Acta Biol. Univ. Daugavp. 9 (1) 2008
ISSN 1407 - 8953

FENETIC ANALYSIS OF *BOMBINA BOMBINA* VENTRAL SPOTS PATTERN IN 8 LOCALIZATIONS IN LATVIA

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Pupins M., Pupina A. 2009. Fenetic analysis of *Bombina bombina* ventral spots pattern in 8 localizations in Latvia. *Acta Biol. Univ. Daugavp., 9 (1): 131 - 136.*

The pattern of ventral spots of 72 individuals of *Bombina bombina* from 8 localizations in Latvia were investigated. The typification of variations was carried out on the basis of the analysis of relative length and the form of the prevailing fragments of orange spots. 15 variations were distinguished, which were united into 5 clusters. The analysis of ventral spots of *Bombina bombina* showed the domination of definite variations in concrete localizations that indicates the high degree of the relationship of individuals. When combining the variations into clusters and localizations in the population, the domination of definite clusters was registered. For the first time the rare in Latvia variation was noted: one large monolithic orange spot practically without any black spots.

Key words: *Bombina bombina*, Latvia, ventral spots.

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INTRODUCTION

The use of *Amphibia* pattern individuality is a widespread method in recognizing individuals (Donnelli 2003). Forming of *Bombina bombina* (Linnaeus 1761) ventral spots pattern stops after the first wintering (Masalykin 1989, cited in: Novitsky et al 2001). 12 ventral phenocomplexes with numerous variations, which can be used as markers, are distinguished (Novitsky et al. 2001). It is possible to determine the genetic specificity by the frequency of separate phens' versions (Novitsky et al. 2001; Nürnberger et al. 1995). The spottiness of *Bombina bombina* belly is connected with the variability of gene-markers (Yanchukov et al. 2002). Ventral spots of *Bombina sp.*, whose pattern remains permanent for the whole life, serve for the individual labelling of animal (Gollmann, Gollmann 2002; Streich et al. 1997).

The majority of *Bombina bombina* populations in Latvia (*Bauska*, *Ilgas*, *Ainavas*, *Spulgu*) are extremely small in number and during several years only 1-9 vocalizing males were registered there (Pupiņa & Pupiņš 2007). In 2007, under the complete investigation of all known *Bombina bombina* populations in Latvia, 228 vocalizing males *Bombina bombina* were registered in total (Pupiņa & Pupiņš 2007). The ratio of *Bombina bombina* males and females in population in neighbouring to Latvia Belarus composes 1:1,18 (Drobenkov et al. 2005). Thus, the probable total number of known adult *Bombina bombina* in Latvia can be estimated to 497 individuals in 2007. Our studies of ecology and *Bombina bombina* migrations in Latvia required the individual recognition of *Bombina bombina* individuals; therefore it was of current importance to investigate and fix the *Bombina bombina* phenocomplexes, which are characteristic for the

populations that dwell on the northern boundary of the area, in Latvia.

MATERIAL AND METHODS

The study was carried out on the territory of Latvia during 2006-2007. *Bombina bombina* from 8 localizations, representing all 5 constantly registered *Bombina bombina* populations in Latvia were examined (Pupiņa, Pupiņš 2007) (Fig.1.).

During the study ventral phenocomplexes of adult *Bombina bombina* (n=72) were registered and analyzed, which makes 14,49% of a probable total number of adult *Bombina bombina* (n=497) in Latvia.

The mapping of ventral phenocomplexes of *Bombina bombina* was carried out by photographing. While photographing *Bombina bombina* were fixed in one's hand or in transparent plastic box of 15x10 cm with water level of 1 cm. The length and nature of fragments form were accepted as the basis of variations typification during the analysis of ventral phenocomplexes.

