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Diversity and Relationships of Vertebrate Fauna of Pastrina Hill, a Poorly Studied Hot-spot Karstic Region in North-western Bulgaria

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Abstract: Currently, Pastrina Hill is amongst the poorly studied territories in Bulgaria. For more than a century, only few publications concerning the local vertebrate fauna have been published. The present study was carried out during a period of seven years (2008–2014). Local biodiversity was compared with neighbouring regions in respect to species diversity and richness. Additionally, expected richness was evaluated using the rarefaction method. Our results demonstrated that 48% of the ichthyofauna, 78% of the batrachofauna, 86% of the herpetofauna, 17% of the avifauna, and 88% of mammalian fauna had not been previously reported for the region. From biogeographical point of view, the study area clearly shows more similarity to the Southern Danubian Plain than to the Western Stara Planina mountainous region. During the study period, the expected species richness for the ichthyofauna, batrachofauna and herpetofauna as well as the expected ratio of local-to-regional species richness have been achieved. This demonstrates the great importance of the area and determines its role as a biodiversity hot-spot. This finding should be accounted for ed in the management plan of the Pastrina Site of Community Importance (SCI BG0001037)where are situated.

Key words: species richness, similarity, herpetofauna, avifauna, mammalian fauna, Pastrina Hill, Natura2000, Bulgaria

Introduction

Identification of priority areas for biodiversity protection is essential for the conservation of natural resources. Faunistic studies of vertebrate fauna were defined as an essential and primal step in determining conservation measures and activities in protected areas and their surroundings. The fundamental knowledge includes species distribution, vulnerability status, anthropogenic threats and levels of protection (Jenkins et al. 2013). In particular, the species richness is of primary importance when studying community ecology and when targets are set toward conservation and management of biodiversity (Colwell, Coddington 1994). It is a useful tool for evaluating the overall functioning of ecosystems (Gamfeldt *et al.* 2008). In this respect, vertebrates have been a priority object both for reasons of popular interest and the availability of data (Myers 1988, 1990, Myers *et al.* 2000, Lamoreux *et al.* 2006).

At national level in Bulgaria, biodiversity studies have been limited to a set of works that incorporated distributional data for single (e.g. avifauna, Iankov 2007) or selected vertebrates groups (Golemanski *et al.* 2015). At the regional level, the published studies on vertebrate fauna that provide biodiversity data are limited and focus mainly on faunistic data, e.g. Kresna Gorge (Beron 2001) and Rhodopi Mountains (Beron, Popov 2004, Beron 2006). Moreover, only a few regions have been studied including both biodiversity and distributional data with respect to natural protection and management, like Special Protection Areas Ponor and Besaparski Ridove (Nikolov *et al*. 2014). Respectively, there are still many poorly studied regions even for vertebrate fauna, such as Pastrina Hill, and a serious bias in this respect is currently present at the country level. For more than a century, few publications concerning the local vertebrate fauna of Pastrina Hill have appeared. A single article about the ichthyofauna was published (Trichkova *et al*. 2009) and there were no case studies on other vertebrate groups. Generalised data about avifauna were included in the national atlas of breeding birds (Iankov 2007) and for selected ichthyofauna, avifauna and mammalian fauna representatives in the National Red Data Book (Golemanski *et al*. 2015). These two sources summarised data collected before 2007 and 2011, respectively. Only single, sporadic species records were published, e.g. single observations of bat (Beron 1972) or amphibian species (Wielstra *et al*. 2013, Dufresnes *et al*. 2015); the only exception is the recently studied devastating effect of fires on local populations of *Testudo hermanni* that provided extended distributional data (Slavchev *et al*. 2014).

The main goal of the present work is to provide detailed faunistic data about the vertebrates of Pastrina Hill and to evaluate the importance of the biodiversity of the studied area in terms of maintaining the vertebrate diversity of neighbouring regions.

Material and Methods

The study territory covers Pastrina, an insulated karstic hill of 98–557 m a.s.l., in North-western Bulgaria, in the northern part of the central Balkan Peninsula. The region is situated northeast of the town of Montana and is located between Ogosta, Shugavitsa and Botunya Rivers. Since 2007, the greater part of the hill is part of the network of protected areas Natura2000 (Pastrina Site of Community Importance, or Pastrina SCI, BG0001037; Fig. 1). It covers 3551.58 ha, which is a medium- to small-sized protected area for Bulgaria. The study area is surrounded by the villages of Lipen, Stubel, Krapchene, Nikolovo, Dolno Belotinci, Erden, Ohrid and Palilula.

The climate is temperate continental with cold winters and hot summers. The average temperature is –2°C in January and 25°C in July. Summer temperatures of 35–40°C are common (Kopralev 2002).

The survey in Pastrina region was carried out for seven years (2008-2014), between March and October. A total of 101 tracks were performed during the study period, with mean length of 4.12 km (in total 416 km) and 870 GPS point locations were collected. Data on ichthyofauna were collected by active fishing and by surveying fishermen's catch, regularly throughout the season. Amphibian and reptile species were studied by visual encounter surveys (special attention was paid at suitable microhabitats, e.g. under rocks and logs), supplemented with acoustic searching for anurans. Additionally, funnel traps and deep netting were used for newts and amphibian larvae. Amphibians' eggs clumps and masses were also used for species identification. Birds and some mammal species were observed with a binocular. For mammals, footsteps or other kinds of evidences (artefacts) for their activity were also searched. For all groups, road kill specimens were collected and identified as well as detectable animal remains (including bones, shed snake skins).

Geographic coordinates for each observation were determined in the field with a GPS receiver Garmin eTrex Vista (manufacturer specified accuracy ± 5 m). Coordinates were recorded as latitude and longitude in decimal degrees, and referred to the WGS84 (World Geodetic System of 1984) datum. Data were attributed to standard 10×10 km cells on the national UTM grid. The study area covers four grid cells: FP80, FP81, FP90 and FP91.

Taxonomy and identification of ichthyofauna were according to Kottelat, Freyhof (2007). For herpetofauna, the classification adopted by Stojanov *et al*. (2011) was used, with minor exceptions: in the case with the *Hyla arborea* species complex, *H. orientalis* was proven to occur in the study area (locality Montana in Gvoždík *et al*. 2015; village Dobrusha and Pastrina Hill after Dufresnes *et al*. 2015). No data are available about the taxonomic status of the *Bufotes* species at the national level; for this reason, the recorded members of this genus were presented as "*B. viridis* complex". Regarding the intraspecific differentiation of *Natrix natrix,* we follow Kindler *et al*. (2013) concerning the need of an updated taxonomic scheme but the encountered stripped specimens (formerly *N. natrix persa*) were treated separately in the analyses. The taxonomy of birds was according to Ivanov *et al.* (2009). Mammalian systematics was according Popov, Sedefchev (2003) and Peshev *et al*. (2004), with some lattermost considerations.

