robusta* in mollusks taken from two water bodies where anomalous water frogs were found.

Key Words

amphibian morphological anomalies, *Strigea robusta*, trematode

Introduction

The Eastern European Plain is inhabited by three native species of the genus *Pelophylax* (Lada 1995): two parental species, the marsh frog *P. ridibundus* (Pallas, 1771) and the pool frog *P. lessonae* (Camerano, 1882), and their hybridogenetic hybrid, the edible frog *P. esculentus* (Linnaeus, 1758). These species inhabit a wide range of biotopes: the marsh frog tends to inhabit large open water bodies, riverbeds and large reservoirs, while the pool frog inhabits small forest water bodies and sometimes large

water bodies with woody vegetation along banks. Edible frogs live usually syntopic with the parental species.

Living in various aquatic habitats makes water frogs vulnerable to a wider range of trematodes, the larval stages of which parasitize freshwater mollusks. European water frogs serve as hosts for approximately 40 species of trematodes in the Middle Volga River drainage (Kirillov et al. 2012). Some species can be highly pathogenic to water frogs. For example, species of the genus *Echinostoma* Rudolphi, 1809 lead to renal dysfunction and metamorphic oedema in tadpoles (Fried et al. 1997; Koprivinkar et al.

2006; Holland 2010; Orlofske et al. 2017; Billet et al. 2020), *Codonocephalus urnigerus* (Rudolphi, 1819) causes castration of frogs (Ivanov et al. 2012), and *Holostephanus volgensis* (Sudarikov, 1962) leads to scoliosis in tadpoles (Vershinin and Neustroeva 2011).

Strigea robusta (Szidat, 1928) is also a highly pathogenic trematode for amphibians. It has a complex 3-host life cycle that includes planorbid snails as the first intermediate hosts (mollusks of the genera *Planorbis* Müller, 1774, *Planorbarius* Duméril, 1806, *Anisus* Studer, 1820, *Bathymophalus* Charpentier, 1837, *Gyraulus* Charpentier, 1837, and *Segmentina* Fleming, 1818), amphibians as the second intermediate hosts, and anatid birds as the definitive hosts. The infection of *S. robusta* causes the so-called anomaly P in some species of anuran amphibians: these are widespread mass morphological anomalies described for the genera *Pelophylax* Fitzinger, 1843, *Bufo* Rafinesque, 1815 and *Bufo* Garsault, 1764 (Rostand 1971; Yakovlev 1984; Ouellet 2000; Dubois 2017; Svinin et al. 2019a, b, 2020, 2022).

The anomaly P has a mild and severe form. The mild form includes symmetrical polydactyly (supernumerary digits) on the hindlimbs and forelimbs, and sometimes both (but never on forelimbs only), while severe forms of the anomaly P are manifested as strong changes of the hind- and forelimb morphology and includes symmetrical hindlimbs flexions (taumely), brachymely (shortened parts of limbs), polydactyly, hyperplasy of tissues in the inguinal region, outgrowths, spikes and local hemorrhages (Rostand 1971; Dubois 2017). Thus, this disease can be considered a case of strigeiosis in amphibians. Modifications of limb morphology and reduced locomotion lead to a decrease in survival rates of tadpoles and juveniles and might have a significant impact on population dynamics (Dubois 2017).

The distribution of *S. robusta* covers Germany, Czech Republic, Romania, Lithuania, Ukraine, Kazakhstan, Turkmenistan, Kyrgyzstan, and Russia, where the helminth was recorded in Kaliningrad Province, the Volga River drainage, Western and Eastern Siberia (Sudarikov 1984). According to the wide distribution of the parasite

S. robusta, the anomaly P syndrome can appear in various parts of water frog species ranges.

Herein, we provide new observations of the anomaly P syndrome, which were observed during a long-term study of water frogs in Mari El Republic (the Volga River drainage, Russia) and adjacent territories in the period 2008–2022. In this study, we used DNA flow cytometry and molecular analyses for the reliable determination of water frog species. Also, we provided data on the occurrence of various trematode species, as well as the prevalence of *S. robusta* in planorbid snails in the biotopes where we observed the anomaly P.

