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New records of the anomaly P syndrome in two water frog species (*Pelophylax ridibundus* and *P. lessonae*) in Russia

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Abstract

The "anomaly P" was described in Palearctic water frogs of the genus *Pelophylax* by Jean Rostand as complex morphological anomalies of water frogs, including polydactyly, brachymely, hind limb oedema, bone outgrowths, spikes, flexions and additional limbs in the inguinal region. In 2016, the anomaly P syndrome was rediscovered in central Russia, confirming the hypothesis concerning its wider distribution. Here, three new records of this syndrome in two species of western Palearctic water frog from Russia are described.

Key Words

morphological anomalies, anomaly P, polydactyly

In the 1950s, the famous French writer and biologist Jean Rostand discovered morphological anomalies of an unknown etiology in water frogs of the genus *Pelophylax*, which he named "the anomaly P" (Rostand 1971). Recently, it is also known as "Rostand's anomaly" (Dubois 2017). The anomaly P is one of the longest studied cases of amphibian anomalies in Europe (Dubois 1989; 2017; Ouellet 2000). The anomaly P syndrome refers to morphological anomalies of water frogs that have light and severe forms of manifestation. The light form of the anomaly is symmetrical polydactyly. Specimens suffering from severe forms of the anomaly have brachymely, polydactyly, hind limb oedema, bone outgrowths, spikes, flexions and additional limbs in the inguinal region (Dubois 2017). The anomaly P, in comparison with deformities caused by trematode *Ribeiroia ondatrae* (Johnson et al. 2001; 2002), had no additional full limbs and can have small autopod elements in inguinal regions only. The most important difference is symmetry of bilateral traits: number of structures and their shapes are usually the same on both sides of the body. Rostand (1971) showed that this is not inherited (after laboratory crosses of abnormal specimens), but caused by a certain factor in the environment. He grew larvae from clutches taken from water bodies with deformed frogs in laboratory conditions and did not encounter any anomalies. He tried to influence the tadpoles with various chemicals, increased water salinity and ultraviolet light exposure. However, none of these studies led to similar anomalies (Rostand 1971; Dubois 2017).

Over a long period of research, Rostand (1971) found several additional records of frogs with the anomaly P in France. Severe forms of the anomaly P were revealed in Trévignon and Penloc'h (Concarneau, Finistére), Lingé (Indre), "Marais Vendéen" and Saint-Philbert-de-Grand-Lieu (Loire-Atlantique) (Fig. 1). One locality with severe forms was also found in Morocco and one case was registered in Amsterdam (Hillenius 1959 in Rostand 1971). Polydactyly was much more dispersed and found in different places across France, including Champdieu (Loire), Soustons, Léon, Aureilhan (Landes), Bordeaux (Gironde), Luxeuil-les-Bains (Haute-Saône), Faverois (Territoire-de-Belfort), Fénetrange (Moselle) and Tassenières (Jura) (Dubois 1984). Rostand (1971) suggested the hypothesis that the infectious agent causing the anomaly P was more widespread than it was initially expected when he discovered his first anomalies from the ponds in Concarneau.

The anomaly P syndrome was studied for 20 years until its disappearance in the 1970s. This happened with localities in Trévignon and Saint-Philbert-de-Grand-Lieu. Hence, it was no longer possible to study anomalies in detail with the aim of finding its causative agent (Dubois 2017). The anomaly was encountered under field experiments when tadpoles were reared together with fish (Rostand and Darré 1968). These authors (Rostand and Darré 1969) also observed the same effect in a laboratory when feeding tadpoles with the contents of fish intestines. The experiments were not repeated owing to the disappearance of the anomaly in the "ponds with monsters". Finally, Rostand (1971) suggested a hypothesis for the induction of the anomalies by a teratogenic virus transmitted by fish or some components of their diet.

For half a century, the anomaly could not be found despite the searches undertaken (Dubois 1979; 1984). However, in 2016, we rediscovered Rostand's anomaly P (Svinin et al. 2019), confirming the hypothesis concerning its wider distribution (Rostand 1971). Water frogs with the anomaly were found in Ostrovtsovskaya Lesosteppe, Privolzhskaya Lesosteppe Nature Reserve, Penza Region, Russia.

Here, we describe three additional new localities from Russia where the anomaly P syndrome was observed in two species of western Palearctic water frog (Fig. 1). All these new localities have very different conditions. The anomaly P was found in both natural and anthropogenic ponds, small lakes and huge reservoirs, in canopy and open landscapes, nature reserves and within settlements.