According to the geographical regionalisation (Petrov 1997), the study area (Pastrina Hill) belongs to the Western Stara Planina natural region, category 1 (High Mountains) and subcategory 1a (coniferous and mixed coniferous–broadleaved forests and alpine zone). In addition, the faunistic data from Pastrinal Hill were compared as well to neighbouring

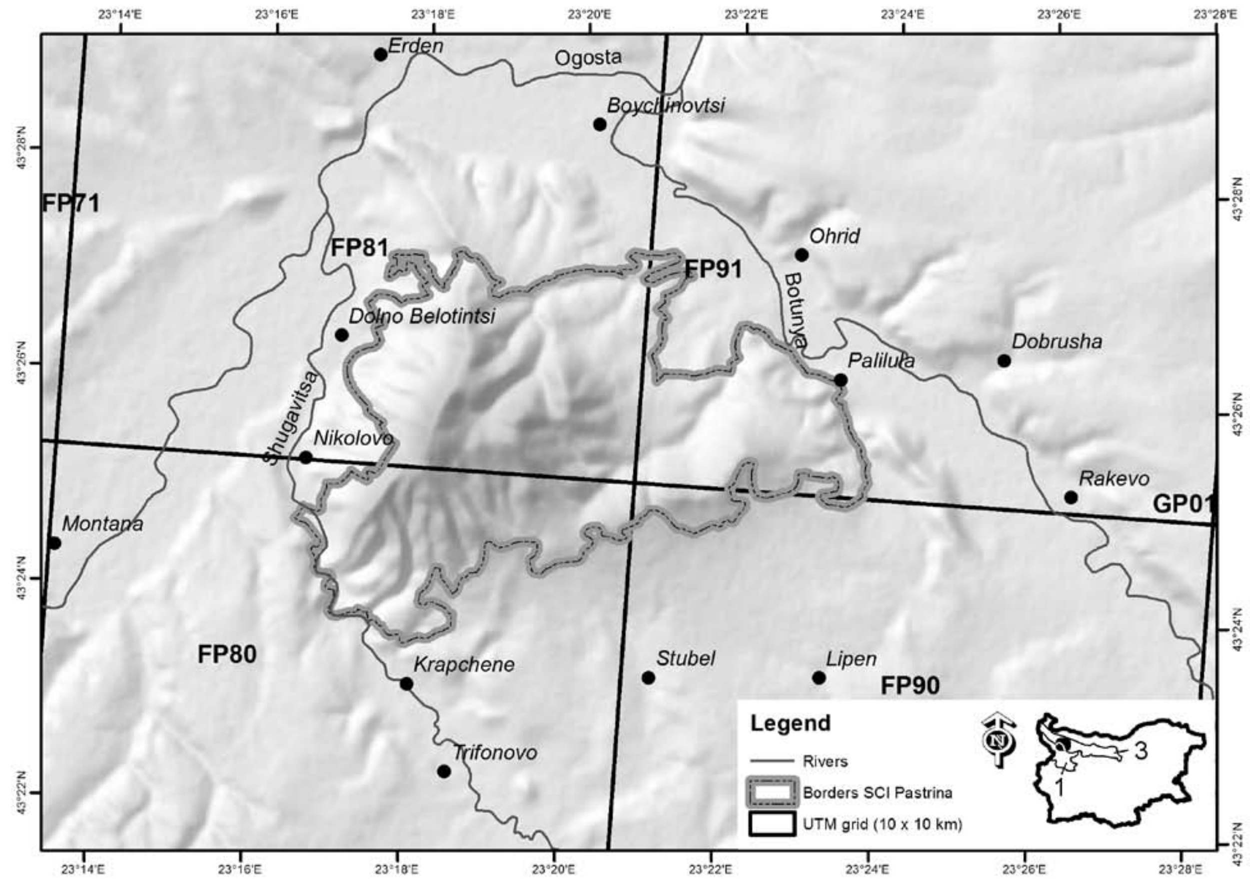

Fig. 1. Map of the study area. Western Stara Planina natural region, category 1 and Southern Danubian Plain, category 3 (according to Petrov 1997) are presented in the legend with number 1 and 3 respectively; the study area is denoted with black dot

Southern Danubian Plain, category 3 (Hilly Lands in the Lowland Plains Dominated by Farmlands and Pastures) as defined in Petrov (1997).

Species richness (S_{obs}) among vertebrate classes expressed as number of species encountered per grid cell was compared with the available published data sources attributed to covered gird cells, or with the geographic region (sensu Petrov 1997) as a whole (S_{comp}).

For general comparison and representation of avifauna composition the scheme presented by Iankov (2007) was adopted and slightly modified: instead of species with up to ten observed specimens in one UTM grid cell certain number of specimens were used (category A); next categories were directly implemented: from 10 to 99 specimens (B); from 100 up to 999 specimens (C) and from 1000 and more specimens (D).

Fauna data entries were categorised as follows: not confirmed (NC); verified (V); verified and newly found (VN), in case of new localities found instead of previously known for a particular gird cell; newly found for Pastrina (N).

Beta diversity was defined sensu Wittaker *et al.* (1960), following the clarification made by Tuomisto (2010 a,b) and regional-to-local diversity ratio was used: $\beta_{Mt}=\gamma/\alpha_t$, where γ was regional and α was local richness.

Analyses were performed using R software (version 3.1.2, R Core Team 2013). Shannon diversity index (H') was used to express the diversity for studied UTM grid cells (except for UTM FP90, due to data deficiency). Data sets for studied grid cells, presented in a national bird atlas (Iankov 2007) were then compared with H' values for the data from the present study using the permutation method provided by the R package *vegan* (Oksanen *et al.* 2015). Bootstrap confidence intervals (95% CI) for each value were computed with the same package. In order to avoid algorithm biases in calculations for compared pairs, 3×10^4 random permutations were generated (Lehsten, Harmand 2006).

For comparing the faunal similarity and the attribution to certain natural regions as defined in Petrov (1997), the dataset for the neighbouring natural re-

Table 1. List of the ichthyofauna species in Pastrina Hill region. For abbreviations, see Material and Methods. Already published records – ([1]) Trichkova *et al.* (2009); ([2]) Golemanski *et al.* (2015), before 1985; ([3]) Golemanski *et al.* (2015), after 2003

Taxon	UTM	Status
Rhodeus cericeus (Pallas, 1776)	FP80[1], FP91[1]	V
Gobio gobio (Linnaeus, 1758)	FP80[1], FP91[1]	V
Romanogobio kesslerii (Dybowsky, 1862)	FP80[2], FP81[2], FP90[2], FP91[2]	NC
Romanogobio uranoscopus (Agassiz, 1828)	FP80[2]	NC
Barbus barbus (Linnaeus, 1758)	FP80[1,2], FP81[2], FP90[2], FP91[2]	V
Barbus petenyi Risso, 1827	FP80[1]	NC
Carassius gibelio Bloch, 1782	FP80[1], FP91	V
Cyprinus carpio Linnaeus, 1758	FP81, FP91	N
Abramis brama (Linnaeus, 1758)	FP81	N
Alburnoides bipunctatus (Bloch, 1782)	FP80[1], FP91	V
Alburnus alburnus (Linnaeus, 1758)	FP80[1], FP91	V
Aspius aspius (Linnaeus, 1758)	FP81[2], FP91[2]	NC
Chondrostoma nasus (Linnaeus, 1758)	FP81, FP91	N
Squalis cephalus (Linnaeus, 1758)	FP80[1], FP91	V
Scardinius erythrophthalmus (Linnaeus, 1758)	FP81, FP91	N
Tinca tinca (Linnaeus, 1758)	FP81, FP91	N
Pseudorasbora parva (Temminck & Schlegel, 1846)	FP91	N
Cobitis sp.	FP80[1]	
Cobitis elongata Heckel & Kner, 1858	FP91	N
Cobitis elongatoides Băcescu & Mayer, 1969	FP91	N
Sabanejewia balcanica (Karaman, 1922)	FP80[1,3], FP81[3], FP91[3]	NC
Barbatula barbatula (Linnaeus, 1758)	FP80[1,3], FP81[2], FP91[2]	NC
Silurus glanis Linnaeus, 1758	FP81, FP91	N
Esox lucius Linnaeus, 1758	FP81, FP91	N
Lepomis gibbosus (Linnaeus, 1758)	FP80[1], FP91	V
Gymnocephalus cernua (Linnaeus, 1758)	FP91	N
Perca fluviatilis Linnaeus, 1758	FP81, FP91	N