Materials and methods

A total of 68 localities from the Middle Volga River drainage were studied (Fig. 1, Table 1). The list of *Pelophylax* samples in which we analyzed anomalies was the same ($n = 1337$) as it was presented for morphological analysis in our previous study (Svinin et al. 2021). An absence of polyploidy in local populations of *P. esculentus* makes it possible to simplify the preliminary identification of these three species by examination of external morphology only (Svinin et al. 2021). However, the reliable determination of these species is possible with the use of molecular or cytogenetic methods (Plötnner et al. 2008; Biriuk et al. 2016; Dufresnes et al. 2017, 2018). Water frog species were partially identified with the use of multiplex PCR and DNA flow cytometry (Table 1). We used DNA flow cytometry in order to precisely identify species, as well as the ploidy of hybrids. Details of this method were previously published (Borkin et al. 1987; Vinogradov et al. 1990, 1991). Additionally, the identification of water frog species was performed using the previously described multiplex PCR method (Ermakov et al. 2019; Svinin et al. 2021), which makes it possible to analyze the species-specific polymorphism of gene fragment of the intron-1 of the nuclear serum albumin (*SAL-1*). The

Table 1. Occurrence of the anomaly P syndrome in water frogs from the localities examined.

#	Localities	Coordinates	System	Species	Year	N	Adult.	Juv. + tad.	Polydactyly (%)	Severe forms (%)
1	Gusevo village	56.962°N, 47.737°E	L	<i>P. lessonae</i>	2014	1	1	–	100	–
					2020	97	1	96	20.6	3.1
2	Shusher settlement	56.673°N, 47.262°E	REL	<i>P. ridibundus</i>	2011–2019	19	19	–	–	–
				<i>P. lessonae</i>		110	110	–	0.9	–
				<i>P. esculentus</i>		20	20	–	–	–
3	Medvedevo settlement	56.642°N, 47.753°E	REL	<i>P. lessonae</i>	2013–2017	2	2	–	–	–
				<i>P. esculentus</i>		10	10	–	–	–
				<i>P. ridibundus</i>		60	60	–	–	–
				<i>P. lessonae</i>	2021	6	3	3	–	–
				<i>P. esculentus</i>		32	1	31	3.1	–
<i>P. ridibundus</i>		25	–	25	–	–				
4	Yoshkar-Ola, Turunovo	56.625°N, 47.980°E	L	<i>P. lessonae</i>	2013	27	27	–	7.4	–
5	Yoshkar-Ola, Chikhaydarovo	56.606°N, 47.891°E	REL	<i>P. ridibundus</i>	2011–2019	92	92	–	2.2	–
				<i>P. esculentus</i>		1	1	–	–	–
6	Yoshkar-Ola, Forest park “Sosnovaya Rozhsha”	56.616°N, 47.925°E	R	<i>P. ridibundus</i>	2008–2021	142	142	–	0.7	–
7	Chermyshevo settlement	56.195°N, 46.514°E	REL	<i>P. ridibundus</i>	2012–2017	64	64	23	1.6	–
				<i>P. lessonae</i>		25	25	1	4.0	–
				<i>P. esculentus</i>		41	41	14	–	–