Locality 1. Edrovo village (57.916640N, 33.625937E, 196 m alt.), Novgorod Region, 2 August 1999. Here, the pool frog (*P. lessonae*) inhabits an artificial pond. Twenty metamorphosed individuals were collected. Two metamorphosed individuals had severe forms of the anomaly P. Four adults were without anomalies. Samples are stored in the herpetological collections of the Zoological Institute of the Russian Academy of Sciences. A re-investigation of the same pond on 17 August 2019, showed the presence of the carnivorous invasive fish *Perccottus glenii* and the complete absence of water frog tadpoles.

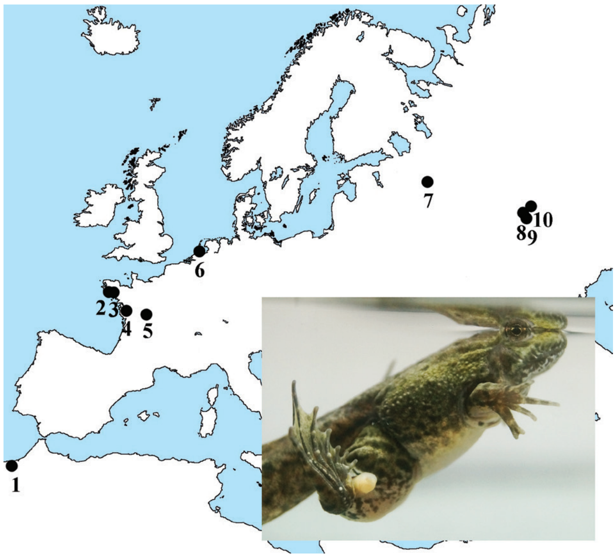

Figure 1. Distribution of severe cases of Rostand's anomaly P: 1 – Port Lyautey near Kenitra, Morocco; 2, 3 – Trévignon and Penloc'h, Finistère, France; 4 – Lingé, France; 5 – Saint-Philbert-de-Grand-Lieu, France; 6 – Amsterdam, Netherlands; 7 – Edrovo village, Russia; 8 – Poperechenskaya Steppe, Russia; 9 – Ostrovtsovskaya Lesosteppe, Russia; 10 – Surskoe Reservoir, Russia (photo: *Pelophylax ridibundus* individual with severe cases from Ostrovtsovskaya Lesosteppe).

Locality 2. Poperechenskaya steppe (53.049986N, 44.294268E), part of Privolzhskaya Lesostep' Nature Reserve, Penza Region, Russia. It is located in the upper part of the Khoper River drainage basin (the same as Ostrovtsovskaya Lesosteppe, where the anomaly P was previously found). Despite the fact that these two parts of the reserve are situated approximately 20 km from each other, they were very distant regarding their watercourses. The pond in Poperechenskaya steppe was made by beavers (*Castor fiber*), is located in a steppe landscape and is inhabited by the marsh frog, *P. ridibundus.* In June 2019, a total of 12 tadpoles were caught. All tadpoles had severe forms of the anomaly P (Fig. 2). One of five subadult frogs had symmetrical polydactyly.

Locality 3. Surskoye Reservoir (53.032927N, 45.290373E), Penza Region, Russia. The water area of the reservoir is 110 km^2. The investigated habitat is a small backwater of the reservoir. It was inhabited by the marsh frog, *P. ridibundus.* In June 2019, we caught 110 tadpoles. Of these tadpoles, 104 were normal, two had polydactyly and four had severe forms of the anomaly P. Six adult frogs were caught showing no abnormalities.

The polydactyly of water frogs has attracted scientists for a long time. One of the first cases of polydactyly was discovered by French zoologist Isidore Geoffroy Saint-Hilaire in 1832 (Geoffroy Saint-Hilaire 1832; Rostand 1971). In the recent area of the Russian Federation, polydactyly was described in 1896 by N.A. Kholodkovsky, who observed specimens of water frogs with polydactyly, both on fore and hind limbs from "Khrenovskoy Bor" (Kholodkovsky 1896). Recently, the bibli-

Figure 2. Specimens with the anomaly P found in Surskoe reservoir: A, B – polydactyly, C, D – severe cases of the anomaly P syndrome.

ography provided by Rothschild et al. (2012) includes approximately 40 publications (together with articles written by Jean Rostand) in which polydactyly in water frogs has been described.

Following Rostand (1971), we consider the anomaly P phenomenon more widespread. Indeed, many cases of symmetric polydactyly, especially those presented in both the fore and hind limbs, probably relate to the anomaly P. Polydactyly, however, may be caused by completely different factors. For example, polydactyly and other morphological limb abnormalities can be a result of the influence of radiation and chemicals (Rostand 1971; Vershinin 1989; Ouellet 2000; Henle et al. 2017). At present, it is difficult to separate these cases, especially in field. However, as a rule, polydactyly caused by the infectious agent of the anomaly P had the following characteristics: symmetry (1), a gradient from 6 to more digits (2), presence on both the hind and fore limbs (3) and mass occurrence (4).

