

Data on the Populations of *Bombina variegata* (Amphibia: Anura: Bombinatoridae) from Cozia National Park and Its Surrounding Areas (Vâlcea County, Romania)

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Received: November 11, 2016; Accepted: May 17, 2017; Available online: June 29, 2017; Printed: June 30, 2017

Abstract. The results of surveys upon the *Bombina variegata* populations in the Cozia National Park are presented. 52 location points were found. The species is widespread in the investigated area, is resilient to some extent to the current level of natural and human disturbance, is moderately anthropophilic and shows evidence of previous introgressive hybridization with *B. bombina*. The habitats of the various populations in the area show different quality, connectivity and dynamics. These data should be useful for any management strategy for *Bombina variegata* in the National Park and its surrounding areas.

Key words. Cozia National Park, *Bombina variegata*, populations, distribution, habitat, dynamics, introgressive hybridization.

INTRODUCTION

The Cozia National Park is a part of both the National Park network and the Natura 2000 network of Romania. As a Natura 2000 Site of Community Importance, the Cozia National Park is dedicated to the protection via conservation-oriented management of a number of Natura 2000 species (species on the Annex II of the 92/43/EEC Directive), among which the Yellow-Bellied Toad, *Bombina variegata*. This species is widely distributed in Europe (Speybroeck et al., 2016) and Romania, still locally common in many montane and submontane areas of Romania (Cogălniceanu et al., 2000; Cogălniceanu et al., 2013), but also declining in other areas (Cogălniceanu et al., 2000; Iftime, 2005). Good knowledge of the population particulars for a species within a protected area is necessary for its management, including distribution, habitat requirements and characteristics, metapopulational dynamics, population connectivity, hybridization and the reaction to human and natural changes in its habitat. We try to improve upon the previous knowledge of *Bombina variegata* in the Cozia National Park (researched herpetologically by us in the recent past – Iftime & Iftime, 2006, 2007) by highlighting these aspects.

MATERIAL AND METHODS

This paper is based upon field work performed in September 2014, May and July 2015 and May 2016, mostly within the grant PN-II-PT-PCCA-2013-4-1489, but also making use of independently obtained data. Qualitative observations were undertaken using the active transects method (after McDiarmid, 1992, and Heyer et al., 1994, in Cogălniceanu, 1997), the transect being 4 m wide. The geographical

coordinates for all locations were acquired by GPS. On some transects (Valea lui Stan, Spinu, Lotrișor-Narătu, Păușa, Căciulata, Olt valley, Lotrișor-Căpățâni) photographs of the ventral pattern were taken for a number of specimens, ensuring recognition for sampling studies within the same project. The morphological *Bombina variegata*-like vs. *Bombina bombina*-like character states were analyzed in order to evidence possible past introgressive hybridization using character tables (Tabs 1, 2), each containing a set of 10 characters – after Gollmann, 1984; Szymura & Barton, 1991; Stugren, 1980; Ghira & Mara, 2000; Covaciu-Marcov et al., 2003; Sas et al., 2005, modified by us (Iftime & Iftime, 2007 – Tabs 1, 2). Each *Bombina bombina*-like character state is counted as 0 and each *B. variegata*-like as 1; thus for each set of characters a score is obtained for each specimen. The average of these two scores is obtained for each specimen, ranging between 0 (“pure” *B. bombina*) and 10 (“pure” *B. variegata*). The median average, maximum and minimum values and quartiles are calculated for each population from the scores of pertaining specimens.

RESULTS

We found *Bombina variegata* in 52 points in and around the Cozia National Park. The distribution points, with their localities (grouped by major topographic feature: i.e. valley or another area of contiguity, that empirically separate local [sub] populations), visit year(s), GPS coordinates, and data pertaining to reproduction (whether it was observed at a given point), altitude and ecological characteristics (altitude, habitat type, persistence of habitat) are given in table 3; a map showing the points is given in fig. 1.

The average character score for each locality/population investigated in this respect is given in table 4.

DISCUSSION

Our results show that *Bombina variegata* has a wide distribution in and around Cozia National Park (Fig. 1). However, as can be easily observed (Figs 1, 2), the populations of this species are mostly found at relatively low altitudes (350–650

Table 1

First set of characters and states used to determine a score reflecting introgressive hybridization in *Bombina* species.