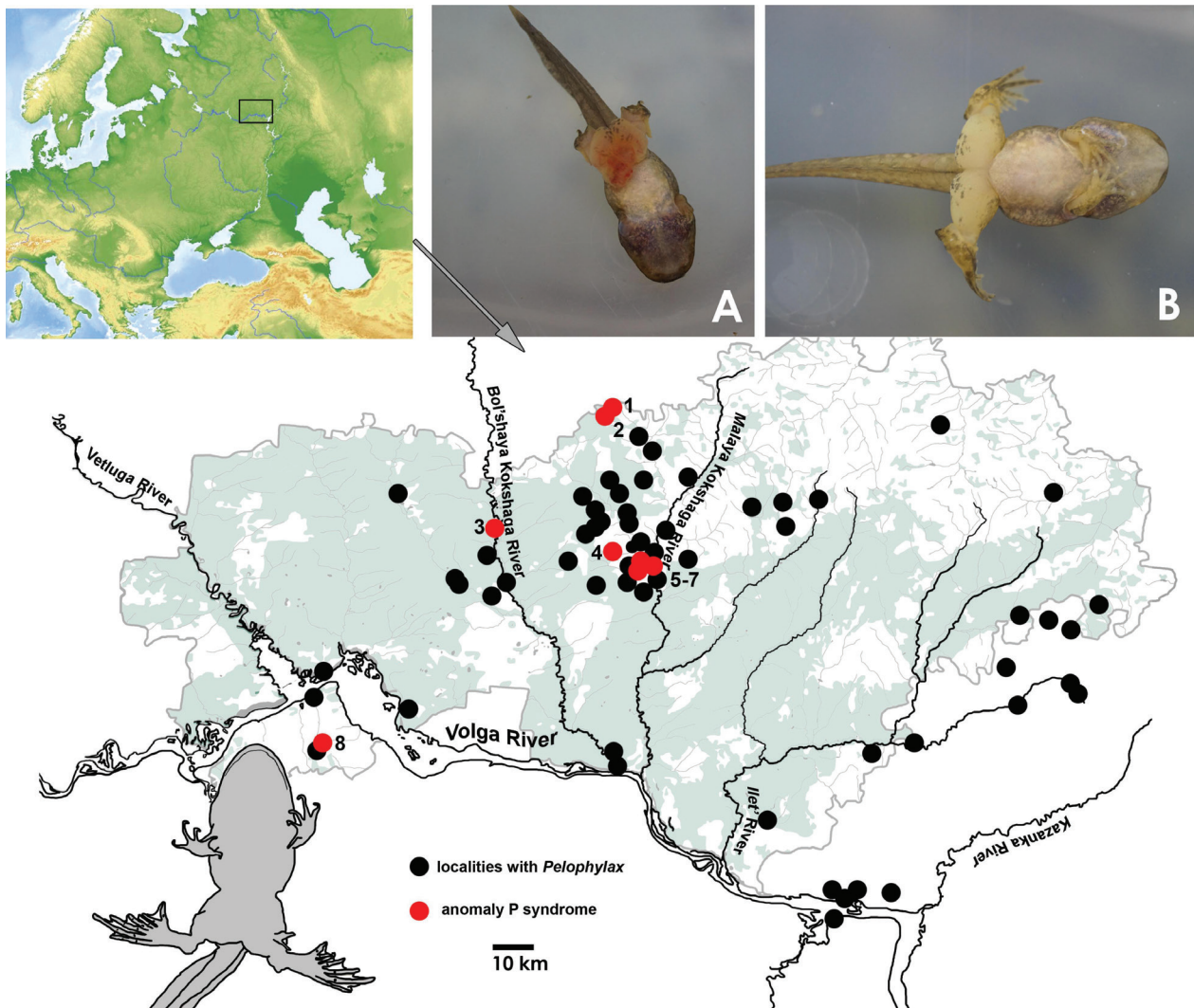


Figure 1. Studied localities ($n = 68$) in the Middle Volga River drainage, where live three *Pelophylax* species (black dots; Svinin et al. 2021), and 8 localities with the anomaly P syndrome (red spots). **A, B.** Severe forms of the anomaly P in the pool frog (*P. lessonae*) from Gusevo village (Mari El Republic, Russia). Borders of Mari El Republic are designated by gray line and forests by light green fill.

basic principle of the method is a separation of amplification products that have three different lengths (due to differences in primers: one forward general and three species-specific reverse primers) in three species of water frogs (*P. cf. bedriagae*, *P. ridibundus* and *P. lessonae*) living in European Russia. Because of hybrid origin of *P. esculentus*, it has usually two bands on electrophoregrams after the multiplex PCR (Ermakov et al. 2019).

In the Middle Volga region, three species of *P. esculentus* complex form various population systems, and we classified them into five main types according to the standard classification system used in water frog studies (Plötner et al. 2010, 2012; Fayzulin et al. 2018): only parental species (R type, *P. ridibundus* only; L type, *P. lessonae* only), one parental species and hybridogenetic hybrids (L-E type, *P. lessonae* and *P. esculentus*; R-E type, *P. ridibundus* and *P. esculentus*) and all three taxa living together (R-E-L type, *P. ridibundus*, *P. lessonae* and *P. esculentus*).

Mollusks were collected from Gusevo and Medvedevo ponds in Mari El Republic where we found the anomaly P (Fig. 2). The mollusks *Planorbis corneus* (Linnaeus,

1758) in Gusevo and *Planorbis planorbis* Linnaeus, 1758 in Medvedevo were chosen for screening of trematode cercariae. A total of 895 *Planorbis corneus* and 706 *Planorbis planorbis* were examined (Table 2). Snails were transferred into small glass containers with a volume of 50 ml. The emergence of cercariae was stimulated by heating the containers with a lamp for 1–2 h (Faltýnková et al. 2007). The 0.05% neutral red stain was used for their vital staining (5–10 min). The final species identification of cercariae was performed after studying stained preparations using a stereodissecting microscope Zeiss Discovery V.8 (Carl Zeiss AG, Oberkochen, Germany) and microscope Axio Imager.A2 (Carl Zeiss AG, Oberkochen, Germany). The species identification of trematode cercariae was performed using keys provided by Combes (1980) and Faltýnková et al. (2007). Identification of *S. robusta* cercariae (hosts are mollusks *Planorbis planorbis* from Medvedevo) was carried out using molecular analysis in our previous laboratory experiments (Svinin et al. 2022).

