ANALYSES REGARDING SOME *Bombina* GENUS'S POPULATIONS IN THE ARAD COUNTY, ROMANIA

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Abstract. Our study took place in may 2006, and was made on a *Bombina* sp. population nearby Cermei locality, a field region, and on 4 populations from Mădrigeşti, a mountain region, from Arad county. We analyzed 20 characters belonging to both species on porpose to establish the affiliation of the studied populations. The Cermei's population belongs to B. bombina species, however were signed the presence of some characters expressed like *B. variegata* . The same think about the Mădrigeşti's population which belong to B. variegata species, there was identified, noticed some characters expressed like *B. bombina.*

Key words: affiliation, character, *Bombina bombina, Bombina variegata*

Rezumat. Analiza unor populaţii ale genului Bombina din judeţul Arad, România. În perioada lunii mai 2006 în judeţul Arad, am studiat o populaţie de *Bombina* sp. din regiunea de câmpie Cermei, şi 4 populaţii din regiunea montană Mădrigeşti. Am urmărit 20 de caractare pentru a stabili afilierea populaţiilor studiate. Populaţia de la Cermei aparţine speciei B. bombina, prezentând caractere de *B.variegata*. Populaţiile din regiunea Mădrigeşti aparţin speciei B. variegata, prezentând caractere ale speciei B. *bombina.*

Cuvinte cheie: afiliere, caractere, *Bombina bombina, Bombina variegata*

INTRODUCTION

Bombina bombina is a typical species for the field fauna living in the low regions from the east and center Europe (ARNTZEN, 1980), generally going up to 400 m height. *Bombina variegata* species is spread in the high regions, the inferior area being at 100 m height, here is present just in a exceptional way (MADEJ, 1964). In general, appears up to 150 m height, and it can arrive at 1870 m (STUGREN & GHIRA, 1987).

Bombina bombina and *Bombina variegata* species are not isolated from the reproduction point of view, and there were noticed hybrid populations in all regions where the 2 species have contact (SZYMURA, 1993). In the speciality literature were identified cases when the characters of a species appears at the other species, despite their far localization from the hybrid zone(STUGREN, 1980, GHIRA & MARA, 2000, COVACIU-MARCOV et al., 2002, 2003), and the presence of these characters can't be caused by the cross-breeding phenomenon.

We attended to establish the affiliation of the populations to the 2 species, based on the known characters, and also the existence of a species characters in the other.

MATERIALS AND METHODS

Our study was realized in may 2006 on 33 samples from Cermei and 143 samples from the 4 habitats in Mădrigeşti.

To establish the affiliation of the studied populations we used the method of analyzing some morphologic and chromatics characters of the two species. We analyzed 20 characters using 2 grids, each grouping 10 characters. The used characters are the most important diagnose characters of the two species and different authors used them (STUGREN, 1980, GOLLMANN et al., 1993, SZYMURA & BARTON, 1991, GHIRA et al., 2003).

The first grid analyses the morphology, the dimensions and the ratios of light ventral spots. These are red at *Bombina bombina* species and yellow at *Bombina variegata* species. (FUHN, 1960). The grid analyses the confluence or separate degree between the light ventral spots, from different parts of the body identifying 10 chromatics groups (Table no. 1). The grid was made by Szymura, Gollman and then by many others authors (SZYMURA & BARTON, 1991, GOLLMANN, 1984, GOLLMANN et al., 1993) . If the light spots are separated between by dark pigment then the character belongs to *Bombima bombina* species, and if the light spots are united, the light pigment appears uniformly the characters belongs to *Bombina variegata* species.

The second grid analysis 10 characters and was made by Stugren (1980) and modified by Ghira & Mara (2000) (Table no. 2).

Each character receives a mark: 1 if is *Bombina variegata*, 0 if is *Bombina bombina*. Summing the obtained mark's for each character, a certain individual can receive on each grid a score ranging from 0 to 10, these score equal to 0 means that the individual is a pure *Bombina bombina*, the score equal to 10 indicating a pure *Bombina variegata* individual, and the values closed to these indicates *Bombina bombina* and *Bombina variegata – like* individuals. The intermediate scores of 4, 5, 6 indicates hybrids.