gions for batrachofauna and herpetofauna (Stojanov *et al*. 2011; personal data), avifauna (Boev *et al.* 2007) and for mammal fauna (Popov 2007a) were compared with data obtained from the study area, as well as ichthyofauna similarities between the rivers Ogosta, Botunya and Shugavitsa were expressed by means of Chao's Abundance-based Jaccard (S_{cj}) and Sørensen (S_{cs}) indices (sensu Chao *et al*. 2005). Coleman's classic area-based sampling model was used to express the estimated species richness ($S_{est;}$ Colwell *et al*. 2012). Both similarity indices and rarefaction model were evaluated with EstimatesS software (Colwell 2013).

Results and Discussion

Presently, a rich vertebrate fauna occurs in Pastrina Hill region. Based on intensive field studies most of the expected vertebrate fauna that inhabits the neighbouring regions was found.

The ichthyofaunal list comprised 26 species (Table 1); 12 species were newly found. Nine species being published in previous studies were confirmed (Trichkova *et al*. 2009, Golemanski *et al*. 2015) and were found in new localities; five species were not found in the region.

Several fish species that are common and typical for the upstream parts of the rivers species (such as *Cottus gobio*, *Phoxinus phoxinus*, *Salmo* sp. and the introduced *Oncorhynchus mykiss* and *Salvelinus fontinalis*, both present in Ogosta dam), previously recorded for the upstream river Ogosta and its tributaries (Michailova 1970, Karapetkova 1994, Stefanov, Trichkova 2015) were missing in the studied region. These results show much more similarity to the ichthyofauna of the Danubian Plain than to that of Western Stara Planina. Some species that were not reported by Trichkova *et al*. (2009) for Ogosta (site 31) and Shugavitsa Rivers (site 35; both sampling sites fall within UTM FP80), were present prior in Ogosta River (Karapetkova 1994) and were categorised as mainly limnophilic and eurytopic Danubian species, present mainly in the estuaries of the tributaries; these include *Abramis brama, Cyprinus carpio, Scardinius erythrophthalmus, Tinca tinca, Misgurnus fossilis, Silurus glanis, Gymnocephalus baloni, G. cernuus, G. schraetser, Sander lucioperca* and *Benthophilus stellatus*. At least part of them were found from Botunya (UTM FP91) and some in Ogosta Rivers (UTM FP81), e.g. *A. brama, C. carpio, S. erythrophthalmus, T. tinca*, *S. glanis, G. cernuus.* There is a possibility that the populations of these species have appeared due to the fish stocking in the region. The species diversity in Botunya River was higher than both Ogosta and Shugavitsa Rivers (25 vs. 21 and 11 species), but sharing nearly all species except *A. brama* and *Romanogobio uranoscopus* that were found only in Ogosta River. Respectively four species were found only in Botunya River (*Pseudorasbora parva*, *Cobitis elongatoides*, *Gymnocephalus cernua* and *Carassius gibelio*, this last one was present also in Shugavitsa River). Shared species with Ogosta River were 19 (76%; obtained similarity S_{cj}=0.74/ S_{cs}=0.85). Shugavitsa River was with higher similarity to Botunya (S_{cj}=0.44/S_{cs}=0.61) than to Ogosta (S_{cj}=0.38/S_{cs}=0.55) River, without having common streams, being both tributaries of Ogosta. Close proximity of urban waters coming from the town of Montana seems to influence negatively the ichthyofauna diversity and could be the main reason for the observed lower species richness in Ogosta River. Data on the ecological status based on macrozoobenthos, general physical and chemical quality elements also revealed the decreasing water quality in Ogosta River, downstream of the town of Montana and upstream of Erden Village, where the influence of Montana town and the many small farms in the river basin led to the deterioration of the river status category to bad (Site 5 in Stoyanova, Traykov 2014). Results demonstrated also the great importance of the tributaries serving as a source for the repopulation of the main rivers. Nevertheless, Botunya River was polluted by wastewater from the chemical industry and local purification station in Vratsa town (Vasileva *et al*. 2008) that points out of the possible high importance of the secondary or higher tributary classes as well.

Nine amphibian species were found in Pastrina Hill area. For comparative analyses, batrachofaunal data from the neighbouring natural regions Western Stara Planina natural region, category 1 and Southern Danubian Plain, category 3 (Petrov 2007) was also stated, respectively sharing the same species with the second one but with respect to the first one containing *Bombina bombina* instead of *Rana temporaria* and *Ichthyosaura alpestris* that were missing in the lower altitudes. Nine species (seven newly reported) of amphibians have been found in Pastrina Hill region (Table 2), thus sharing 82% (obtained similarity S_{cj}=0.82/S_{cs}=0.90) with the lowland region vs. 73% (S_{cj}=0.62/S_{cs}=0.76) with the corresponding mountainous region.

The amphibian species with the highest conservation status at European level, *Triturus cristatus*, was found at two new localities in the study area (Table 2). The locality published in Wielstra *et al*. (2013), within the town of Montana was already destroyed (2010/2011). This taxon was recently found in Bulgaria, at the southernmost distribution of this species (Tzankov, Stojanov 2008). As of 2015, only seven known localities in North-western Bulgaria

Table 2. List of the batrachofauna species in Pastrina Hill region. For abbreviations see material and methods. Already published records – ([1]) Wielstra *et al.* (2013); ([2]) Gvoždík *et al.* (2015); ([3]) Dufresnes *et al.* (2015)

Taxon	**UTM**	**Status**
Lissotriton vulgaris (Linnaeus, 1758)	FP81, FP91	N
Triturus cristatus (Laurenti, 1768)	FP 80, FP81[1], FP91	VN
Bombina bombina Linnaeus, 1761	FP81, FP91	N
Bombina variegata (Linnaeus, 1758)	FP81	N
B. bombina x *B. variagata* hybrids	FP81	N
Bufo bufo Linnaeus, 1758	FP80, FP81, FP91	N
Bufotes viridis complex	FP80, FP81, FP91	N
Hyla orientalis Bedriaga, 1890	FP80, FP81[2], FP91[3]	VN
Pelophylax ridibundus (Pallas, 1771)	FP80, FP81, FP91	N
Rana dalmatina Fitzinger in Bonaparte, 1839	FP80, FP81, FP91	N