Character	<i>Bombina bombina</i>	<i>Bombina variegata</i>
Light ventral coloration	Red, orange, deep yellow	Yellow, light yellow
Colour of the upper part of the first finger and of the finger tips	Black	Yellow
Dorsal coloration	Greenish-grey	Brownish-grey
Tarsal and palmar light spots, to each other	Separated	United
Ventral coloration	Light spots on a dark ground	Dark spots on a light ground
Relation of head length and width	Length > width	Length < width
Ventral and lateral warts	White dots around warts	No white dots around warts
Dorsal dark spots	Symmetrical	Irregular
Dorsal warts	Blunt	Sharp, giving rugous aspect
Report of tibio-tarsal articulations when stylopode and zeugopode are parallel	Not touching	Touching

Table 2

Second set of characters and states used to determine a score reflecting introgressive hybridization in *Bombina* species.

Character (Light spots on the...)	<i>Bombina bombina</i>	<i>Bombina variegata</i>
Throat-throat	Separated	United
Throat-chest	Separated	United
Chest-chest	Separated	United
Chest-shoulder	Separated	United
Shoulder-arm	Separated	United
Chest-abdomen	Separated	United
Abdomen-abdomen	Separated	United
Abdomen-basin	Separated	United
Basin-basin	Separated	United
Basin-thigh	Separated	United

m a.s.l.), a rare occurrence at 1115 m a.s.l. breaking this pattern – while in many other Romanian montane areas *Bombina variegata* is found commonly at much higher altitudes (Iftime & Iftime, 2014). This can be explained by the mostly inhospitable nature of the upper reaches of the Cozia and surrounding mountains: very steep, rocky areas that are either quite dry and exposed or washed by very quick-flowing streams that do not afford suitable habitat for *Bombina variegata*. This situation has the double effect of confining *Bombina variegata* to the lower areas and creating discrete valley populations, more or less effectively separated by intervening ridges (some marginal populations, such as those from Spinu and the Pripoare-Dângești area, do not fit into this pattern and are definitely not beyond linking to each other, as the terrain there is a series of rolling foothills with slow-flowing rivulets). However, where conditions are proper *Bombina variegata* can and does occur higher up and even breeds there (e.g. at Stânișoara Monastery, 736 m a.s.l.).

The habitat types are in relation to the above-mentioned aspect. Most of them are road-side ditches, stream-side ponds being also quite numerous (Fig. 3); obviously small stagnant waterbodies are the most favourable habitats. Streams and small rivers are also good enough, provided their flow is not much too strong. Very steep angles of slopes are unfavourable for either of the above. We were, however, unable to observe any *Bombina* in the large, slow-flowing, dammed Olt River; it seems to be avoided, for toads living in its proximity, in small-river habitats contiguous to the Olt dam lakes, never appeared to venture in these last.

The metapopulational dynamic is also evident. Of the habitats supporting *Bombina variegata* that were investigated over more than one year, less than a quarter (Fig. 4) were not persistent over the whole investigation period. The causes are both natural and artificial (Tab. 3). Some water points dry up despite not especially dry years; others form anew, and others are colonized anew by *Bombina variegata*. Also, major floods (such as occurred in 2014) seem to have a tremendous impact: after the torrential flooding, in September 2014, the Băiașu valley was apparently devoid of *Bombina variegata* and the habitats were washed downstream. In 2015 a single element of the presence of *Bombina variegata* was found – a dead specimen – and in 2016 a breeding population was formed in a place where in 2014 and 2015 there was

Table 3

Locations investigated with their geographical coordinates and characteristics. Data on species' reproduction.