Mass and symmetrical polydactyly in water frogs was repeatedly described in Europe (Table 1). In six locations, frogs had polydactyly both on fore and hind limbs; thus, they were very similar to the anomaly P manifestation. Machado et al. (2010) found several cases that undoubtedly belong to the anomaly P. In the marshy areas of Upper Swabia in southern Germany, during 1981, anomalies were found in 13 of 192 specimens (7%) and labelled as "Seelenhofer Ried". Such a variant of polydactyly was observed in a marsh frog from a pond near Mordovo village and from a pond near the city of Penza in Russia (Zaks 2008; Fayzulin 2012). Three specimens of a total twenty-three (13%), from a pond in the village of Bol'shaya Lipovitsa in Russia, had polydactyly on the hind and forelimbs (Kozhevnikova and Lada 2016).

Symmetric polydactyly on the hind limbs was noted in the village of Fedorovka (2 of 15 specimens, i.e. 13%) and the Botanical Garden of Samara (1 individual from 19, i.e. 5%) in the Samara Region of Russia (Fayzulin et al. 2018). It was observed in a pond near the village of Alba in Belarus: 46 of 161 individuals (29%) captured between 1977 and 1979 had polydactyly on their hind limbs (Borkin and Pikulik 1986). In 1983, a specimen with symmetrical polydactyly (eight digits) on the hind limbs was found in the village of Staritsa within the Tambov region in Russia (Lada 1999). Mass cases of polydactyly were found in Minai village and Stanytsia Luhanska suburbs of Ukraine (Kurtyak 2005; Marushchak and Muravynets 2018). A case of symmetrical polydactyly (6 digits on the hind limbs) was also found in Romania at Gherţa Mică (Sas and Kovacs 2006).

Additional cases of asymmetric polydactyly were revealed in the following localities in the Samara Region of Russia (Fayzulin 2012; Fayzulin et al. 2018): the village of Klimovka (1 from 16, i.e. 6%), the village of Upper Suskan (1 from 17, i.e. 6%), the Gorniy settlement (1 from 10, i.e. 10%) and the city of Ufa (1 from 26, i.e. 4%).

Our records extend the knowledge of the distribution of the infectious agent and most probably, within these ranges, new regions of "hotspots" with a high occurrence of the anomaly P will be discovered. The hind-limb anomalies in anurans impair locomotor performance (Zamora-Camacho and Aragón 2019), which is likely related with reduced survival (Heinen and Hammond 1997). We also never observed severe cases of Rostand's anomaly P in adult individuals in wild populations, because individuals showing strong deformities may have been eaten by predators or died after metamorphosis due to injuries and open wounds (our and Rostand's laboratory observations; Rostand 1971). High frequency of anomalies can decrease survival of individuals and lead to reduction of local populations size (Dubois 2017). The monitoring of water bodies with high abnormality rates will detect variation in frequency of abnormal individuals and determine the most significant environmental factors causing such differences. It is expected that the detailed study of such localities will throw light on the nature of the teratogenic agent of the anomaly P.

Table 1. Distribution of polydactyly and heavy cases of the Rostand's anomaly in European water frogs.