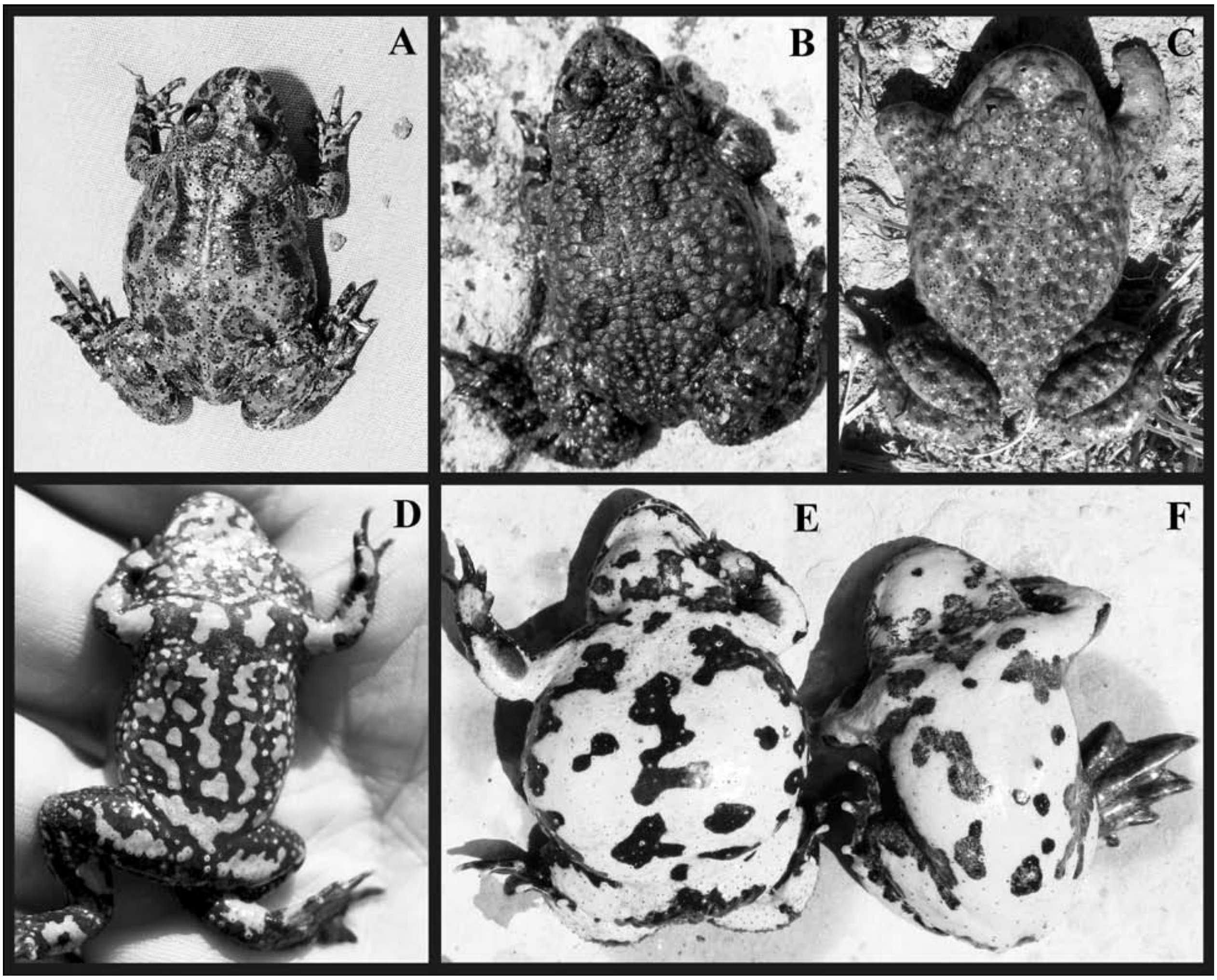

Fig. 2. Comparison between males of *B. bombina* (A, underside D), hybrid *B. bombina* x *B. variegata* (B, E) and *B. variegata* (C, F). Note the upper view of the hybrid is more similar to that of *B. bombina* in respect to colour pattern, head proportions and skin tubercles type (smoothed); the underside is more similar to *B. variegata* with predominant yellow colour

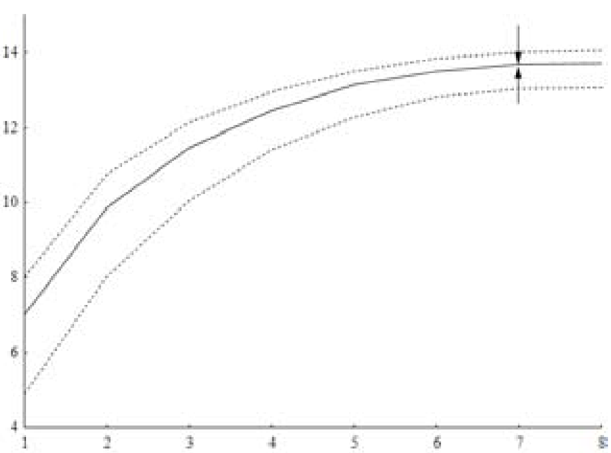

Fig. 3. Rarefaction curve expressing the model values (±SD), representing the consecutive field seasons (years) versus estimated species richness for amphibians. Arrows denote the $S_{obs}=S_{est}$

exist, placing a spotlight on the greater importance of the study area. In Pastrina Hill both *Bombina bombina* and *B. variegata* species were found: *B. bombina* at four locations, *B. variegata* at two. After 2010, hybrid specimens were found regularly in one of the wetlands in the study area (Fig. 2).

This is one of the very few recorded places in Bulgaria where both species lives in syntopy as is the case near the village of Brusen (Beshkov *et al*. 1967; not confirmed after mid-eighties; V. Beshkov, pers. comm.) or produce fertile hybrids as in Chernelka Gorge (Jameson *et al*. 1982; not confirmed recently). One species, *Salamandra salamandra,* was not found during our study but could be potentially present. A possible reason was the lack of suitable breeding places, e.g. permanent streams or temporary ones standing sufficient time. Concerning the batrachofauna's species richness, the plateau of the

Table 3. List of the herpetofauna species in Pastrina Hill region. For abbreviations see material and methods. Already published records – ([1]) Slavchev *et al.* (2014); ([2]) Vergilov, Tzankov (2014)

Taxon	UTM	Status
Testudo hermanii (Gmelin, 1789)	FP80[1], FP81[1], FP91[1]	V
Emys orbicularis (Linnaeus, 1758)	FP91	N
Ablepharus kitaibelii Bibron & Bory de Saint-Vincent, 1833	FP80, FP81[2], FP91	VN
Anguis colchica (Nordmann, 1840)	FP80, FP91	N
Darevskia praticola (Eversmann, 1834)	FP91	N
Lacerta viridis Laurenti, 1768	FP80, FP81, FP91	N
Podarcis muralis (Laurenti, 1768)	FP80, FP81, FP91	N
Podarcis tauricus (Pallas, 1814)	FP80, FP81, FP91	N
Coronella austriaca Laurenti, 1768	FP81, FP91	N
Dolichophis caspius (Gmelin, 1779)	FP81, FP90, FP91	N
Zamenis longissimus (Laurenti, 1768)	FP81, FP91	N
Natrix natrix Linnaeus, 1758	FP80, FP81, FP91	N
Natrix tessellata (Laurenti, 1768)	FP80, FP91	N
Vipera ammodytes (Linnaeus, 1758)	FP80, FP81, FP91	N

Coleman's model (i.e. $S_{obs}=S_{est}$) was reached before the sixth field season (Fig 3).