RESULTS

The obtained in the study examples of the investigated sampling spots (n=72) *Bombina bombina* were split into 15 variations or phenomorphs (Tab.1). During the analysis of the phenomorphs' occurrence frequency in the inspected samplings of *Bombina bombina* from different localizations the tendencies toward the domination of *Katriniski*: *Crown 4*; *Briezi*: *Loop*, *Strip*, *Pebble*; *Spulgu*: *Loop*; *Behova 1*: *Crown 3*; *Turaidas*: *Thin hook*, *Wave*; *Ozolaine*: *Hieroglyph* and *Splinter*; *Ilgas Meliorative channel*: *Hieroglyph*, *Loop*, *Thin hook*; *Ilgas Round pond*: *Hieroglyph* were reviled (Fig.2).

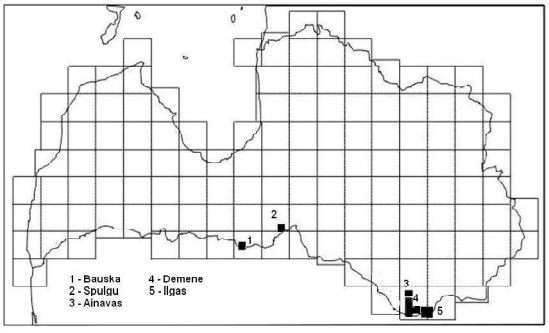


Fig.1. The distribution of 5 examined populations *Bombina bombina* in Latvia.

Similar phenomorphs (Fig.3.) were grouped in clusters (Tab.2).

In the study a tendency towards the domination of different clusters in *Bombina bombina* samplings from different localizations was noted: *Briezi*, *Spulgu*, *Ozolaine*, *Ilgas Meliorative channel*: Cluster 2; *Katriniski* and *Behova 1*: Cluster 3; *Ilgas Round pond*: Cluster 1; *Turaidas*: Cluster 1 and Cluster 4. Cluster 5 is very rare; the single instance is registered in localization *Ozolaine* (Fig.4., 5.).

During the analysis of the frequency of clusters occurrence in samplings from examined populations a tendency towards the domination of clusters was registered: population *Bauska* (*Turaidas*): Cluster 2 and Cluster 4; population *Demene*: Cluster 3; populations *Medumi*, *Ilgas* and *Spulgu*: Cluster 2 (Fig.6.).

During the analysis of the frequency of clusters occurrence in *Bombina bombina* samplings from examined populations a tendency towards the domination of clusters was registered: population *Bauska* (*Turaidas*): Cluster 2 and Cluster 4; population *Demene*: Cluster 3; populations *Medumi*, *Ilgas* and *Spulgu*: Cluster 2 (xx.att.). In all populations a tendency towards the subdomination is noted Cluster 1 and Cluster 4, an exception is the population *Spulgu*, where Cluster 4 is not registered. Cluster 3 is registered only in nearby populations *Demene* and *Ilgas* (Fig.7).

Summarizing the study data of *Bombina bombina* samplings (n=72; 14,49 % from all known adult *Bombina bombina* in Latvia), the following ratio

Table 1. Variations of phenocomplex of *Bombina bombina* ventral part (phenomorphs)

Variation	Title	Description of spots	Length of fragments mm
1.	*Hieroglyph*	Spots remind of hieroglyphs.	
2.	*Loop*	Secluded spots with a black spot inside.	
3.	*Hieroglyph fragment*	Smaller elements of hieroglyphs.	
4.	*Strip*	Relatively straight lines.	7-11
5.	*Wave*	Wavy spots.	5-10
6.	*LZ*	L-,Z-like spots.	5-6
7.	*Splinter*	Spots remind of splinters.	3x4
8.	*Pebble*	Roundish small spots.	3x4
9.	*Crown 1*	Spots remind of crowns, combs.	7-10
10.	*Crown 2*	Spots remind of crowns, combs	5-6
11.	*Crown 3*	Spots remind of crowns, combs	3-4
12.	*Crown 4*	Spots remind of crowns, combs	2-3
13.	*Hook*	Spots remind of hooks.	2-3
14.	*Thin hook*	Spots remind of thin hooks.	1-3
15.	*Mono*	Spot is monolithic, occupies the largest part of a belly.	

of clusters' occurrence in the samplings singled out during the study was established: Cluster 2: 40,28%; Cluster 3: 23,61%; Cluster 1: 18,6%; Cluster 4: 16,67%; Cluster 5: 1,39% (Fig.8).