After this, we calculated the average score of all individuals of each population, for each grid, and then the average of the two grids, obtaining a number (%) which express the affiliation of the population. The values closed to 0 indicates a *Bombina bombina* population and values closed to 100 indicates a *Bombina variegata* population. The final

mark indicates the amount of *Bombina variegata* species features in every studied population. The method allows to change some features in percentage and the statistic interpretation of these.

The studied habitats are represented by 3 pools and a brook from Mădrigesti at 450 meters height and a pool nearby Cermei locality, at 105 m height.

The first habitat from Mădrigesti is represented by a permanent big pool of 10 m^2 with approximate 1 m deepness, situated next to the road and a beech forest with rich vegetation (reed and scouring rush). In this habitat we found besides Bombina species newts species as well, and for this the habitat was named the pool with newst. The second habitat located beyond the road, on the field being a temporary pool, of about 3 m^2, with reduce deepness and oozy substratum. The third habitat from Mădrigesti is represented by a brook located in a wooded zone, next to the pool with newts. The deepness of the weather reach maximum 30 cm, the pollution degree is reduced, the following of the weather being relatively fast. The forth habitat is a temporary pool, with reduced size, nearby the pool with newts; it shrinks in the forest, named the pool under the forest, with a small deepness and oozy substratum.

The Cermei's habitat is represented by a pool located in a field region; it has a size of about 70 m^2 , but strongly polluted. The vegetation of the pool is thick (reed and scouring rush), being present invertebrates and vertebrates as well.

Table 1. Grid 1 of differentiation of the European species of the *Bombina* gender (the characteristics of the ventral pattern) (SZYMURA & BARTON, 1991, GOLLMANN, 1984)
Tabel 1. Grila 1 pentru diferențierea speciilor europene ale genului *Bombina* (caracteristicile modelului ventral) (SZYMURA & BARTON, 1991, GOLLMANN, 1984)

Characteristic (light spots on):		***Bombina bombina***	***Bombina variegata***
1	Chin-chin	Separated	United
2	Chin-chest	Separated	United
3	Chest-chest	Separated	United
4	Chest-shoulder	Separated	United
5	Shoulder-arm	Separated	United
6	Chest-abdomen	Separated	United
7	Abdomen–abdomen	Separated	United
8	Abdomen – basin	Separated	United
9	Basin–basin	Separated	United
10	Basin–thigh	Separated	United

Table 2. Grid 2 of differentiation of the European species of the *Bombina* gender (after STUGREN, 1980, modified by GHIRA & MARA, 2000)
Tabel 2. Grila 2 pentru diferențierea speciilor europene ale genului *Bombina* (după STUGREN, 1980, modificat de GHIRA & MARA, 2000)

Character		***Bombina bombina***	***Bombina variegata***
1	**.** Colour of open ventral spots	*Red, orange, yellowish*	*Yellow*
2	Colour of upper part of the first finger and the top of fingers	*Black*	*Yellow*
3	Dorsal colouring	*Black*	*Pale grey*
4	The relation tarsian and plantar open spots	*Separated*	*United*
5	Ventral colour	*Orange spots on black background*	*Black spots on yellow background*
6	The relation between the length and width of the head	*Length > width*	*Length < width*
7	**.** The drawing of lateral and ventral parts	*White spots around the verrucae*	*Without white spots around the verrucae*
8	The drawing of the dorsal part	*Regulated black tubercles*	*Black scattered verrucae*
9	Dorsal verrucae	*Lens – shaped squatted*	*Sharp, rough*
10	The ratio of tibia – tarsian joints when the stylopode and the zeugopode are parallel	*Not touching*	*Touching*

RESULTS

The studied population from Cermei is a *Bombina bombina* population, presenting the major amount of the studied features by us, expressed just like at the fire-bellied toad. For these, the studied population presents a percentage of 12,27 % features which expresses like at *Bombina variegata* (Fig.1).

