Nature Conservation 50: 1–22 (2022)
doi: 10.3897/natureconservation.50.86002
https://natureconservation.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Distribution and conservation status of threatened endemic amphibians within the Aspromonte mountain region, a hotspot of Mediterranean biodiversity

Giuseppe Martino[1], Andrea Chiocchio[1], Antonino Siclari[2], Daniele Canestrelli[1]

1 *Department of Ecological and Biological Science, Tuscia University, Largo dell'Università s.n.c., 01100 Viterbo, Italy* **2** *Città Metropolitana di Reggio Calabria, Piazza Italia, 89100, Reggio Calabria, Italys*

Corresponding author: Andrea Chiocchio (a.chiocchio@unitus.it)

Academic editor: Franco Andreone | Received 29 April 2022 | Accepted 27 July 2022 | Published 12 September 2022

https://zoobank.org/DCDD662A-DC90-4DB7-B9BE-2DBEB7EF3A87

Citation: Martino G, Chiocchio A, Siclari A, Canestrelli D (2022) Distribution and conservation status of threatened endemic amphibians within the Aspromonte mountain region, a hotspot of Mediterranean biodiversity. Nature Conservation 50: 1–22. https://doi.org/10.3897/natureconservation.50.86002

Abstract

Amphibian biodiversity loss in recent years has exceeded that of all other groups of vertebrates. In this context, biodiversity hotspots represent priority targets for conservation in amphibian populations. However, little information is available on the distribution and conservation status of amphibian species within most biodiversity hotspots. Here, we characterized the distribution and conservation status of four threatened endemic amphibians (*Bombina pachypus*, *Salamandra salamandra gigliolii*, *Salamandrina terdigitata*, and *Rana italica*) in the Aspromonte Mountain region, a biodiversity hotspot in southern Italy where the conservation status of amphibians is almost unexplored. We conducted an intensive field survey of 507 potential breeding sites spanning over 2,326 km^2. We found that all four species were widespread in the study area. We observed 337 species occurrences: 63 for *S. s. gigliolii*, 29 for *S. terdigitata*, 84 for *B. pachypus*, and 161 for *R. italica*. Species distribution analysis revealed that *S. s. gigliolii* and *R. italica* populations had an extended and homogenous distribution. Conversely, *S. terdigitata* showed a dispersed pattern, with long distances among breeding sites, and *B. pachypus* an aggregated pattern, associated with the availability of suitable artificial habitats. On the other hand, we reported a decrease in *B. pachypus* occurrence in its natural habitats, which reflects a negative trend of its populations. Overall, our results provide an encouraging framework for the conservation of amphibian populations in this area, but highlight the low coverage of threatened amphibian populations in protected areas, highlighting the need for a reassessment of conservation policies and spatial conservation planning for the Aspromonte region.

Keywords
Amphibian decline, Apennine yellow-bellied toad, biodiversity conservation, biodiversity hotspot, fire salamander, Italian peninsula, Italian stream frog, spectacled salamander

Introduction

Amphibians are declining worldwide owing to habitat degradation, pollution, climate change, and emerging diseases (Blaustein and Kiesecker 2002; Collins and Storfer 2003; Stuart et al. 2004; Collins 2010; Blaustein et al. 2011; Catenazzi 2015; Scheele et al. 2019). Nearly half of all amphibian species are experiencing regional or local decline, and approximately one-third are threatened by extinction. In Europe, 23% of amphibian species are included in the threatened categories of the International Union for Conservation of Nature European Red List (Temple and Cox 2009; Sindaco 2016). Although the extinction rate in amphibians exceeds that of any other group of vertebrates (Stuart et al. 2004; McCallum 2007), conservation policies have made little progress in halting or reversing these trends (Grant et al. 2016).

