RESEARCH UPON SOME *BOMBINA VARIEGATA* POPULATIONS (AMPHIBIA) FROM JIU GORGE NATIONAL PARK, ROMANIA

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Abstract. All of the *Bombina variegata* populations studied from the Jiu Gorge National Park also presented characters of the sister species, *Bombina bombina*. The weight of the features of the sister species is higher at the southern limit of the park, in the areas with lower altitudes, that are found at the contact with the flat relief sectors from the alluvial plain of the Jiu River. The weight of the *B. variegata* characters increases with the altitude. Moreover, the weight of the features is also influenced by the habitat morphology. Thus, the weight of the *B. variegata* characters is always higher in the smaller-sized habitats. Considering all of the analysed populations, the weight of the *B. variegata* features is lower than in the case of the populations from The Iron Gates.

Keywords: *Bombina variegata*, different habitats, characters.

Rezumat. Cercetări asupra unor populaţii de *Bombina variegata* (Amphibia) din Parcul Naţional Defileul Jiului, România. Toate populaţiile de *Bombina variegata* studiate in Parcul Naţional Defileul Jiului au prezentat caractere ale speciei *Bombina bombina*. Ponderea caracterelor speciei congenere este este mai mare la limita sudică a parcului, în zone cu altitudini joase, aflate in contact cu sectoarele cu relief plat din lunca râului Jiu. Ponderea caracterelor de *B. variegata* creşte odată cu altitudinea. În plus, pondrea caracterelor este influenţată de morfologia habitatului. Astfel, pondrea caracterelor de *B. variegata* este intotdeauna mai mare în habitatele de dimensiuni reduse. Luând în considerare toate populaţiile analizate, pondrea caracterelor de *B. variegata* este mai scazută decât în cazul populaţiilor de la Porţile de Fier.

Cuvinte cheie: *Bombina variegata*, habitate diferite, caractere.

INTRODUCTION

In Romania, *Bombina variegata* is an almost threatened species, being firstly affected by the deterioration of the habitats which is a result of the clearings (IFTIME, 2005). In this respect, the protected natural areas have a special importance in the conservation of this species, and of other herpetofauna species as well. The Jiu Gorge National Park (JGNP) is a protected area, which preserves many natural surfaces where numerous *B. variegata* populations are present (COVACIU-MARCOV et al., 2009a). *B. variegata* also holds a distinct theoretical importance. Therefore, both in Europe and Romania, a congenera species is spread alongside of *B. variegata*, respectively *B. bombina*. The two species have vicariant areas, *B. bombina* occupying the plains, while *B. variegata* the higher areas (FUHN, 1960). *B. bombina* and *B. variegata* are not reproductively isolated, hybridizing in the contact sectors of their areas (SZYMURA, 1993). The hybridization area is situated at the limit between the plains and hills, being a very long one (SZYMURA, 1993). The hybridization area also comprises Romania's territory, hybrid populations being recently signalled in different areas from the country (GHIRA et al., 2003; COVACIU-MARCOV et al., 2003a, 2004, 2005, 2006, 2009b; SAS et al., 2005, FERENŢI et al., 2008). However, outside of the hybridization areas and at a distance from them there are present populations of a species that also display characters of the congenera, although it is missing from the region (STUGREN, 1980; COVACIU-MARCOV et al., 2002, 2003b, 2007; KOVACS & COVACIU-MARCOV, 2009). Such situations have been recently recorded at some *B. variegata* populations from western Romania. However, these studies lack from JGNP and research upon the two species of the *Bombina* genus have not been realised in Gorj County. Thus, the present study presents preliminary data regarding some *B. variegata* populations from JGNP in order to establish the presence of certain *B. bombina* features.

MATERIALS AND METHODS

The research was performed in May 2008. We analysed five populations, investigating 189 toads. We captured the amphibians either directly by hand or with the help of a net in the case of the larger habitats. After we had finished the study, the frogs were released in their habitats. The analysed region is represented by the Jiu Gorge National Park.

The studied populations occupied different habitats, situated at different altitudes.

The first habitat lies near the tunnel from Bumbeşti. It is represented by a canal of approximately 50 m long, situated alongside the rail road, starting from the tunnel. The canal has concrete margins, having a width of approximately 50 cm and a depth of 30 cm. It is abundant in aquatic vegetation, silt being present at the level of the substratum.

The second habitat is situated in Meri Railway station, 6 km upstream the first. It is represented by a system of puddles which occupy a part of the 4th and 5th lines, which are presently unused, as well as a ditch that separates line 5 from the surrounding terrains. The ditch is represented by two bogging areas that have a surface of several m². The habitat is a permanent one that has its own spring. Generally, the water depth is of 30 cm, but in the canal it surpasses 70 cm. At the level of the lines, the aquatic vegetation is missing. The substratum is covered by a thin layer of silt. The toads were captured from the flooded lines, where they were hiding under the crossbars or under the rocks.