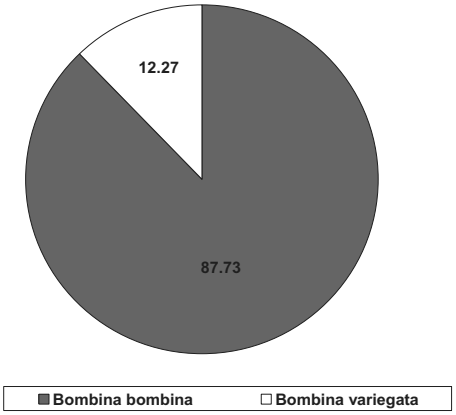

Figure 1. The amount of characters of *B. variegata* in the Cermei's *Bombina bombina* population
Figura 1. Ponderea caracterelor de *B. variegata* la populația de *Bombina bombina* de la Cermei

After analyzing the characters from the first grid (Table no.3) we have noticed the expression of some features just like at *Bombina variegata* represented by C1 (3 %), C3 (3 %), C6 (3 %), C7 (1,5 %), C10 (3 %), dominating the C5 feature. The C2, C4, C8 and C9 characters were typical of *Bombina bombina*. In the second grid the C3 and C9 features are presented in the same way like at the population they belong (0%), the rest of the characters in some cases express like *Bombina variegata*, with the C6 feature dominating, this one being mostly responsible of the major amount of the *Bombina variegata* species features at the *Bombina bombina* population.

Table 3. The amount of Bombina variegata characters in the Cermei's Bombina bombina population
Tabel 3. Ponderea caracterelor de *B. variegata* la populația de *Bombina bombina* de la Cermei

	Habitat	Cermei's Pool
	Total no. of samples	33
G1%	Character1	3.00
	Character 2	0.00
	Character 3	3.00
	Character 4	0.00
	Character 5	46.90
	Character 6	3.00
	Character 7	1.50
	Character 8	0.00
	Character 9	0.00
	Character 10	3.00
G2%	Character 1	6.00
	Character 2	21.20
	Character 3	0.00
	Character 4	1.50
	Character 5	10.60
	Character 6	90.90
	Character 7	3.00
	Character 8	15.10
	Character 9	0.00
	Character 10	39.30

Regarding the Mădrigesti habitats, the *Bombina variegata* populations also presented characters which express just like at the other species, represented in the pool with newts by 22,07 %, in the pool from the field with 22,26 %, in the brook 20,69 % and in the pool under the forest 23,7 %.

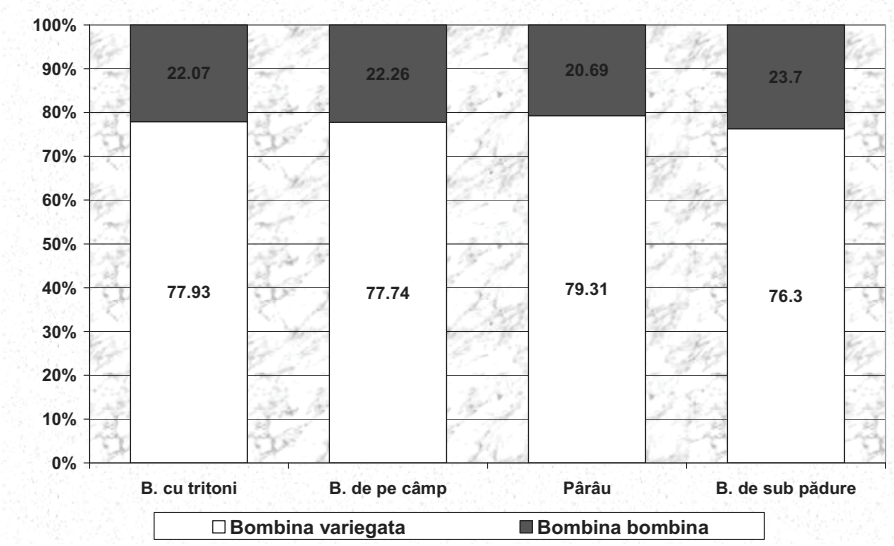

Figure 2. The amount of the *B. variegata* characters in the Mădrigeşti's *Bombina variegata* population
Figura 2. Ponderea caracterelor de *B. variegata* la populațiile de *Bombina variegata* de la Mădrigeşti

Table 4. The amount of *B. variegata* characters in the Mădrigeşti's *Bombina variegata* population
Tabel 4. Ponderea caracterelor de *B. variegata* la populațiile de *Bombina variegata* de la Mădrigeşti