The Italian Peninsula is one of the most important biodiversity hotspots in the Palearctic region and hosts a highly diverse amphibian fauna, comprising about half of all amphibian species in Europe (Sindaco et al. 2006; Temple and Cox 2009; Rondinini et al. 2013), as well as many endemic evolutionary lineages. A generalized decline has been detected in the Italian populations of many amphibians, including some endemic taxa such as the Apennine yellow-bellied toad (*Bombina pachypus*), the Italian stream frog (*Rana italica*), the fire salamander (*Salamandra salamandra gigliolii*) and the spectacled salamanders (*Salamandrina terdigitata* and *S. perspicillata*) (Barbieri et al. 2004; Lanza et al. 2007; Andreone et al. 2009). Despite this widespread decline, monitoring schemes aimed at establishing demographic trends and conservation statuses are scattered and mostly limited to a few amphibian populations (e.g. Italica S. 2011). In particular, the conservation status of amphibians in the southernmost part of the Italian Peninsula is severely under-investigated. It is worth noting that this part of the peninsula holds the most of the intraspecific diversity for several species (Canestrelli et al. 2010; Bisconti et al. 2018a; Chiocchio et al. 2019), including most Italian amphibians investigated to date (Canestrelli et al. 2006a, 2006b, 2008, 2012, 2014; Chiocchio et al. 2021). Consequently, a thorough understanding of the conservation status of amphibian populations in southern Italy is a priority for establishing effective conservation plans at both local and regional scales.

In this study, we aimed at characterizing the distribution and conservation status of the threatened amphibians inhabiting the Aspromonte Massif, a mountain region in the southernmost part of the Italian Peninsula (Table 1). The Aspromonte Massif is an extended and heterogeneous mountain region surrounded by the sea and characterized by a high diversity of habitats spanning dry grasslands and shrublands to oak, pine, and beech forests, including a wide range of humid areas that host many amphibian species. Recent studies identified the Aspromonte region as a glacial refuge and hotspot of genetic diversity for many temperate species (Todisco et al. 2010; Canestrelli et al. 2010), including amphibians (Canestrelli et al. 2006a; Iannella et al. 2018). Despite the biogeographic and conservation value of this area, surveys on the distribution and conservation status of amphibians are scarce and discontinuous over space and time (Tripepi et al. 1999; Tripepi and Sperone 2007; Temi and Agristudio 2018). The only exhaustive study carried out in this area to clarify the conservation status of an endangered amphibian, the Apennine Yellow-bellied toad, highlighted the strong discordance between the historical and current distribution of the investigated species (Zampiglia et al. 2019). This evidence, coupled with the scarcity of data for other threatened amphibians, suggests the need for a thorough investigation of the current distribution of amphibians in this area.

The objective of this study is to provide an updated and detailed reference framework concerning the distribution, habitat preferences, and conservation status of threatened amphibians in southern Italy. To achieve this, we apply a fine-scale field survey to map the current presence of threatened amphibians in the Aspromonte region. We will focus on four amphibian species endemic to the Italian Peninsula, which have shown local or regional decline because of habitat reduction/alteration or pathogen outbreaks: *Bombina pachypus*, *Rana italica*, *Salamandrina terdigitata*, and *Salamandra salamandra gigliolii* (see Table 1).

Table 1. Conservation status of the taxa investigated in this study. [1]Costa et al. (2021); [2]Grasselli et al. (2019); [3]Zampiglia et al. (2013); [4]Canestrelli et al. (2013); [5]Zampiglia et al. (2019); [5]Fagotti et al. (2019).

Taxon	Endemic	IUCN Italy	IUCN Europe	Habitat Directive Annexes II or IV	Pathogen threats
Salamandra salamandra gigliolii	Yes	LC	LC		Yes[1,2,3]
Salamandrina terdigitata	Yes	LC	LC	II, IV	Yes[1]
Bombina pachypus	Yes	EN	EN	II, IV	Yes[4,5]
Rana italica	Yes	LC	LC	IV	Yes[3,5]

Methods

Study area

The Aspromonte is a mid-to-high mountain region (maximum altitude 1,957 m asl), located at the southern boundary of the Italian Peninsula (Fig. 1) and characterized by a high diversity of habitats and singular climatic conditions. The seasonal distribution of rainfall has typical Mediterranean features, with less rainfall in the summer months than in the winter months (Colacino et al. 1997). The mountainous belts are characterized by abundant precipitation during autumn, winter, and spring, and streams and brooks are mainly perennial. Most humid habitats in the hilly and coastal strips show dependence on seasonal precipitations. In mountainous areas, the average winter temperatures are quite low, with minimum temperatures occurring in January and February and frequently dropping below 0 °C. Conversely, in the coastal area,