The third habitat is found near Meri quarry, being represented by a system of puddles situated inside the forest, on an abandoned road. The water depth is of maximum 20 cm, while the maximum diameter is of 1 m², and some of the puddles have their own springs.

The fourth habitat is also represented by a system of temporary puddles, situated on a forest road. In comparison to the previous one, the road has some traffic, the puddles being situated in a glade, lacking their own springs. The water depth is of maximum 30 cm, their form being elongated due to the passing of the wheels of the vehicles, the turbidity being high.

The fifth habitat is situated at just 30 m from the previous one. It is however different, being a quasi-permanent puddle of approximately 15 m², situated in a low depression. The habitat is called "the newt pond" because it represents the most important reproducing habitat for the newts from the entire JGNP (COVACIU-MARCOV et al., 2009a). During spring, the water depth reaches 1 m, but once with the approaching of the warm season it strongly decreases.

The method we used for establishing the affiliation of the studied populations was represented by the analysis of the main morphologic and chromatic characteristics of the two species, which are grouped in two grids. The used characteristics are the most important diagnose - characteristic of the two species and several authors used them (STUGREN, 1980; GHIRA & MARA, 2000; GHIRA et al., 2003; GOLLMANN et al., 1993; SZYMURA & BARTON, 1991). We analysed 20 features, using two grids, each grouping 10 characteristics. The first grid analysed the morphology, the dimension and the ratios of light ventral spots, coloured in red at *B. bombina* and in yellow at *B. variegata*. The degree of confluence or separation of different ventral spots is scored for 10 chromatic groups (Table 1). If the light spots are separated among them by black pigment the character belongs to *B. bombina*. When the spots are united among them and the light pigment on the body appears uniformly, the character belongs to *B. variegata*. The second grid analyses 10 features as well and was used by STUGREN (1980) and modified by GHIRA & MARA (2000) (Table 1).

Both of the grids use a binary system (0. 1). For both grids each feature receives a mark: 1 if it is expressed like *B. variegata* and 0 if it is expressed like *B. bombina*. Summing the marks obtained for each characteristic, a certain individual can receive on each grid a score ranging from 0 to 10; the score equal to 0 means a pure *B. bombina*, the score equal to 10 means a pure *B. variegata*. After assessing the score for each individual, we calculated the average score of all individuals of each population, for each grid and then the average of the two grids. This method allows the transfer of the features into percentages and their statistical interpretation. The final mark indicates the amount of *B. variegata* features.

Table 1. The two grids of differentiation of the European species of *Bombina*.
Tabel 1. Grilele de diferențiere ale caracterelor celor două specii europene de *Bombina*.

	Character	***Bombina bombina***	***Bombina variegata***
	Grid 1 (the characteristic of the ventral pattern)		
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
	Grid 2 (after Stugren, Ghira & Mara, modified)		
1	Colour of light ventral spots	Red, orange, yellowish	Yellow
2	Colour of the top of fingers	Black	Yellow
3	Dorsal colour	Black	Pale grey
4	Relation between tarsal and plantar light spots	Separated	United
5	Ventral colour	Orange spots on black background	Black spots on yellow background
6	Relation between the head length and width	Length > width	Length < width
7	The drawing of the lateral and ventral parts	White spots around the verrucae	Without white spots around the verrucae
8	Aspect of the dorsal black tubercles	Regulated	Scattered or absent
9	The aspect of the dorsal verrucae	Lens-shaped, squatted	Sharp, rough
10	Ratio of tibia-tarsian joints when the legs segments are parallel	Not touching	Touching

RESULTS AND DISCUSSIONS

None of the five *B variegata* populations from JGNP present exclusive characters from this species. The weight of the features of the sister species, *B. bombina*, was different between the populations, varying between 66.7% in the case of the population from Bumbeşti tunnel and 78.27% at the population from the road-puddles from Comandă (Table 2). However, the weight of the characters proves that all five populations belong to *B. variegata*, covering most

of its features. These results confirm the previous data according to which the only species from the Bombina genus that is present in JGNP is *B. variegata* (COVACIU-MARCOV et al., 2009a). Meanwhile, the obtained data are in concordance with the altitude of the area and the aspect of the habitats.

Table 2. The affiliation of the studied populations.
Tabel 2. Afilierea populațiilor analizate.