All herpetofauna species except two recently cited (Slavchev *et al*. 2014, Vergilov, Tzankov 2014) were newly recorded (Table 3). Pastrina Hill species richness was a little bit lower Sobs=14 (all species matched) than in both neighbouring regions (18 and 16 species respectively in the mountainous and the lowland regions), thus the similarities with the lowland region was just a little higher (S_{cj}=0.88/S_{cs}=0.93 vs. S_{cj}=0.78/S_{cs}=0.88).

Among the reptile species in the region with the highest conservation status was *Testudo hermanni*. This species was found sporadically all across the area but local population was heavily impacted by large scale fires and the currently established overall abundance values were extremely and critically low (reaching up to 0.1 ind./ha: Slavchev *et al*. 2014). It must be pointed out that although *T. graeca* was mapped to occur in the region, the map presented in the National Red data book (Beshkov 2015) was overestimated and the closest known credible localities were in the surroundings of the villages of Beli Izvor (Beshkov *et al*. 1967) and Dolno Ozirovo (late 80ies, original source by Rumen Todorov, identified by Vladimir Beshkov; late 90[ies]: Georgi Stoyanov, unpublished data, confirmed by Andrey Stoyanov; both cases were based on remains accumulated by raptors; both falling in UTM FN99), which are also isolated from the core species distribution according to the map of the recent geographical range provided by Stojanov *et al*. (2011). *Darevskia praticola* was found with a single specimen within the protected area (26.03.2011). Outside Pastrina hill, neighbouring to this territory in an insulated forest patch of predominantly oak near Todorina padina place, a population with a relatively high abundance was found (31 specimens/km, 05.07.2009). *Darevskia praticola* could serve as an indicator species for certain microclimate conditions in forests, e.g. specific soil and air humidity, taking into account his evident absence in other type of forests in the region and specifically in sparse oak forest patches. Several threats such as fires and forest cutting heavily contribute to the observed high degree of erosion in the region that directly correspond and seem to be the main cause for the scattered distribution of this species. Other reptiles with similar requirements were also very rarely encountered. *Coronella austriaca* was recorded at only two locations in the area with a single specimen (17.04.2010, 26.05.2010). Similarly, only five specimens of *Zamenis longissimus* were encountered during the whole study period (29.05.2009, 17.04.2010, 22.04.2011 and 22.04.2014).

Among all the observed *Natrix natrix* specimens in the study site (n=16), only two were striped (formerly *N. n. persa*; 12.5%), both coming from a garden pond in Dobrusha village (06.2012 and 05.05.2013). The observed pattern among both colour forms contradicted to the former model proposed by Beshkov (1986) which states that north of the Balkan Mountains and below 300 m a.s.l. striped specimens prevailed (54.5% and respectively 80.5%).

In the survey area the nominate subspecies of *Vipera ammodytes* have been encountered, thus

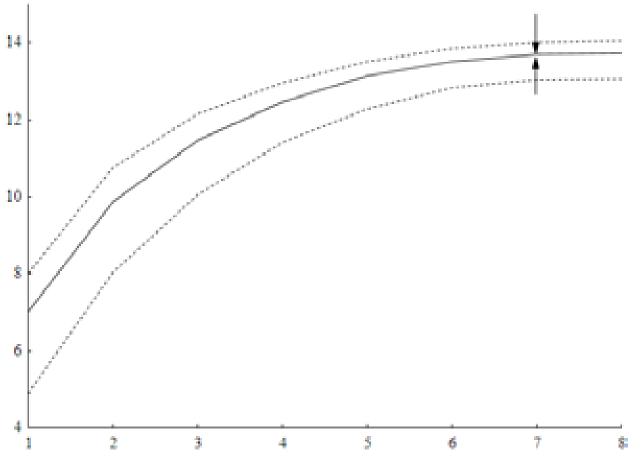

Fig. 4. Rarefaction curve expressing the model values (±SD), representing the consecutive field seasons (years) versus estimated species richness for reptiles. Arrows denote the observed $S_{obs}=S_{est}$

tracing the northeastern border of its distribution (Stojanov *et al*. 2011).

No herpetofauna species was expected to occur in addition to the known ones in the study area, and the corresponding plateau of the Coleman's model (i.e. $S_{obs}=S_{est}$) was reached by the seventh field season (Fig 4).

The given numbers of nesting birds according to the proposed regionalisation (regions 1 and 3 in Petrov 1997) was 147 and respectively 115 species (Boev *et al*. 2007). The study area clearly has shown a greater similarity with this latter region despite its well-expressed relief structure (S_{cj}=0.62/S_{cs}=0.77; 77 or 65% shared species). The dominant open habitats and sparse forests also contribute to a higher similarity with this region. Respectively, the similarity with the corresponding mountainous region was a bit lower (S_{cj}=0.42/S_{cs}=0.59; 71 or 46% shared species). The observed differentiation in respect to the neighbouring lowland region was lower (β=1.28) than that to the mountainous one (β=1.74).

When comparing the avifauna diversity before 2007 (datasets presented in Iankov 2007) and that from the study period (2008–2014; Table 4), statistically significant differences were found between all compared pairs: UTM FP80 (H'_{2007}=4.32, CI = 4.19–4.44; $H'_{2008–2014}$=3.50, CI = 3.31–3.69, p=0.03), UTM FP81 (H'_{2007}=4.49, CI = 4.37–4.61; $H'_{2008-2014}$=3.81, CI = 3.64–3.97, p=0.03) and UTM FP91 (H'_{2007}=3.50, CI = 3.31–3.69; $H'_{2008-2014}$=4.43, CI = 4.32–4.56, p=0.01).

The obtained results have revealed a negative tendency in respect to the species richness for UTM FP80 and FP81, and positive for grid cell UTM FP91. Respectively, the corresponding similarity (here interpreted as valuable confirmation) between explored pairs was relatively low, i.e. S_{cj}=0.32/S_{cs}=0.48, 26 (32%) confirmed species for UTM FP80, S_{cj}=0.31/S_{cs}=0.48, 32 (31%) confirmed species for UTM FP81 and S_{cj}=0.32/S_{cs}=0.49, 29 (33%) confirmed species for UTM FP91. These observations should be interpreted with caution, given that even for a seven-year study period the avifauna list is likely incomplete, particularly with respect to the rare species. On the other hand, the nearly equal levels of species confirmation for grid cell with decreased or respectively increased species richness suggested possible relevance of the observed results. Moreover, the observed tendencies at the continental scale revealed a rapid and non-random change in avifauna community composition, most likely driven by anthropogenic activities (Le Viol *et al*. 2012).