Locality/population, visit year(s)	GPS Coordinates	Reproduction	Altitude and ecological characteristics	
Valea lui Stan 2014-16	N45 20.100 E24 11.836	Yes (eggs, larvae and/or metamorphs present)	354 m; ponds on stream side; mixed deciduous forest and grassy clearing	
	N45 20.227 E24 11.796	Inaparent (adults and/or subadults only)	431 m; ponds on stream side; mixed deciduous forest and grassy clearing	
Spinu 2014-16	N45 22.682 E24 24.061	Yes (eggs, larvae and/or metamorphs present)	527 m; slow-flowing stream; rural mosaic	
	N45 18.227 E24 16.970	Yes (eggs, larvae and/or metamorphs present)	271 m; ponds on river side; mixed deciduous forest	
Lotrișor-Narătu 2014-16	N45 17.277 E24 14.140	Yes (eggs, larvae and/or metamorphs present)	661 m; road-side ditch; beech and mixed forest; dried 2016	
	N45 18.188 E24 16.963	Inaparent (adults and/or subadults only)	309 m; ponds on river side; mixed deciduous forest	
	N45 17.566 E24 14.625	Yes (eggs, larvae and/or metamorphs present)	619 m; ponds on river side; mixed deciduous forest	
	N45 17.190 E24 14.102	Inaparent (adults and/or subadults only)	689 m; road-side ditch; deciduous forest, mainly beech	
	N45 17.483 E24 14.968	Inaparent (adults and/or subadults only)	757 m; road-side ditch; deciduous forest, mainly beech	
	Păușa valley 2014-16	N45 16.127 E24 19.894	Inaparent (adults and/or subadults only)	335 m; stream in mixed deciduous forest
		N45 16.551 E24 20.123	Inaparent (adults and/or subadults only)	378 m; stream in mixed deciduous forest
		N45 16.671 E24 20.145	Inaparent (adults and/or subadults only)	392 m; stream in mixed deciduous forest
N45 16.841 E24 20.100		Yes (eggs, larvae and/or metamorphs present)	409 m; ponds on stream side, tail of dam lake in mixed deciduous forest	
N45 17.172 E24 19.903		Inaparent (adults and/or subadults only)	447 m; road-side ditch; mixed deciduous forest	
Stânișoara monastery 2016		N45 18.098 E24 20.371	Yes (eggs, larvae and/or metamorphs present)	736 m; spring ponds; grassy clearing
Căciulata valley 2014-16	N45 15.478 E24 18.689	Yes (eggs, larvae and/or metamorphs present)	377 m; puddles in road and stream in mixed deciduous forest	
Olt valley 2014-16	N45 20.341 E24 16.764	Inaparent (adults and/or subadults only)	331 m; road-side ditch; mixed deciduous forest; dried 2015	
	N45 23.036 E24 18.022	Inaparent (adults and/or subadults only)	358 m; road-side spring pond; mixed deciduous forest; dried 2015	
	N45 20.171 E24 16.801	Inaparent (adults and/or subadults only)	280 m; stream in rural mosaic	
	N45 22.424 E24 18.042	Inaparent (adults and/or subadults only)	314 m; ponds on river side; mixed deciduous forest and grassy clearing	
	N45 20.516 E24 16.834	Inaparent (adults and/or subadults only)	307 m; large roadside pond; mixed deciduous forest	
	Lotrișor (Căpățâanii) 2014-16	N45 23.597 E24 16.376	Inaparent (adults and/or subadults only)	440 m; ponds on stream side; mixed deciduous forest and grassy clearing
N45 24.243 E24 14.793		Inaparent (adults and/or subadults only)	526 m; road-side ditch; mixed deciduous forest; dried 2015	
N45 24.069 E24 14.940		Yes (eggs, larvae and/or metamorphs present)	507 m; ponds on stream side; disturbed gravel bed	
N45 23.839 E24 15.548		Yes (eggs, larvae and/or metamorphs present)	424 m; ponds on stream side; mixed deciduous forest and grassy clearing	
Băiașu valley 2014-16	N45 22.164 E24 20.841	Inaparent (adults and/or subadults only)	357 m; large backwater stream-side pond, mixed deciduous forest and grassy clearing; formed 2015	

Table 3 (continued)