Locality	Bumbeşti tunnel	Meri Railway station	Meri quarry	Comandă puddles from the road	Comandă newt puddle
No. of studied individuals	27	34	38	44	46
Altitude (m.)	315	363	346	816	817
Average grid 1	59.1	60	73.60	72.39	71.36
Average grid 2	74.3	79.70	81.70	84.16	82.04
Average of grids	66.7	69.85	77.65	78.27	76.7

Generally, there is an increase of the weight of the *B. variegata* characters together with the increase of the altitude at which the habitats of the respective populations are situated. Thus, the lowest weight of the *B. variegata* features was registered at the population from Bumbeşti tunnel, which is situated at 315 m altitude. Starting from this habitat, as the altitude increases, the weight of the *B. variegata* features also increases. The fact is expected, as the phenomenon has been registered in the case of other regions from the country, being in concordance with the requirements of the two species (COVACIU-MARCOV et al., 2004, 2005, 2006, 2007, 2009b).

The increase of the weight of the *B. variegata* features together with the increase of the altitude is not however homogenous and linear. Thus, the population from Meri quarry is situated at a slightly lower altitude than the one from Meri Railway station, and still the weight of the *B. variegata* characters is almost 9% higher (Table 2). Also, the two populations from Comandă are practically situated at the same altitude and at 30 m apart from one another, but in the puddles from the road the weight of the *B. variegata* characters is approximately 1.5% higher.

Situations in which the differences between the weights of the characters of two populations are not directly and exclusively influenced by altitude have been previously signalled (COVACIU-MARCOV et al., 2009b, c; KOVACS & COVACIU-MARCOV, 2009). In the lack of the altitudinal cause, the only explanation for the difference between the populations is offered by the habitat morphology. In the hybridization areas, the characteristics of the habitats greatly influence the aspect of the hybrid populations (MACCALLUM et al., 1998). Thus, the habitat from Meri quarry is a typical one for *B. variegata*, species attached to the small-sized aquatic habitats (COGĂLNICEANU et al., 2000), being represented by a series of temporary puddles situated in the forest. Unlike this habitat, the one from the railway station is larger, has a muddy substratum and is not situated in a forested area. Regarding the habitat from the railway station, the *B. bombina* phenotypes are favoured, which present characters such as the darker back colour. This allows the toads to camouflage themselves in the dark mud. The presence of both types of allele for all of the features at the two species was indicated in the past (STUGREN, 1980), these being differently expressed, depending on the situation. The explanation for the presence of both types of allele is most likely offered by the successive contact theory and implicitly by gene exchange at the level of the different interglacial periods (MAXON & SZYMURA, 1979). Moreover, at Meri Railway station the level of anthropogenic interference is very high; both in the canal and in the bogging area from its level, there are present the poultry of the locals (DOBRE et al., 2007). Their presence can also explain the position of the toads in the areas between the lines where they find shelter and birds cannot have access to.

The habitats from Comandă are framed in the same direction, where the somehow higher weight of *B. variegata* characters is registered in the temporary puddles. Probably, there is an active selection of the adequate habitat. Only 30 m separate the two ponds from Comandă, while *B. variegata* can move around over 1 km (HARTEL, 2008). Regarding the hybridization areas, the differences between the populations can also be caused by the migration of some individuals in the habitats that suit their ecological requirements (VINES et al., 2003).

The somehow high weight of the *B. bombina* features, at certain populations, can be very surprising at a first glance due to the relatively high altitude at which they are found. Therefore, the purest population of *B. variegata* that we studied, situated at 816 m altitude presents more *B. bombina* characters than a population situated at just 140 m altitude, on the Vodița Valley, Mehedinți County (COVACIU-MARCOV et al., 2007). The comparison is also available between the other populations from JGNP and other populations situated around The Iron Gates. The fact is unusual, as normally with the increase of the altitude there is an increase of the *B. variegata* features, which is very obvious at JGNP. Thus, although the populations from The Iron Gates are found at a lower altitude than the ones from JGNP, they do not have to be interpreted as a series found on a simple ascending altitudinal gradient. Even if approximately 100 km separate the populations from the two areas and they are found on an ascending altitudinal line, they actually represent two distinct units, with a different history. Therefore, the populations from The Iron Gates are situated in extremely typical habitats for this species, despite the low altitude. In addition, populations of the sister species have not been identified in the surrounding areas; *B. variegata* descends here at the lowest altitude in the country (COVACIU-MARCOV et al., 2009d). Meanwhile, the refuge of the *B. variegata* group which colonised most of the Romanian Carpathians was found in The Iron Gates region (HOFMAN et al., 2007). The populations from the territories of the former refuges are

purer than the ones situated on the colonization routes, fact which is also available for the sister species, *B. bombina* (GROZA et al., 2007).