We used a classification of morphological anomalies according to Rostand (1971), Nekrasova (2008), Vershinin

Table 2. Prevalence (%) of larval trematodes in two species of planorbid snails from two ponds with the anomaly P.

Species of trematodes	<i>Planorbis corneus</i> Gusevo				<i>Planorbis planorbis</i> Medvedevo		
	2020	2021	2022	Total	2020	2021	Total
<i>Australapatemon</i> sp.	–	–	–	–	34.4	14.8	25.4
<i>Bilharziella polonica</i>	–	–	0.9	0.2	–	–	–
<i>Echinostoma</i>	–	–	–	–	6.6	7.7	7.1
<i>revolutum</i> complex							
<i>Haematoloechus</i> sp.	–	2.2	7.8	2.7	28.1	17.5	23.2
<i>Notocotylus ephemera</i>	1.4	1.2	0.5	1.1	–	–	–
<i>Notocotylus regis</i>	–	–	–	–	0.5	0.3	0.4
<i>Paralepoderma</i>	–	–	–	–	1.0	–	0.6
<i>cloacicola</i>							
<i>Rubestrema</i>	27.2	16.4	7.8	18.5	–	–	–
<i>exasperatum</i>							
<i>Strigea robusta</i>	–	–	–	0.0	0.8	–	0.4
<i>Tylodelphys excavata</i>	–	2.8	2.3	1.6	–	–	–
Two species in one snail (double invasion)							
<i>Australapatemon</i> sp. + <i>Paralepoderma</i> <i>cloacicola</i>	–	–	–	–	0.3	–	0.1
<i>Australapatemon</i> sp. + <i>Haematoloechus</i> sp.	–	–	–	–	2.9	0.3	1.7
Without infection	71.4	77.5	80.7	75.9	25.5	59.4	41.1
Number of mollusks	353	324	218	895	381	325	706

(2015), Henle et al. (2017a, b), and Dubois (2017). Mild (light, benign) forms of the anomaly P are symmetrical (or, rarely, asymmetrical) polydactyly on the hindlimbs or both on the hindlimbs and forelimbs. Severe (heavy) forms are anomalies, which include in addition to polydactyly (rare without), brachymely (shortening of the limbs), taumely (inversion of the hindlimbs), and bone outgrowths.

Results

Abnormality rates in water frogs

We found frogs with symmetrical polydactyly in seven out of 68 studied localities (10.3%; Table 1). We registered severe cases of the anomaly P in one locality only (Gusevo pond). Among 96 individuals of *P. lessonae* caught in August 2020, 24.0% had the anomaly P: 20 frogs had polydactyly (20.6%) and three had the severe form of the

anomaly P (3.1%; see Fig. 1). Additional records of symmetrical polydactyly (severe cases absent) were observed in Chermyshevo village, Shusher settlement, Medvedevo settlement, and three localities in the city of Yoshkar-Ola: Chikhaydarovo district, Turunovo district, and the forest park “Sosnovaya Roscha” (Table 1). The occurrence of frogs with polydactyly was 0.7–20.6%.

The anomaly P was observed in all three local water frog species: *P. ridibundus* from Chermyshevo village, Chikhaydarovo district, and “Sosnovaya Roscha”; *P. lessonae* from Turunovo district, Shusher and Gusevo settlements, and Chermyshevo village; and *P. esculentus* from Medvedevo settlement. The anomaly was found in three types of water frog population systems (R, L, and R-E-L). In the Chermyshevo, the anomaly P syndrome was found in both parental species (Table 1).

Prevalence of *Strigea robusta* in two ponds with the anomaly P

Trematodes were studied in mollusks from two ponds (Gusevo and Medvedevo), where we found frogs with the anomaly P (Table 2). Among 895 mollusks *Planorbis corneus* from Gusevo, 24% were infected by larvae of six species of trematodes. The cercariae of *S. robusta* were not found in mollusks. Among 706 mollusks *Planorbis planorbis* from Medvedevo, 59% were infected by larvae of six species of trematodes. In June 2020, three mollusks out of 381 were infected by *S. robusta* (0.8%; Table 2). In June 2021, *S. robusta* was absent among 325 mollusks. In this year, we observed here the complete disappearance of the anomaly P in a new generation of water frogs (59 metamorphs), while the percentage of polydactyly among adult frogs was 25% (Table 1).