Locality/population, visit year(s)	GPS Coordinates	Reproduction	Altitude and ecological characteristics
	N45 22.200 E24 21.575	Yes (eggs, larvae and/or metamorphs present)	417 m; road-side ditch, mixed deciduous forest and grassy clearing; formed 2016
Pripoare-Dăngești 2015-16	N45 19.577 E24 25.394	Inaparent (adults and/or subadults only)	627 m; road-side ditch, mixed beech and coniferous forest; drained 2016
	N45 18.365 E24 25.106	Yes (eggs, larvae and/or metamorphs present)	675 m; spring ponds, pasture
	N45 18.604 E24 25.400	Yes (eggs, larvae and/or metamorphs present)	702 m; trough for watering cattle, mixed beech and coniferous forest
	N45 19.212 E24 25.489	Inaparent (adults and/or subadults only)	719 m; ponds on stream side, mixed beech and coniferous forest
	N45 19.484 E24 25.389	Yes (eggs, larvae and/or metamorphs present)	694 m; ponds on stream side, mixed beech and coniferous forest
	N45 20.269 E24 25.128	Inaparent (adults and/or subadults only)	611 m; road-side ditch, mixed beech and coniferous forest; drained 2016
	N45 20.442 E24 24.996	Yes (eggs, larvae and/or metamorphs present)	597 m; road-side ditch, mixed beech and coniferous forest, grassy clearing; drained 2016
	N45 20.605 E24 25.104	Yes (eggs, larvae and/or metamorphs present)	591 m; ponds on stream side, mixed beech and coniferous forest; drained 2016
	N45 18.956 E24 25.398	Yes (eggs, larvae and/or metamorphs present)	727 m; road-side ditch, mixed beech and coniferous forest, grassy clearing
	N45 18.495 E24 25.370	Yes (eggs, larvae and/or metamorphs present)	664 m; ponds in pasture, at edge of mixed forest
	N45 19.892 E24 25.377	Yes (eggs, larvae and/or metamorphs present)	660 m; ponds on stream side, mixed beech and coniferous forest
Pătești (Sălătruc) valley 2016	N45 16.936 E24 23.039	Yes (eggs, larvae and/or metamorphs present)	553 m; spring pond in pasture
	N45 17.005 E24 22.922	Yes (eggs, larvae and/or metamorphs present)	553 m; pond in small landslide in clearing
	N45 17.555 E24 22.506	Yes (eggs, larvae and/or metamorphs present)	645 m; road-side ditch, mixed beech and coniferous forest, grassy clearing
	N45 17.860 E24 22.465	Inaparent (adults and/or subadults only)	687 m; road-side ditch, mixed beech and coniferous forest
	N45 18.129 E24 22.261	Inaparent (adults and/or subadults only)	723 m; road-side ditch, mixed beech and coniferous forest
Lotrișor (Cozia) 2014-16	N45 22.242 E24 18.527	Inaparent (adults and/or subadults only)	373 m; road-side ditch; mixed deciduous forest; formed 2015
	N45 22.143 E24 18.668	Inaparent (adults and/or subadults only)	383 m; road-side ditch; mixed deciduous forest
	N45 21.821 E24 19.079	Yes (eggs, larvae and/or metamorphs present)	418 m; road-side ditch; mixed deciduous forest
Near "La Leurde", Pleașa ridge 2015	N45 19.344 E24 22.287	Inaparent (adults and/or subadults only)	1115 m; road-side ditch, mixed beech and coniferous forest
Doabra valley 2016	N45 20.939 E24 14.638	Inaparent (adults and/or subadults only)	330 m; stream in rural mosaic
	N45 21.002 E24 14.617	Inaparent (adults and/or subadults only)	337 m; road-side ditch, mixed deciduous forest, grassy clearing
Vasilatu valley 2016	N45 21.096 E24 12.628	Inaparent (adults and/or subadults only)	358 m; road-side ditch, mixed deciduous forest, grassy clearing
	N45 22.067 E24 12.486	Yes (eggs, larvae and/or metamorphs present)	401 m; road-side ditch, mixed deciduous forest, grassy clearing
	N45 22.568 E24 12.570	Inaparent (adults and/or subadults only)	421 m; road-side ditch, mixed deciduous forest, grassy clearing