DISCUSSION

The examined quantity of *Bombina bombina* (n=72) composes 14,49% of the probable total number of adult *Bombina bombina* (n=497) in Latvia, which makes it possible to draw primary conclusions about the nature of phenomorphs in Latvia and the expansion of singled out phenomorphs and clusters in Latvia.

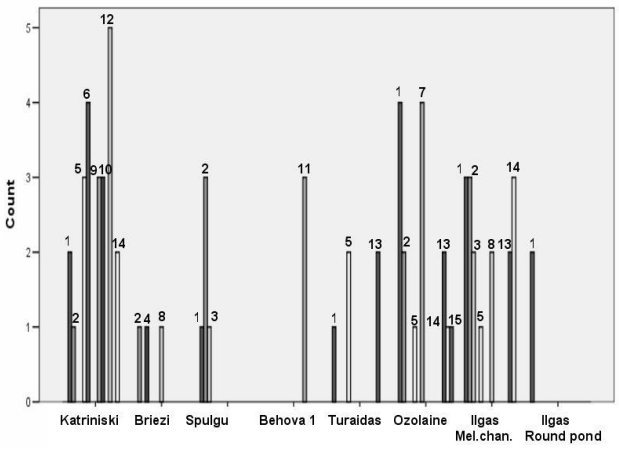


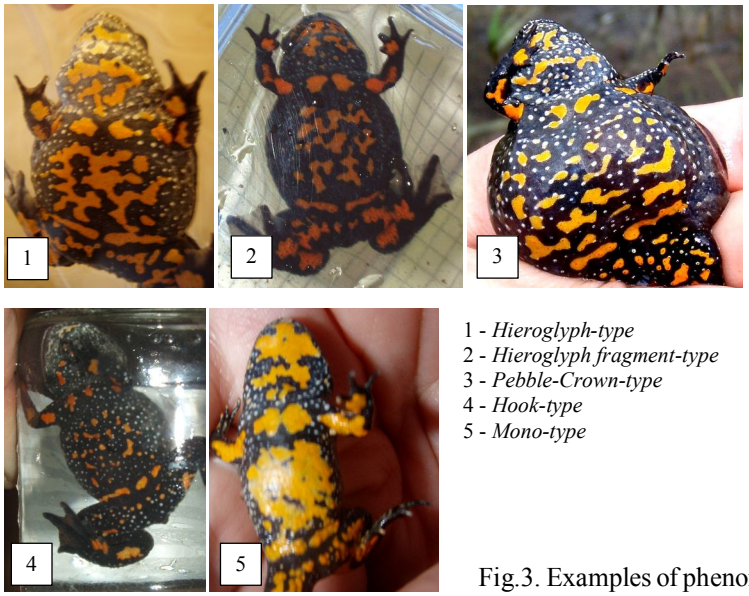
Fig.2. The frequency of ventral phenomorphs (1-15) occurrences in *Bombina bombina* samplings (n=72) from different localizations in Latvia

The main aim of the study was pattern description of the mostly widespread phenomorphs of *Bombina bombina* ventral complexes in Latvia for the use of an individual pattern of belly spots as markers for the distinguishing of *Bombina bombina* individuals. Such method is used by many researchers, also using computer programmes (Streich et al. 1997, Voros et al. 2007).

The tendency towards the domination of different phenomorphs variations in samplings from different localizations and populations was noted in the study that can be explained by homing of the metamorphized individuals of *Anura* (Ogurtsov 2003). The mosaic territorial distribution of *Bombina bombina* phenomorphs in Belarus was noted by R.Novitsky (Novitsky et al. 2001), explaining this by the genetic isolation of some parts of the area, especially on the boundary of specific area. Since the population of *Bombina bombina* in Latvia is relatively small, the existing

Table 2. Clusters of phenomorphs.