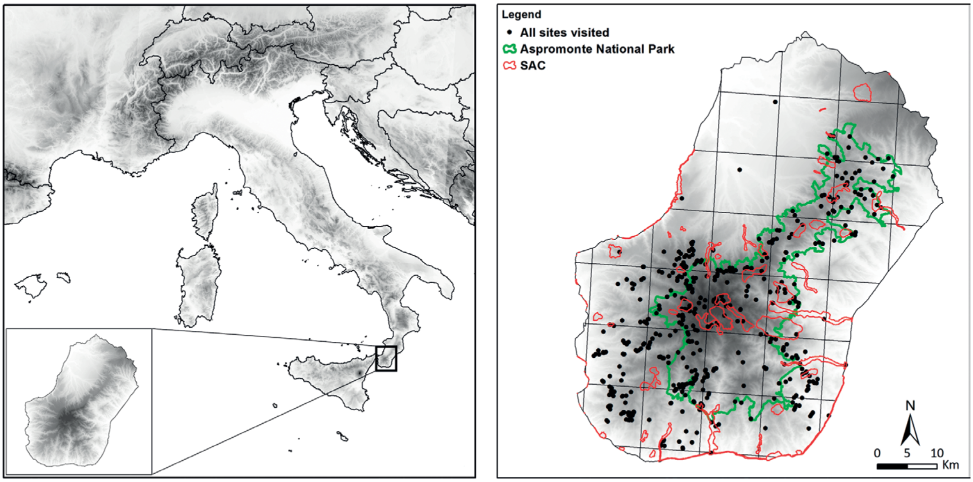

Figure 1. Geographic location of the study area. Location of the Aspromonte massif within the Italian peninsula (left), and the distribution of the sites investigated during the field survey (right); black dots indicate occurrences, green line indicates the ANP boundary, red lines indicate SAC boundaries.

the average summer temperatures exceed 40 °C in July and August. In general, the western and eastern sides are characterized by almost opposite microclimatic features, that is, wetter and cooler on the western side and warmer and drier on the eastern side. Brullo et al. (2001) identified two distinct macro-bioclimates in Aspromonte (divided into different thermotypes): a Mediterranean rain-seasonal oceanic bioclimate between 0 and 1,100 m asl and a temperate oceanic bioclimate between 1,100 and 1,957 m asl.

Climatic and orographic diversification have generated ecological gradients and highly heterogeneous vegetation typologies (Spampinato et al. 2008). In endemism, relict species, and ancient woods, the Aspromonte represents a hotspot of plant and animal biodiversity at the species, genetic, and community levels (Spampinato et al. 2008; Zampiglia et al. 2019; Piovesan et al. 2020). Biodiversity conservation within the Aspromonte region is undertaken by the Aspromonte National Park (ANP), together with 57 Natura 2000 areas, 55 of which are Special Areas of Conservation (SACs) and two are Special Protection Areas (SPAs).

Field survey

The identification of potential presence sites for all the investigated species was obtained by consulting the available literature (Ruffo and Stoch 2005; Lanza et al. 2007; and reference therein) and analyzing cartographic data. Cartographic data were retrieved from the Italian Military Geographic Institute (Military Geographic Institute) and satellite images to identify potential aquatic habitats. In line with other studies (e.g. Sindaco et al. 2006; Brandmayr et al. 2017), we considered the original standard grid defined by the European Community as a cartographic reference (reference system ETRS89-LAEA Europe - Lambert Azimuthal Equal Area projection), a universal transverse mercator (UTM) grid with cells of 10 × 10 km mesh (N = 45, 2.804 km^2) and WGS84 projection.

Field surveys were conducted from spring 2016 to autumn 2021, during the period of activity of the investigated species, focusing on the species' reproductive season, according to the phenology of each species, i.e. from March to October. We applied standard methodologies, such as the visual encounter survey (VES) and calling survey (CS), as reported by Heyer et al. (1990) and Sindaco (2016). We also followed all the relevant guidelines to avoid disease spreading during monitoring activities (Phillott et al. 2010). We examined adults, subadults, larvae, and eggs. Each site was visited three times. The field survey involved both natural habitats and artificial environments (Suppl. material 2: Table S2). Investigation of *B. pachypus* covered a large part of the Aspromonte Massif (19 UTM grids, for a total amount of 1,822 km^2) whereas investigations of the other three species were almost exclusively in the ANP and adjacent territories (18 UTM grids, for a total amount of 913 km^2). Each site was georeferenced using a GPS device and subsequently transferred to a geographic information system (GIS) platform (ArcMap 10.7.1, ESRI Inc., CA, USA). As *B. pachypus* and *S. terdigitata* are mentioned in Annexes II and IV of the EU Habitat Directive, we added abundance estimates of populations using visual counts for these two species. All procedures were approved by the Italian Ministry of Ecological Transition and the Italian National Institute for Environmental Protection and Research (ISPRA; permit number: 7727, 15-04-2016).