According to the faunistic analyses on mammals performed by Popov (2007b), the study area was attributed to Western and Central mountainous subdistrict (region Ia in the original source). A total of 45 species (including two Chiroptera species) were presented in neighbouring subregions 2a and 5 (after Popov 2007a), corresponding respectively to the already mentioned regions 3 and 1a (after Petrov 1997). The study area showed a nearly equal similarity with both compared subregions (S_{cj}=0.53/S_{cs}=0.70 and S_{cj}=0.52/S_{cs}=0.69), respectively sharing 24 (55%) vs. 23 (54%) species. That corresponds to its intermediate position but it must be pointed out that it could be a result of sampling insufficiency. The total number of identified mammal species in the area (n=25) was nearly two times lower than that io the the neighbouring main large subregions. Here also the range scale effect must be taken into account, as the study territory was considerably smaller than the mentioned subregions used for comparison. Only for three species a particular published data coming from the study area were available (Table 5). A single record of *Rhinolophus ferrumequinum* was published by Beron (1972). In another part of the area one dead specimen of *R. ferrumequinum* was found (06.04.2013). The study area (karstic with well expressed relief form) offered a potential habitats for a list of bat species that inhabit the neighbouring geographic region, biogeographic units 1a (seven species) and 2a (22 species; Benda *et al*. 2003), but additional more specific research is needed. The lack of deep caves in the study area could also be a reason for the lower bats richness, as deep caves were often preferred wintering places, instead of shallow caves and artificial mines according to a comparable study in adjacent Western Stara Planina (Pandurska, Beshkov 1998).

Table 4. List of the avifauna species in Pastrina Hill region. For abbreviations see material and methods. Already published records – ([1]) Golemanski *et al.* (2015), before 1985; ([2]) Golemanski *et al.* (2015), after 1985; ([3]) Golemanski *et al.* (2015), after 2003. New data compare to data from Iankov (2007) were bolded and italicised

Taxon	FP80	FP81	FP90	FP91	RDB	Status
Tachybaptus ruficollis (Pallas, 1764)		13			FP81[3]	NC
Podiceps cristatus (Linnaeus, 1758)		4			FP81[3]	NC
Phalacrocorax carbo (Linnaeus, 1758)	1/***1***	1		1		VN
Ixobrychus minutus (Linnaeus, 1766)		10				NC
Nycticorax nycticorax (Linnaeus, 1758)		20		1		VN
Ardeola ralloides (Scopoli, 1769)		10			FP81[3]	NC
Egretta garzetta (Linnaeus, 1758)		25		2		VN
Casmerodius albus Linnaeus, 1758				2		N
Ardea cinerea Linnaeus, 1758	***3***	1		4		VN
Ardea purpurea Linnaeus, 1758		1			FP81[2]	NC
Ciconia nigra (Linnaeus, 1758)	1	1		1/***2***	FP80,81,91[3]	V
Ciconia ciconia (Linnaeus, 1758)	15	8	15	8/***2***		V
Anas crecca Linnaeus, 1758				3		N
Anas platyrhynchos Linnaeus, 1758	***B***	8		B		VN
Anas querquedula Linnaeus, 1758		2			FP81[3]	NC
Aythya ferina (Linnaeus, 1758)		1				NC
Aythya nyroca (Gueldenstaedt, 1770)		5			FP81[2]	NC
Aythya fuligula (Linnaeus, 1758)		1				NC
Neophron percnopterus (Linnaeus, 1758)	1				FP80[1]	NC
Aquila chrysaetos Linnaeus 1758	1				FP90[3]	NC
Circaetus gallicus (Gmelin, 1788)	1	1		1		N
Circus pygargus (Linnaeus, 1758)				5		N
Circus aeruginosus (Linnaeus, 1758)	1/***1***	***1***				VN
Accipiter gentilis (Linnaeus, 1758)		2		4		N
Accipiter nisus (Linnaeus, 1758)	2	1/***1***		2		VN
Accipiter brevipes (Severtzov, 1850)	1	2				NC
Buteo buteo (Linnaeus, 1758)	4	5/***2***		B		VN
Buteo rufinus (Cretzschmar, 1827)	1/***1***	***2***		2		VN
Aquila pennata (Gmelin, 1788)				1		N
Falco tinnunculus Linnaeus, 1758	9/***1***	12	2	12		V
Falco subbuteo Linnaeus, 1758		1/***1***			FP81[1]	V
Perdix perdix (Linnaeus, 1758)	B/***B***	***20***	B	30/***B***		V
Coturnix coturnix (Linnaeus, 1758)	C/***B***	C/***B***	C	C/***B***		V
Phasianus colchicus Linnaeus, 1758	B/***B***	B/***B***	B	***B***		VN
Rallus aquaticus Linnaeus, 1758		4				NC
Porzana porzana (Linnaeus, 1766)		4			FP81[2]	NC
Crex crex (Linnaeus, 1758)		20				NC
Gallinula chloropus (Linnaeus, 1758)	A	B		8		VN
Fulica atra Linnaeus, 1758		B				NC
Charadrius dubius (Scopoli, 1786)	1	2		***2***	FP81[2]	VN
Vanellus vanellus (Linnaeus, 1758)	A	10				NC
Gallinago gallinago (Linnaeus, 1758)				1		N
Tringa ochropus Linnaeus, 1758	1				FP80[2]	NC
Actitis hypoleucos (Linnaeus, 1758)	1			3		VN

Table 4. Continued

Taxon	**FP80**	**FP81**	**FP90**	**FP91**	**RDB**	**Status**
Larus michahellis Naumann, 1840	10	1				NC
Columba livia forma domestica Gmelin, 1789	C/***B***	B/***B***	B	***B***		V
Columba palumbus Linnaeus, 1758	1	1		4		VN
Streptopelia decaocto (Frivaldszky, 1838)	200/***B***	C/***B***	300	C/***B***		V
Streptopelia turtur (Linnaeus, 1758)	C	C		1		VN
Cuculus canorus Linnaeus, 1758	20/***10***	15/***20***	30	30/***B***		V
Bubo bubo (Linnaeus, 1758)	1	***1***				VN
Athene noctua (Scopoli, 1769)	15/***2***	A	10/***4***	15/***5***		V
Asio otus (Linnaeus, 1758)	A	1/***1***				VN
Caprimulgus europaeus Linnaeus, 1758	1					NC
Tachymarptis melba (Linnaeus, 1758)		4		1		N
Upupa epops Linnaeus , 1758	10	A	A	***4***		VN
Alcedo atthis (Linnaeus, 1758)	A	A	1	***9***		VN
Merops apiaster Linnaeus, 1758	60/***B***	B/***B***		C		VN
Coracias garrulus Linnaeus, 1758		1		1	FP81[3]	VN
Jynx torquilla Linnaeus, 1758	A	A		2		VN
Picus viridis Linnaeus, 1758	A	A	A	A/***7***		V
Picus canus Gmelin, 1788				1		N
Dendrocopos major (Linnaeus, 1758)	3	1		4		N
Dendrocopos syriacus (Hemprich & Ehrenberg, 1833)	B	B	15	15/***1***		V
Dendrocopos minor (Linnaeus, 1758)	A	1		1		VN
Galerida cristata (Linnaeus, 1758)	B/***B***	B/***B***	B	B/***B***		V
Lullula arborea (Linnaeus, 1758)	1	1				N
Alauda arvensis Linnaeus, 1758	C/***C***	C/***C***	C	C/***C***		V
Riparia riparia (Linnaeus, 1758)	B			B		VN
Hirundo rustica Linnaeus, 1758	C/***C***	C/***C***	C	C/***C***		V
Cecropis daurica (Linnaeus, 1771)	A/***1***	A				V
Delichon urbicum (Linnaeus, 1758)	900/***B***	C/***C***	B	B/***B***		V
Motacilla flava Linnaeus, 1758	C	C/***B***		C/***B***		V
Motacilla alba Linnaeus, 1758	15/***6***	B/***1***		A/***B***		V
Anthus campestris (Linnaeus, 1758)				1		N
Troglodytes troglodytes (Linnaeus, 1758)	A	A		3		VN
Erithacus rubecula (Linnaeus, 1758)	B	B		B		VN
Luscinia megarhynchos Brehm, 1831	C/***B***	C/***B***	100	C/***B***		V
Saxicola rubetra (Linnaeus, 1758)		1		1		N
Saxicola torquatus (Linnaeus, 1766)	A	A				NC
Oenanthe isabellina (Linnaeus, 1758)	1					N
Oenanthe oenanthe (Linnaeus, 1758)	A	A/***3***		B		VN
Turdus merula Linnaeus, 1758	C/***C***	B/***B***	B	B/***B***		V
Turdus philomelos Brehm, 1831	B	A/***B***		9		VN
Locustella fluviatilis (Wolf, 1810)		3				NC
Locustella luscinioides (Savi, 1824)		2				NC
Acrocephalus palustri (Bechstein, 1798)		B				NC
Acrocephalus scirpaceus (Hermann, 1804)		15				NC
Acrocephalus arundinaceus (Linnaeus, 1758)	A	B		8		VN
Sylvia curruca (Linnaeus, 1758)	A	5	A	A		NC