	Habitat	The pool with newts Mădrigeşti	The pool from the field Mădrigeşti	The brook Mădrigeşti	The pool under the forest Mădrigeşti
	Total no. of samples	40	40	40	23
G1%	C1	100.00	100.00	100.00	95.60
	C2	3.70	12.50	6.20	8.60
	C3	17.50	7.50	12.50	13.00
	C4	83.70	78.70	82.50	82.60
	C5	100.00	100.00	100.00	100.00
	C6	46.20	53.70	46.20	41.30
	C7	98.70	100.00	98.70	100.00
	C8	95.00	100.00	95.00	100.00
	C9	100.00	100.00	97.50	100.00
	C10	100.00	98.70	100.00	100.00
G2%	C1	100.00	100.00	100.00	100.00
	C2	100.00	100.00	100.00	100.00
	C3	100.00	97.50	100.00	73.90
	C4	76.20	76.20	78.70	71.70
	C5	100.00	100.00	100.00	100.00
	C6	80.00	82.50	100.00	91.30
	C7	30.00	5.00	0.00	4.30
	C8	72.50	75.00	85.00	56.50
	C9	100.00	100.00	97.50	100.00
	C10	57.50	67.50	90.00	82.60

In the first grid the 2 and 3 characters are responsible for the appearance of the *Bombina bombina* characters in the *Bombina variegata* populations, and the most stable characters, constantly expressed just like at *Bombina variegata* species are C1, C5, C7, C8, C9 and C10 (>95 %) (Table no.4).

In the second grid the smallest values are noticed in the case of the character number 7, which don't overtake 30% (The pool with newts), the brook has all it's individuals, presenting the feature just like at *Bombina bombina* (0%).

The characters 1, 2, 5 and 9 express just like at *Bombina variegata*, with values higher than 97 %. The characters 4, 6 and 8 at some individuals have expressed either just like at *Bombina bombina* or just like at *Bombina variegata*.

DISCUSSION

The height and the characteristics of the Cermei's habitat are favorable to the fire- bellied toad, being present a population of *Bombina bombina*, even though there are meet some characters expressed just like at *Bombina variegata*.

Our results are similar to those obtained by Stugren (STUGREN, 1980) , which identified features of *Bombina variegata* species at the Russian Field's *Bombina bombina* populations, placed at large distance from the yellow-bellied toad area or hybridization zone. Also, there were studied 6 populations from more localities placed in the Ier Field identifying at all studied populations the existence of a reduce percent (generally under 10 %) of characters which express just like at *Bombina variegata* (COVACIU-MARCOV et al., 2002). In opposition whit our results, some studied *Bombina bombina* populations from the Ier Valley, although detain features of the *Bombina variegata* species, these are given by the preponderance of other characters than from this study. For instance, the 4 and 5 characters in the Cermei's population is very closed to *Bombina bombina*, at the Ier Valley's populations the *Bombina variegata* characters are responsible in the *Bombina bombina* populations. Meanwhile in this study the *Bombina variegata* features are thanks to the characters 6 and 10, at the Ier Valley's population the characters 6 and 10 exclusively present *Bombina bombina* features.

In the Cermei's population the amount of the *Bombina variegata* characters at the *Bombina bombina* population is 12,27% a higher value that in the case of Ier Valley study, where this value don't overtake, except one population where the value is 10 % (Chesereu 10,21 %) (COVACIU-MARCOV et al., 2002) . It can be noticed the affiliation of the individuals to *Bombina bombina*, without hybrid individuals or similar to *Bombina variegata*. A single individual present characters expressed in about similar amounts.

The 4 habitat's characters from Mădrigeşti, as well as the height of the localization are favorable to the yellow-bellied toad, even though these populations of *Bombina variegata* we have identified some characters expressed just like at *Bombina bombina*. All the studied *Bombina variegata* populations presents in a percentage characters of *Bombina bombina* species. The amount of expression of the features at the *Bombina variegata* populations presents similar values, the difference between the amount of the characters of the 4 populations being reduced (2 %). The fire-bellied toad characters detain the highest values, over 20 % for each population.