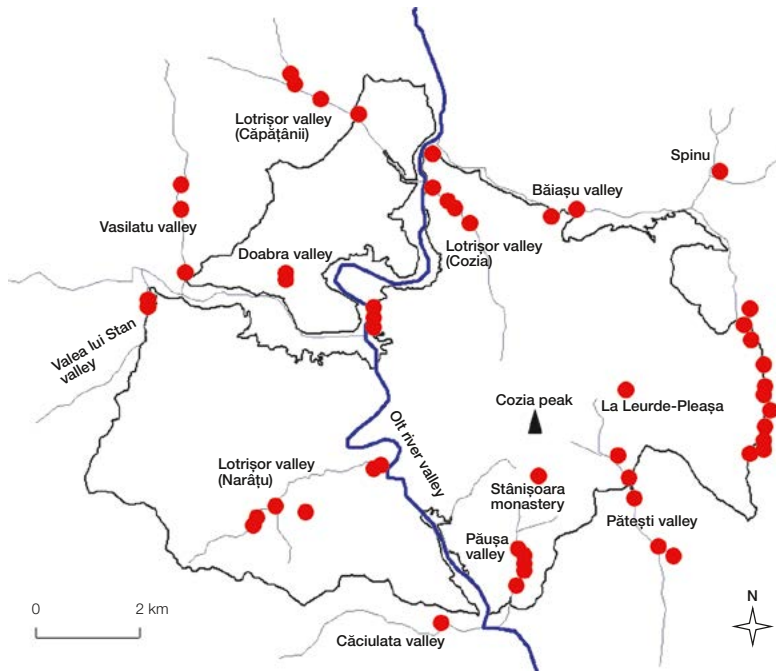


Fig. 1 – Map showing the distribution of *Bombina variegata* (in red) in and around Cozia National Park (Park limits after Ministry of Environment, Water and Forests data, downloaded from <http://www.mmediu.ro/articol/date-gis/434>).

nothing of the sort. Clearly major floods are a disturbing factor, but *Bombina variegata* populations are quickly back – either from locally surviving individuals (more likely, since they were breeding adults) or from dispersion/colonization.

Human impact is manifest in both the destruction and the creation of transient habitats. Along the road from Pripoare to Dăngești, a series of road-side ditches (obviously man-made, in relation to the road) were hosting a rich *Bombina variegata* population up to 2015; many were drained till the spring of 2016 by road improvement works, but new water pits were created in the same process. Also, in the same area, troughs for watering livestock provide a good habitat, stable on the long term, where *Bombina variegata* breeds successfully. On the whole, *Bombina variegata* can be

Table 4

Average character score for each locality/population.

Population	No. of specimens examined	Average morphological score - <i>B. variegata</i> vs. <i>B. bombina</i>
Valea lui Stan	53	8.75
Lotrișor (Narătu)	45	7.5
Spinu	63	9
Păușa	77	8
Căciulata	48	8.5
Lotrișor (Căpățanii)	44	8
Valea Oltului	10	8.4

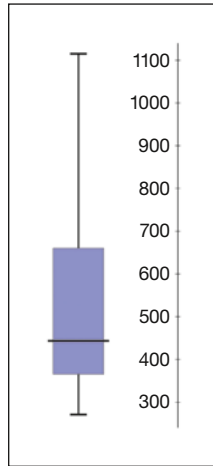


Fig. 2 – Boxplot (showing minimum and maximum, quartiles and median) of the altitudinal distribution of *Bombina variegata* finding points.

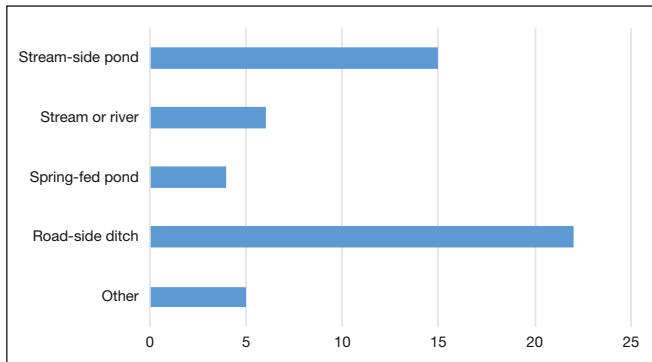


Fig. 3 – Bar diagram of the grouping of the *Bombina variegata* finding points by habitat type.