Cluster	Grouped phenomorphs	Description	Length of fragments mm
1.	1.	*Hieroglyph-type* - the largest spots resemble of complex hieroglyphs.	
2.	2.-7.	*Hieroglyph fragment-type* - spots resemble of hieroglyphs' fragments.	3-10
3.	8.-12.	*Pebble-Crown-type* - spots resemble of pebbles and crowns.	3-10
4.	13.-14.	*Hook-type* - spots resemble of hooks.	1-3
5.	15.	*Mono-type* - spot is monolithic, occupies the largest part of a belly.	



1 - *Hieroglyph-type*
2 - *Hieroglyph fragment-type*
3 - *Pebble-Crown-type*
4 - *Hook-type*
5 - *Mono-type*

Fig.3. Examples of phenomorphs' clusters.

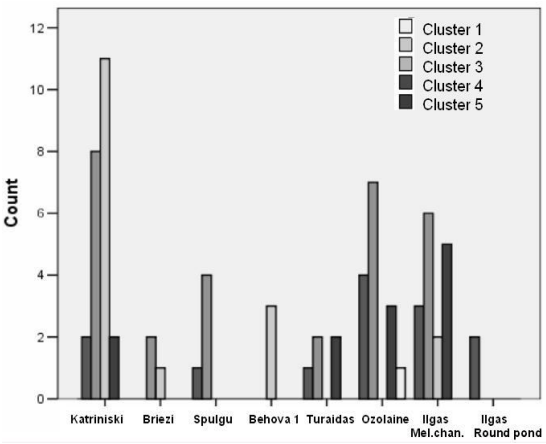


Fig.4. The frequency of ventral phenomorphs' clusters occurrence in *Bombina bombina* samplings (n=72) from different localizations.

data is planned to be supplemented during the following seasons in order to conduct statistical data analysis.

ACKNOWLEDGEMENTS

The research has been executed owing to support of European Structural Funds, Daugavpils University (Project #2004/003/ VPD1/ESF/ PIAA/04/NP/3.2.3.1./0003/0065), Latvian Environmental Protection Fund, Daugavpils Municipality, Latgale Zoo. We thank M.Deicmane (Latvia), H.Drews (Germany), A.Skute (Latvia), S.Kuzmin (Russia), S.Ignatjeva (Latvia), A.Andrejeva