Data analysis

For each presence site, the following environmental information was retrieved: altitude from sea level using the DTM (Digital Terrain Model), with a resolution of 10 m; habitat type according to the CORINE Biotopes (European Commission 1991) and EUNIS codes (APAT 2004); and Horton order of river courses (Horton 1945). The environmental typology surrounding the sites was defined by buffer analysis (see e.g. Plăiaşu et al. 2012; Canessa et al. 2013) with a buffer radius of 100 m (3.14 ha). Each buffer area was superimposed on the land cover map (LC, De Fioravante et al. 2022) to generate an independent set of polygons representing land cover with a resolution of 10 m. The percentage of land cover within each area was calculated. Similarly, altimetry, exposure, and slope were obtained using the DTM layer, with a resolution of 10 m. Data visualization and analysis were performed using ArcMap 10.7.1.

To produce information for conservation management purposes, we estimated the following parameters: the diffusion index (DI, Ragni 2002), the distribution of species within the study area using the minimum convex polygon method (MCP, Mohr 1947), and the pattern of distribution using the average nearest neighbor analysis (ANN, Clark and Evans 1954). DI measures the ratio between the number of cells with the species occurrence out of the total number of cells investigated and spans from 0 (i.e., the species is not present in any cell) to 1 (i.e., the species is present in all the cells investigated). MCP generates a polygon whose angles are the outermost locations of the presence sites. Although MCP tends to overestimate home ranges by including areas not frequently visited by the species (Borger et al. 2006), we employed this method because it allows the identification of the boundaries of the species range in the study area (Burt 1943); the minimum boundary geometry tool was used to obtain MCP. The ANN is a method used to describe the spatial structure of occurrences (Pommerening et al. 2020). It determines the clustering of occurrences in the study area by measuring the distance between each location and its neighboring centroid location and then calculating the average among all the nearest neighbor distances. If the average distance is less than the average for a hypothetical random distribution, the distribution is considered clustered; if the average distance is greater than the hypothetical random distribution, the distribution is considered dispersed (Clark and Evans 1954).

Finally, we estimated species habitat selection in the study area using the Manly standardized habitat selectivity index (α) (Manly et al. 1972, 2002). This index represents the proportional use of a resource (or habitat) divided by the proportional availability of each habitat. The values of α range from 0 to 1: $\alpha = 1/k$, where k is the number of available resources, and indicates that the resource is used randomly and in proportion to the abundance in the environment; $\alpha > 1/k$ indicates positive selection of the resource; $\alpha < 1/k$ indicates resource avoidance. Habitats with the highest α were considered key habitats for the species (Desbiez et al. 2009).

Results

From 2016 to 2021, we visited 507 sites in 27 UTM cells (Fig. 1), corresponding to 46.66% of the study area (mean of 24.14 sites per cell). The investigation involved most of the ANP (245 sites, 48.2%) and 20 of the 55 SACs in the study area. The investigated species were observed in 337 of the 507 visited sites (66.46%), of which 220 were within the ANP; species occurrences were detected in 16 SACs (Suppl. material 2: Table S6). The distribution of species occurrences are summarized in Table 2 and Fig. 2.

Table 2. Summary of the species occurrences within the Aspromonte massif. DI: Diffusion Index; MCP: Minimum Convex Polygon; ANN: Average Nearest Neighbor; OBN; Observed Mean Distance; NNR: Nearest Neighbor Ratio; details in the main text.