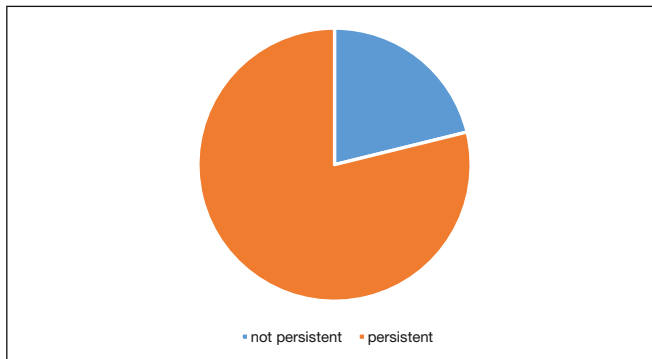


Fig. 4 – Pie diagram of habitat persistence among *Bombina variegata* habitats investigated in more than one year.

said to “hop” from habitat to habitat and from year to year, the metapopulation dynamic, evidenced by the turnover of microhabitats, being apparently quite intense; it readily occupies man-made habitats. However, in intensely human-altered habitats (e.g. on Păușa, Doabra and other valleys) *Bombina variegata* appears at or beyond the edge of human habitation along the water course; in the villages *per se* it was never found, and only twice in “rural mosaic” habitat (hayfields, orchards etc., with close proximity of houses) surrounding the water body (Tab. 3).

Interestingly, not all microhabitats where *Bombina variegata* was seen apparently harboured successful reproduction – on the whole more than half did not (Figs 5, 6). In those we could not see any eggs, tadpoles and metamorphs that would indicate reproduction in the place and not immigration from somewhere else. Of course, this does not mean that in those areas reproduction does not take place at all, but, however, it does indicate lower success and lower recruitment in these areas as compared to places where the successful reproduction is obvious from the large number of larvae and metamorphs. On the Olt valley all points where *Bombina variegata* was found (small ponds more or less distant from the Olt river itself) appeared devoid of successful reproductive activity (Tab. 3). On other valleys, reproductive activity appears to be concentrated in one or a few favourable spots. A good example of this is Păușa

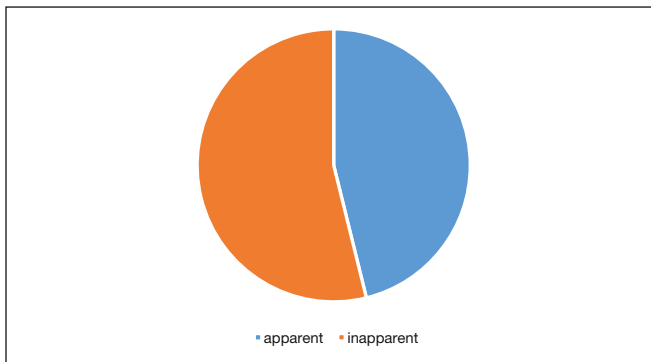


Fig. 5 – Pie diagram of apparent versus inapparent successful reproduction in investigated *Bombina variegata* habitats.

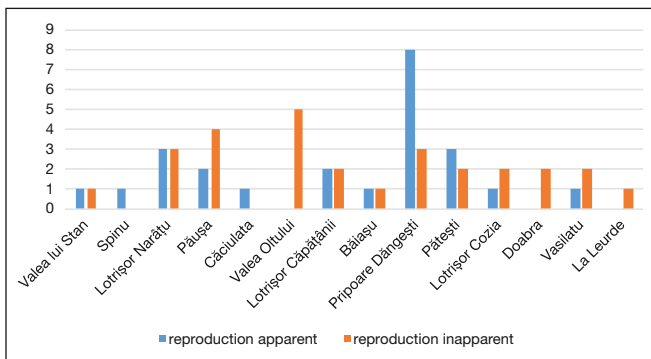


Fig. 6 – Bar diagram of apparent versus inapparent successful reproduction in investigated *Bombina variegata* habitats per population/area.

valley, where in only one place successful reproduction was observed; in all others adults and subadults were seen – which may come from the one reproduction point observed, or from others not detected by us (however, we consider the probability for this to be scarce); or, alternatively, in other years good conditions for breeding may appear in other spots and be exploited. We can, however, note that on the narrow, rocky, relatively steep valleys closer to the Olt valley (and to its affluent the Lotru) good breeding habitats are less common than in the rolling-hill habitats (e.g. Spinu-Pripoare-Dăngești) – fig. 6.