Discussion

With its variety of manifestations, the anomaly P syndrome has a number of specific features that are making its phenotype recognizable. In its mild attenuated form, which is often followed by later detection of severe forms



Figure 2. The localities with the anomaly P from Mari El Republic (Russia): **A.** Pond near Gusevo village; **B.** Pond near Medvedevo settlement.

in the locality (e.g., Medvedevo and Gusevo ponds), it presents as symmetrical polydactyly on the hind limbs or on both the fore- and hind limbs (but never separately on the forelimbs). A certain gradient can be traced from simple polydactyly to more complex cases of polydactyly with more than two extra digits on each side of the body. Severe forms include the same polydactyly (occasionally there are individuals with limb taumely without polydactyly) and, in addition, serious changes in the morphology of limbs. Polydactyly in severe forms often exceeds six digits; there are specimens with a “crown” of 10–20 digits. Even in such cases, polydactyly continues to be symmetrical. However, strict symmetry is not always manifested, and the differences can be one or, less often, two additional digits. In addition to such polydactyly, brachymely, inversion of the limbs, hemorrhages in the inguinal region, bone outgrowths, as well as small distal fragments of the limbs and, rarely, polymely can be found in various individuals. The more complex structures an anomalous tadpole has, the more affected are the forelimbs, presenting in some cases with flipper-like shortened legs (Rostand 1971; Dubois 2017).

Despite the fact that the *S. robusta* parasite is more widespread, severe cases of the anomaly P in water frogs of the genus *Pelophylax* have previously been reported only in France, Morocco, the Netherlands, and Russia (Dubois 1971; Rostand 1971; Yakovlev 1984; Svinin et al. 2019b). However, polydactyly (as a mild form of the anomaly) is one of the most widespread deformities in European water frogs (Henle et al. 2017a; Svinin et al. 2019a). New records in our study expand the occurrence of this phenomenon: these are one of the northernmost records of the anomaly.

Our study of the occurrence of *S. robusta*, as the trematode species responsible for the anomaly P manifestation in amphibians, in two localities with the anomaly P showed its low prevalence in planorbid snails (in Medvedevo – 0.4% and Gusevo – less than 0.1%). The occurrence of *S. robusta* in *Planorbarius corneus* from Ostrovtsovskaya forest-steppe was 0.38% ($n = 1316$; Svinin et al. 2020), while the occurrence was 1.6% in *Planorbis planorbis* from Beloe Lake in Belarus (Akimova et al. 2011). Despite the low occurrence of this parasite in mollusks, the abundance of abnormal individuals of water frogs in Gusevo pond was comparable to Ostrovtsovskaya forest-steppe: 23.7% in Gusevo and 22.9% in Ostrovtsovskaya forest-steppe (Svinin et al. 2020).

It is important to note that the disappearance of polydactylous water frogs in a small pond in Medvedevo is very similar to the situation described by Rostand (1971) in France, where such frogs disappeared from Trevignon, Penloch and Saint-Philbert-de-Grand-Lieu populations. An additional case of such disappearance was observed in the vicinity of Bol'shaya Lipovitsa village in Tambov Province (Russia). In 2018, three years after the study resulting in anomalies (Kozhevnikova and Lada 2016), this population of *P. ridibundus* was re-examined, but no anomalies were revealed (A. O. Svinin, I. V. Bashinskiy, A. G. Goncharov, unpublished data).

There may be several causes for this disappearance in Medvedevo: reconstruction works on a side of the pond (Fig. 2C) and an increase in the number of fishermen who scare away ducks, who are forced to look for other waters for feeding. It is also possible that the infection of water snails occurs from several individuals of ducks that did not arrive at the pond in the year of the study. The disappearance can also be caused by invasive fish *Percottus glenii* Dybowski, 1877 which can selectively eat infected tadpoles (Svinin et al. 2019a). Several additional fish species (*Carassius gibelio* (Bloch, 1782), *Cyprinus carpio* Linnaeus, 1758, *Rutilus rutilus* (Linnaeus, 1758)) have recently been introduced in the pond. Fish fry can eat cercariae, which therefore do not infect tadpoles.

Changes in the migratory activity of birds and the introduction of some fishes can influence the infection pattern of *S. robusta* and, consequently, the distribution of the anomaly P in local populations of frogs. An increase in the abundance of fish fry can also lead to a decrease in the biomass of cercariae, leading to the overall effect of reducing the level of these anomalies in frog populations. However, other environmental factors may also influence *S. robusta* infections and identifying these factors may be a key to understanding the mosaic distribution of anomalies in amphibians.

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