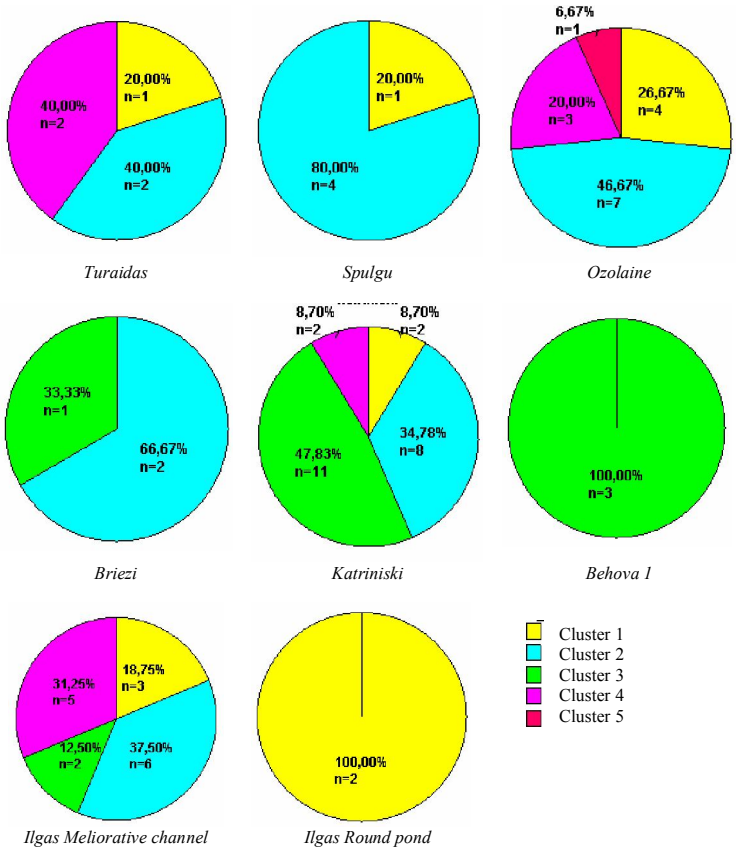


Fig.5. The ratio of registered clusters of ventral phenomorphs in *Bombina bombina* samplings (n=72) from different localizations (n=8).

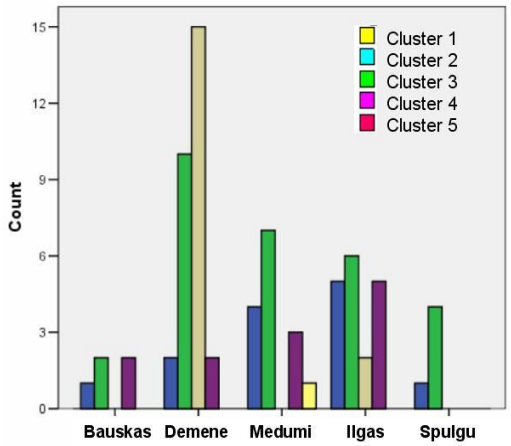


Fig.6. The occurrence of ventral phenomorphs' clusters in *Bombina bombina* samplings (n=72) from the examined populations (n=5)

(Latvia) for the consultations and cooperation.

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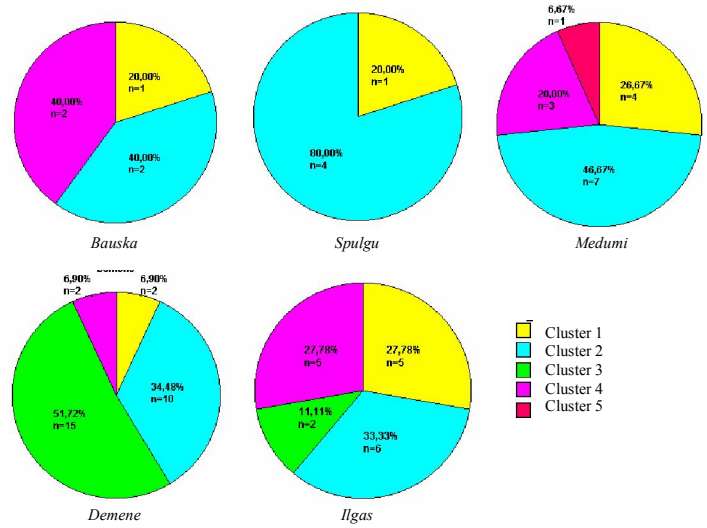

Fig.7. The ratio of ventral phenomorphs' clusters occurrence in *Bombina bombina* samplings (n=72) from the examined populations (n=5).

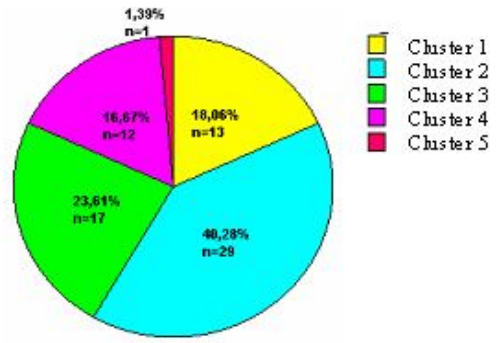

Fig.8. The ratio of different clusters of ventral phenomorphs occurrence in the examined *Bombina bombina* samplings (n=72).

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Received: 30.04.2008.
Accepted: 01.06.2009.