As previously noted (Iftime & Iftime, 2007), the *Bombina variegata* populations from Cozia show traces of past introgressive hybridization with *Bombina bombina*. The scores obtained (Tab. 4, Fig. 7) would place all populations in the “pure” *Bombina variegata* or *B. variegata*-like hybrid category according to the quoted methodology; however, as also noted by us previously (Iftime & Iftime, 2007) there are individuals with lower *B. variegata* score (and conversely higher *B. bombina* score), i.e. around 5, which fall around the typical value of first-generation hybrids (Fig. 7). These reinforce our hypothesis that there was an introgressive hybridization in the past (as it still happens in some foothills of the Southern Carpathians – Iftime & Iftime, 2011). The penetration channel for *B. bombina* and associated gene flow was, likely, along the

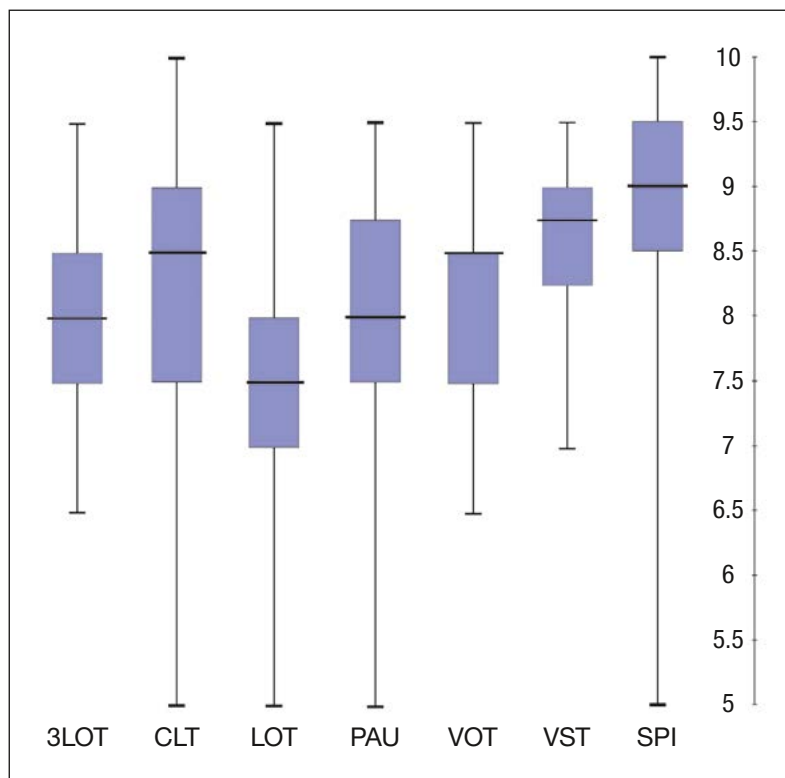


Fig. 7 – Boxplots (showing minimum and maximum, quartiles and median) of the *Bombina variegata* vs. *Bombina bombina* morphological score for populations investigated in this respect. Abbreviations: 3LOT = Lotrișor-Căpățâni; CLT = Căciulata; LOT = Lotrișor-Narătu; PAU = Păușa; VOT = Olt valley; VST = Valea lui Stan; SPI = Spinu.

Olt valley before its damming (Iftime & Iftime, 2007) which is further supported by the higher *B. variegata* score of the populations located further away from the Olt valley (Fig. 7). We may also note that the hybrid score data from Iftime & Iftime, 2007, which were obtained on Păușa valley, were lower than the corresponding current scores: the average was 7.22 in 2007 vs. 8 currently, while the minimum was 4.5 vs. 5 currently. This small increase may pertain to a larger sampling (but one should notice the increase in the minimum), be stochastic, or, alternatively, it may indicate selective forces acting against lower hybrid fitness.

On the whole, the *Bombina variegata* populations in the Cozia National Park are widespread, quite resilient to the current level of natural and human disturbance, moderately anthropophilic (i.e. extensively utilising man-made habitats, but not occurring in dense human settlements) and showing a pattern of previous introgressive hybridization with *B. bombina*. Their habitats show different quality, connectivity and dynamics according to the local topography; whether there is genuine fragmentation or not should be ascertained by more detailed means of investigation. These data should be useful for the conservation-oriented management of the local population of *Bombina variegata* within the National Park and Natura 2000 site context.

ACKNOWLEDGEMENTS

This work was funded by Romanian UEFISCDI Executive Unit for Financing Education Higher Research Development and Innovation, Grant No. 113/2014, code: PN-II-PT-PCCA-2013-4-1489.

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