#	Species	Country	Region	Locality	N / E	Literature
Heavy cases of the anomaly P						
1	"*P. esculentus*"	France	Finistère	Trévignon	47.798869, -3.845854	Rostand 1971; Dubois 1979; 1984; 2017
2	"*P. esculentus*"	France	Finistère	Penloc'h	47.800546, -3.849952	Rostand 1971;
3	"*P. esculentus*"	France	Indre	Lingé	46.755949, 1.083394	Rostand 1971; Dubois 1979; 1984; 2017
4	"*P. esculentus*"	France	Loire-Atlantique	Saint-Philbert-de-Grand-Lieu	47.077709, -1.679565	Rostand 1971; Dubois 1979; 1984; 2017
5	"*P. esculentus*"	Morocco	Rabat-Salé-Kénitra	Port Lyautey near Kenitra	34.306902, -6.578357	R. Lautie, pers. com. in Rostand 1971
6	"*P. esculentus*"	Netherlands		Amsterdam	52.358068, 4.950365	Hillenius 1959 in Rostand 1971
7	*P. ridibundus*	Russia	Penza Region	Ostrovtsovskaya Lesosteppe	52.816111, 44.461111	Svinin et al. 2019
8	*P. lessonae*	Russia	Penza Region	Surskoe Reservoir	53.032927, 45.290373	This publication
9	*P. ridibundus*	Russia	Penza Region	Poperechenskaya steppe	53.049986, 44.294268	This publication
10	*P. ridibundus*	Russia	Novgorod Region	Edrovo settlement	57.916640, 33.625937	This publication
Polydactyly						
Symmetrical polydactyly on both hind and forelimbs						
11	"*P. esculentus*"	Russia	Voronezh Region	Chrenovskoy Bor	51.102176, 40.131986	Kholodkovsky 1896
12	"*P. esculentus*"	France	Ain	Villars-les-Dombes	45.997765, 5.024091	Bonnet and Rey 1937; Rostand 1971; Dubois 1979; 1984; 2017
13	"*P. esculentus*"	Germany	Upper Swabia	Seelenhofer Ried	48.083333, 9.633333	Machado et al. 2010
14	*P. ridibundus*	Russia	Penza Region	Sosnovka	53.1688889, 45.0961111	Zaks 2008
15	*P. ridibundus*	Russia	Samara	Mordovo	53.176002, 49.438077	Fayzulin 2012
16	*P. ridibundus*	Russia	Tambov Region	B. Lipovitsa	52.531394, 41.350586	Kozhevnikova and Lada 2016
Symmetrical polydactyly on hindlimbs						
17	"*P. esculentus*"	France	Loire	Champdieu	45.646192, 4.065011	Rostand 1971; Dubois 1979; 1984; 2017
18	"*P. esculentus*"	France	Gironde	Bordeaux	44.890266, -0.575800	Rostand 1971; Dubois 1979; 1984; 2017
19	"*P. esculentus*"	France	Landes	Soustons	43.764041, -1.337326	Rostand 1971; Dubois 1979; 1984
20	"*P. esculentus*"	France	Landes	Léon	43.897340, -1.320508	Rostand 1971; Dubois 1979; 1984
21	"*P. esculentus*"	France	Landes	Aureilhan	44.227901, -1.215550	Rostand 1971; Dubois 1979; 1984
22	"*P. esculentus*"	Turkey	près d'Istanbul,	Tekirdag	40.926399, 27.400486	Dubois 1979; 1984
23	"*P. esculentus*"	France	Haute-Saône	Luxeuil-les-Bains	47.818387, 6.386234	Dubois 1984
24	"*P. esculentus*"	France	Territoire-de-Belfort	Faverois	47.518561, 7.033751	Dubois 1984
25	"*P. esculentus*"	France	Moselle	Fénétrange	48.841808, 7.003869	Dubois 1984
26	"*P. esculentus*"	France	Jura	Tassenières	46.918473, 5.506221	Dubois 1984
27	*P. esculentus*, *P. lessonae*	Belarus	Minsk	Alba fishery near Nesvizh	53.201240, 26.637838	Borkin and Pikulik 1986
28	*P. ridibundus*	Russia	Tambov Region	Staritsa village	52.606942, 42.797774	Lada 1999
29	*P. esculentus* complex	Russia	Tatarstan	Kazan	55.802554, 49.139874	Zamaletdinov 2003
30	*P. ridibundus*, *P. esculentus*	Moldova		Bugornya	47.958231, 28.823739	Bezman-Moseiko et al. 2014
31	*P. ridibundus*, *P. esculentus*	Moldova		Plot'	47.974755, 29.160899	Bezman-Moseiko et al. 2014
32	*P. ridibundus*	Russia	Samara Region	Fedorovka	53.466, 49.665	Fayzulin et al. 2018
33	*P. ridibundus*	Russia	Samara Region	Samara, Botanical Garden	53.215, 50.179	Fayzulin et al. 2018
34	*P. esculentus*	Romania	Satu-Mare County	Gherța Mică locality	47.933333, 23.233333	Sas and Kovacs 2006
35	*P. ridibundus*	Ukraine	Luhansk region	Stanytsia Luhanska suburbs, Siverskyi Donets river	48.665029, 39.470719	Marushchak and Muravynets 2018
36	*P. esculentus*	Ukraine	Zakarpattia	Mynai village	48.590665, 22.282290	Kurtyak 2005
Asymmetrical polydactyly on hindlimbs						
37	*P. ridibundus*	Russia	Samara Region	Klimovka	53.487, 49.018	Fayzulin et al. 2018
38	*P. esculentus*	Russia	Samara Region	Verhniy Suskan	53.818, 49.311	Fayzulin et al. 2018
39	*P. lessonae*	Russia	Samara Region	Settlement Gornyi	52.997, 51.061	Fayzulin et al. 2018
40	*P. ridibundus*	Russia	Samara Region	Ufa, Lokotki	54.542, 55.931	Fayzulin et al. 2018

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