The brook population presents the highest amount of the *Bombina variegata* characters, witch can be explained by the fact that the brooks are typical habitats for this species. In some populations concerning the hybridization region between the two species and their preference of habitats it can be said that the most pure *Bombina variegata* populations are present in brooks and temporary pools, no matter the height they are situated, with the condition that this has to overtake 150 m. (COVACIU-MARCOV, 2003). The pool with newts present the next highest amount value of *Bombina variegata* characters, thanks to the favorable conditions, this one is the biggest pool, a permanent one, with a rich vegetation. In the pool from the field and the one under the forest are helpful some *Bombina bombina* characters, that's why the amount of this specie's characters increases with 1-2 %.

In the speciality literature are described *Bombina variegata* populations which presents *Bombina bombina* specie's characters. Thus, even though some of the studied populations are placed far away from the hybridization zone placement, nevertheless the amount of the other specie's characters is unexpectedly high, for example the population from Sisteu, placed at over 600m height, in the high zone of the Ses Mountain. At this population, which it should have been pure *Bombina variegata*, the amount of *Bombina bombina* characters expressed are important. This population is placed out and at distance from the cross-breeding zone. (COVACIU-MARCOV, 2003).

For the 4 population from Mădrigeşti and as well as for the studied population from the Beius Depression (COVACIU-MARCOV, 2003) with little differences the same characters are responsible for the *Bombina bombina's* characters in the *Bombina variegata* populations. In can be ascertained the fact that the high amount of the fire-bellied toad characters, this one being more important than the amount of the *Bombina variegata's* characters expressed in Cermei's *Bombina bombina* population. At the Cermei's population the *Bombina variegata's* characters reach just 12,27%. In the study concerning some populations from the Beius Depression, it has been proved the high amount of the fire-bellied toad characters in the *Bombina variegata* populations, this one being more important than the amount of the *Bombina variegata's* characters expressed at the Ier Field's *Bombina bombina* populations (COVACIU-MARCOV, 2003). The studied zones are placed far away from the cross-breeding areas between the two species, thus hybridization can't be a cause for the appearance of one specie's characters in a other specie's populations. The only possible explanation for this could be the presence in their genom of the two species of some allele-genes, of *b* and *v* type, responsible of the codification for both express manners of each characters (STUGREN, 1980), depending on the existent conditions. *Bombina bombina* and *Bombina variegate* divert from the classical concept about species, defined on reproductive isolation (MAYR, 1942, BĂNĂRESCU & BOŞCAIU, 1973). The two species, genetic very closely, (SZYMURA & FARANA, 1978), differentiated in Pliocen (SZYMURA, 1993), moleculars datas place the divergent moment at about 3-5 million years (SZYMURA et. al., 1985, SZYMURA, 1993).

It's hard to explain why it wasn't touched the reproductive isolation, counting the long period of time. An explication could be given by the hypothesis of successive contact of the two species across the Cuaternar period. It is possible that the 2 species have hybrided in the past too (MAXON & SZYMURA, 1979), being able to contact in the warmer interglacials and further to lose contact again. Thus it is explained the appearance of a specie's characters in the

other specie's populations the successive contacts allowing recurrent give and take of genes. In the North- West part of Romania don't exist pure populations, inclusively at those placed at heights and typical habitats appearing characters from the other species (COVACIU-MARCOV, 2003).

In the Mădrigeşti's *Bombina variegata* populations the characters from the other species of *Bombina bombina* appears in a higher amount than in the reverse situation in the case of Cermei's *Bombina bombina* population.

Thus, it is anomaly the fact that a species presents more characters from the other species one, in comparation with the reverse situation. The explanation of this anomaly can't be satisfactory given than the past pf the two species in the actual intergalcial, mostly by the postglacial migration ways of them, and the eventual contact between them. The last glacial periods strongly affected the Europe fauna, the number of the hybridization zones known as far on the continent, being a consequence of these (HEWITT, 1996, 2000). During the last ice age *Bombina variegata* used as a shelter the west zones of Balkanic Peninsula, and *Bombina bombina* had the shelter in the inferior course of the Danubian river (SZYMURA, 1993). Probably the 2 species had a different evolution during the last ice age fact that explains the differences between their characters amounts (COVACIU-MARCOV, 2003).

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