Table 4. Continued

Taxon	FP80	FP81	FP90	FP91	RDB	Status
Sylvia atricapilla (Linnaeus, 1758)	B	B		8		VN
Sylvia communis Latham, 1787	B	90	B	B		NC
Phylloscopus collybita (Vieillot, 1817)		2		1		N
Regulus ignicapilla (Temminck, 1820)				1		N
Aegithalos caudatus (Linnaeus, 1758)		A		1		VN
Poecile palustris Linnaeus, 1758				1		N
Cyanistes caeruleus Linnaeus, 1758	A	B	A	***7***		VN
Parus major Linnaeus, 1758	C/***C***	B/***B***	C	B/***B***		V
Sitta europaea Linnaeus, 1758	A	A		4		VN
Oriolus oriolus (Linnaeus, 1766)	B/***B***	B/***B***	C	B/***B***		V
Lanius collurio Linnaeus, 1758	C	C/***C***	C	C/***C***		V
Lanius minor Gmelin, 1788		A		2		VN
Lanius senator Linnaeus, 1758		2		2		N
Garrulus glandarius (Linnaeus, 1758)	120/***C***	B/***B***	B	B/***B***		V
Pica pica (Linnaeus, 1758)	C/***C***	C/***C***	C	C/***C***		V
Corvus monedula Linnaeus, 1758	B/***B***	C				VN
Corvus corone cornix Linnaeus, 1758	20/***15***	40/***15***	30	50		V
Corvus corax Linnaeus, 1758	1/***C***	1/***C***		B		VN
Sturnus vulgaris Linnaeus, 1758	C/***D***	D/***D***	C	D/***D***		V
Passer domesticus (Linnaeus, 1758)	D/***D***	D/***D***	D	D/***D***		V
Passer hispaniolensis (Temminck, 1820)	C	100	B	B/***2***		V
Passer montanus (Linnaeus, 1758)	C	C	C	C/***C***		V
Fringilla coelebs Linnaeus, 1758	B/***B***	B/***B***	B	B/***B***		V
Carduelis chloris (Linnaeus, 1758)	B/***B***	B/***B***	B	B/***B***		V
Carduelis carduelis (Linnaeus, 1758)	C	C/***C***	C	C/***C***		V
Carduelis cannabina (Linnaeus, 1758)	B			B		VN
Coccothraustes coccothraustes (Linnaeus, 1758)	B	B		B		VN
Emberiza cirlus Linnaeus, 1766				6		N
Emberiza citrinella Linnaeus, 1758	1	2		3		N
Emberiza hortulana Linnaeus, 1758	15	A/***1***		B/***B***		V
Emberiza schoeniclus (Linnaeus, 1758)		A				NC
Emberiza melanocephala Scopoli, 1769		5		4		N
Miliaria calandra Linnaeus, 1758	C/***C***	C/***C***	C	C/***C***		V

Similarly, the number of the encountered small mammal species (e.g. Rodentia) was considerably lower than the species mentioned for the comparative regions [S_{obs}=9, vs. S_{comp}=19 in subregion 2a or S_{comp}=15 in subregion 5 after Popov (2007a)]. No member of Soricomorpha were observed. The lower success rate was obviously due to the lack of targeted research with selective methods, e.g. various traps types (appropriate methods summarised in Popov 2007a). A dead specimen of *Dryomys nitedula* was found on a dirt road close to Dolno Belotintsi village (09.2008) next to a sparse forest. In Todorina Padina place 05.07.2009) freshly killed rodents accumulated by unknown predator were found, comprising two *Microtus* cf. *levis*, determined by the proportion of the tail to the body (as given in Popov, Sedefchev 2003) and one *Apodemus agrarius*. The species *Spermophilus citellus* was observed in two localities. This is an important species as it is commonly preyed upon by many predators (reviewed in Peshev *et al*. 2004), and it has high conservation status and national and international level (Stefanov 2015). Next to the colony near the Ohrid village, a concentration of its potential predators has been repeatedly observed, e.g. snakes (*V. ammodytes, Dolichophis caspius, Z. longissimus*), raptors (*Buteo buteo, Buteo rufinus*)

Table 5. List of the mammals species in Pastrina Hill region. For abbreviations see material and methods. Already published records – ([1]) Beron (1972); ([2]) Golemanski *et al.* (2015), after 1985; ([3]) Golemanski *et al.* (2015), after 2003

Taxon	UTM	Status
Rhinolophus ferrumequinum (Schreber, 1774)	FP 81[1]	V
Erinaceus concolor (Martin, 1838)	FP80, FP81, FP91	N
Talpa europaea Linnaeus, 1758	FP 81	N
Lepus capensis Linnaeus, 1758	FP80, FP81, FP91	N
Spermophilus citellus (Linnaeus, 1766)	FP81[3], FP90[2], FP91[3], FP80	VN
Dryomys nitedula (Pallas, 1778)	FP81	N
Nannospalax leucodon (Nordmann, 1840)	FP81, FP91	N
Apodemus agrarius (Pallas, 1771)	FP80, FP81, FP91	N
Apodemus flavicollis (Melchior, 1834)	FP91	N
Mus cf. *musculus* (Linnaeus, 1758)	FP91	N
Rattus norvegicus (Berkenhout, 1769)	FP81	N
Rattus rattus (Linnaeus, 1758)	FP91	N
Arvicola amphibius (Linnaeus, 1758)	FP91	N
Microtus cf. *levis* Miller, 1908	FP91	N
Canis lupus Linnaeus, 1758	FP81	N
Canis aureus Linnaeus, 1758	FP80, FP81, FP91	N
Vulpes vulpes (Linnaeus, 1758)	FP80, FP81, FP91	N
Martes foina (Erxleben, 1777)	FP80, FP81, FP91	N
Mustela nivalis Linnaeus, 1766	FP81, FP91	N
Mustela putorius (Linnaeus, 1758)	FP81	N
Meles meles (Linnaeus, 1758)	FP81, FP91	N
Lutra lutra (Linnaeus, 1758)	FP80[3], FP81[3], FP90[3], FP91[3]	VN
Felis silvestris Schreber, 1777	FP81, FP91	N
Sus scrofa Linnaeus, 1758	FP80, FP81, FP91	N
Capreolus capreolus (Linnaeus, 1758)	FP80, FP81, FP91	N