Unlike The Iron Gates region, colonizing populations are probably present on the Jiu Gorge, arriving in the region together with the warming of the climate. Meanwhile, it is also possible that these populations came in contact during their migration with *B. bombina*, and thus took features from it even from then. Moreover, even some habitats from JGNP are less characteristic to the species. The fact that *B. bombina* probably highly ascended upstream alongside the Jiu River must not be excluded, fact indicated by the identification of the individual from Bumbeşti tunnel (COVACIU-MARCOV et al., 2009a). The presence, at least in the close past, of some *B. bombina* populations in the nearby areas of the *B. variegata* populations from the southern part of JGNP can be the cause of the relatively high weight of the characters of this species at the *B. variegata* populations from the southern part of JGNP.

All of the studied individuals from all of the populations presented most of the characters from *B. variegata*, with the exception of the population from the first tunnel from Bumbeşti. Regarding this population, an individual had just 22.5% *B. variegata* characters. Thus, even if on a whole this population also belongs to *B. variegata* species, at least the respective individual presented most of its features from the other species. In the case of this population, after the first grid, there were still 3 individuals that presented most of the characters from *B. bombina*, but on a whole this fact was annulled by the higher weight of the *B. variegata* features presented by the second grid or by the other individuals. This situation must also be considered as an indicator of the presence, at least in the close past, of some *B. bombina* populations near the southern margin of JGNP (COVACIU-MARCOV et al., 2009a).

In the case of the other four populations, all of the individuals presented most of their characters as *B. variegata* ones, regarding both grids. We identified two samples that recorded 95% *B. variegata* features at the population from Meri quarry. After the first grid, the respective individuals presented all the characters as *B. variegata* ones. However, there was no individual from any population that presented after both grids all of the features like *B. variegata* ones. Therefore, we did not register even one pure *B. variegata* individual.

Despite the fact that all of the studied populations from JGNP come close, on a whole, to *B. variegata*, there are some characters that are expressed in the case of all of the individuals as *B. bombina* (Table 3). The features are the same that are expressed in the same manner at other *B. variegata* populations from western Romania (COVACIU-MARCOV et al., 2002, 2003b, 2007, 2009b, c). However, there are certain differences towards the populations from the north-western part of the country. Thus, generally the 7th character from the second grid is greatly expressed as *B. bombina* in the case of the populations from JGNP. In the north-western part of the country, the feature is highly expressed as *B. variegata*, especially at the populations from the Oaş Mountains (COVACIU-MARCOV et al., 2009c). These data prove the existence of some regional differences between the populations from different areas of Romania, certain characters having different values in different regions.

Table 3. The features' ratio in the two grids of the studied populations.
Tabel 3. Ponderea caracterelor în cele două grile a populațiilor analizate.

Locality	Bumbeşti tunnel	Meri Railway station	Meri quarry	Comandă puddles from the road	Comandă newt puddle
Feature	GRID 1				
1	88.89	88.23	94.73	100	100
2	0	0	25	0	2.27
3	0	0	28.94	17.39	9.09
4	53.7	70.58	71.05	82.60	90.9
5	98.15	94.11	97.36	100	100
6	7.41	5.88	50	36.95	20.45
7	88.89	88.23	93.42	100	100
8	77.78	82.35	77.63	86.95	90.9
9	81.48	70.58	100	100	100
10	94.44	100	97.36	100	100
Feature	GRID 2				
1	96.3	100	100	100	100
2	100	100	100	100	100
3	100	100	100	100	100
4	46.3	44.11	56.57	63.04	61.36
5	92.59	88.23	86.84	100	100
6	100	100	97.36	36.95	68.18
7	3.7	0	0	0	4.54
8	51.85	70.58	76.31	100	95.45
9	96.3	100	100	100	100
10	55.56	100	100	95.65	90.9

CONCLUSIONS

The *B. variegata* populations from the Jiu Gorge National Park also register, in a relatively high amount, characters of the sister species, *B. bombina*. The amount of the *B. variegata* features differs between the studied

populations, varying from 66.7% to 78.27%. Generally, the amount of the *B. variegata* characters increases with the altitude. This situation is greatly highlighted by the morphology of the habitat, in the temporary, small-sized habitats being expressed more *B. variegata* features. The two factors that determine the increase of the amount of the *B. variegata* features are the general ones, the populations from the studied region being similar, from this point of view, with other populations from north-western Romania. However, the amount of *B. bombina* characters at these populations from JGNP is higher than the one registered at the populations from The Iron Gates region, which are situated at lower altitudes. The differences are probably determined by the different history of the two species from the two areas, as by the actual areas, the contact method between them and the habitat morphology.

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