and occasionally other predators (i.e. *Mustela putorius, Felis silvestris*). *Mustela putorius* was encountered (25.05.2011) in front of a burrow, within a hibernation spot shared by *V. ammodytes, D. caspius, Z. longissimus* and situated nearby the colony. *Felis silvestris* was seen (18.09.2010) at mid-day in an open field with single bushes next to the same colony. Footprints of *Lutra lutra* were observed in the mud of the riverside of Shugavitsa River near Krapchene village (09.2012). Footprints and excrements of *Canis lupus* were found on a forest dirt road (06.04.2013). Then the occasional presence of the species was confirmed by local hunters from Dobrusha village. Individuals of another canid, *C. aureus,* were seen and heard repeatedly in different habitats in the area during the field studies. On an asphalt road close to Dobrusha, a male jackal was found killed by hunters (11.2008). A total of eight active burrows of *Meles meles* were found within the study area. One active specimen was observed in mid-day in a grassy habitat with spare shrubs and trees (25.03.2009). The observed species richness of Carnivora species was very close to the comparative one (S_{obs}=10 vs. S_{comp}=14 for both comparative subregions) and almost all potentially occurring taxa were found, except those rare at national level, e.g. *Mustela eversmanni, Vormela peregusna* (Spassov, Spiridonov 2015 a,b) and currently mostly confined to the mountainous area species, e.g. *Martes martes*, *Ursus arctos* (Spassov, Spiridonov 2015c, Spiridonov, Spassov 2015) for which suitable territories were not detected or the study area did not have sufficient capacity. Same was the situation with Artiodactyla species where two other non-observed species (i.e. *Dama dama, Cervus elaphus*) are unlikely to occur or may occur irregularly in the study area. In fact, the observed richness when mammal groups with lower occurrence rate were excluded (e.g. Rodentia, Soricomoprha and Chroptera) was slightly lower than that from the neighbouring subregions (S_{obs}=15 vs. S_{comp}=22). According to the Coleman's model 13(±2.2) field seasons (years) after the research was started the approximate species rich-

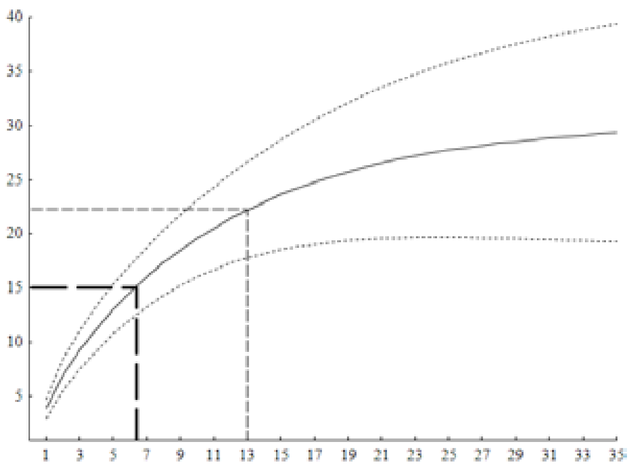

Fig. 5. Rarefaction curve expressing the model values (±SD), representing the consecutive field seasons (years) versus estimated species richness for selected mammal species, largest dashes denote S_{obs}, respectively large dashes denote S_{comp}

ness of the neighbouring regions should be achieved (Fig 5).

From zoogeographical point of view, concerning all vertebrate groups the studied area has shown a greater similarity with the Southern Danubian Plain than with the Western Stara Planina natural region (after Petrov 1997). In support of this conclusion were the composition of the ichthyofauna, batrachofauna and avifauna. The compositions of herpetofauna and mammal fauna were unable to reveal relationships of the study area to one particular region. The observed differences especially for avifauna may be interpreted as faunal changes in process, according and in correspondence to those observed at the continental level (Le Viol *et al*. 2012).

Pastrina Hill area represents a unique biodiversity hot spot at the regional level, having the same ratio of local-to-regional richness (α=γ) for ichthyofauna, batrachofauna, herpetofauna. Two theoretical types of relationships between local and regional richness have been proposed, respectively when the local one is proportional to, but less than, the regional richness (type I) and when local richness attains a ceiling above which it does not rise despite continued increases in regional richness (type II; Cornell, Lawton 1992). Given that most real systems seem to exhibit an underlying type I relationship (review by Gaston 2000), for local avifauna this type was more appropriated. Concerning mammal fauna with respect to the expected species richness and the potential of the studied territory, future research is needed to verify the existence of type II relationship.

The species accumulation it the study area was a slow process. For the best-studied groups, i.e. amphibians and reptiles, the study period was enough for the expected species richness to be achieved. As the existence of well-defined plateau for such type of models was required (Thompson *et al*. 2003), final statements could be evaluated only for these two groups. The present results also impose the necessity of long-term studies when species richness needs to be evaluated even for a relatively small region at a national level. This also reveals the importance and requirement of implementation of a large set of field methods in order the species richness to be studied throughly when various groups are been explored.

Considering the changes in knowledge on conservation, together with threats dynamic processes, the evaluation of priority areas for conservation was postulated to be the main goal of conservation science (Jenkins *et al*. 2013). The study area was heavily affected in two directions. Ground water was heavily impacted by an overall high level of erosion in Pastrina Hill, being among places with highest risk in the whole Ogosta River basin. The groundwater protection especially in karst areas was stated to be in close relation to land use (Orehova *et al*. 2009). These processes occurred in the study area and put a serious threat not only to water related vertebrate fauna, but also led to changes in the environment. At the same time terrestrial fauna suffered from annual fires, sometimes on a large scale, and large territories in Pastrina hHill were devastated regularly (Slavchev *et al*. 2014). Significant role in the overall drought has the ongoing deforestation, a process seriously threatening several species in the study region. The importance and the general effectiveness of protected areas at curtailing deforestation within their boundaries were already demonstrated, but deforestation in surrounding areas is isolating these territories. In this respect land-use zoning and creation of ecological corridors around protected areas were evaluated as effective solutions (Naughton-Treves *et al*. 2005). In addition, it should be mentioned that in spite of their priority status, evidence for significant losses of species richness and biodiversity, within large and well protected natural areas was already demonstrated (Wathen *et al*. 2014). Despite a number of identified serious and important threats, the study area still contains relatively high richness, including a set of priority species implemented in annexes II and IV of the Habitats Directive (European Directive 92/43/EEC). Nevertheless, specific measures have to be undertaken. The existing site of community importance (SCI) urgently needs the adoption and implementation of a management plan.

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