Taxon	Occurrences	Presence cell	Altitude range	DI	MCP (Km^2)	ANN			
						OBN	NNR	z-score	p-value
Salamandra salamandra gigliolii	63	7	718–1774	0.33	239	968.43 m	0,986	-0,197	0,843
Salamandrina terdigitata	29	13	122–1573	0.62	821	3541.27 m	1,284	2,828	0,004
Bombina pachypus	84	17	51–1613	0.81	1191	1418 m	0,753	-4,328	0,000
Rana italica	161	16	118–1822	0.76	1002	944.40 m	0,74	-6,092	0,000

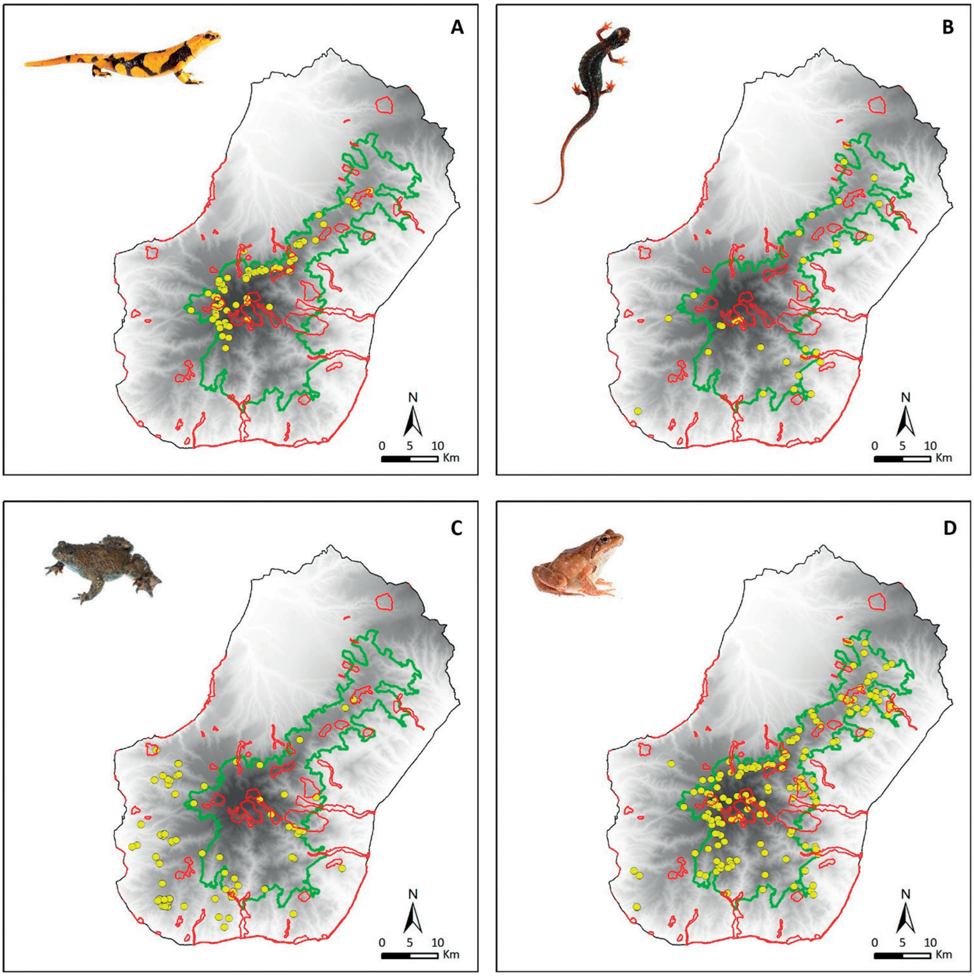

Figure 2. Location of occurrences for each species investigated in this study. Yellow dots indicate species occurrences, green line indicates the ANP boundary, red lines indicate SAC boundaries **A** *Salamandra salamandra gigliolii* **B** *Salamandrina terdigitata* **C** *Bombina pachypus* **D** *Rana italica*.

Fire salamander

Salamandra salamandra gigliolii was observed at 63 sites, mostly within the ANP (Fig. 2A, Suppl. material 1: Table S1). This species is mainly distributed along the western side of the mountain massif. The altitude and habitat distributions are summarized in Fig. 3 and Suppl. material 2: Tables S2–S5. The altitudinal distribution spanned from 718 to 1,774 m asl, with most observations occurring between 1,300 and 1,400 m asl (39%). The species was found almost exclusively in forest environments, such as beech forests (LC code 21115, 67.49%) and oro-

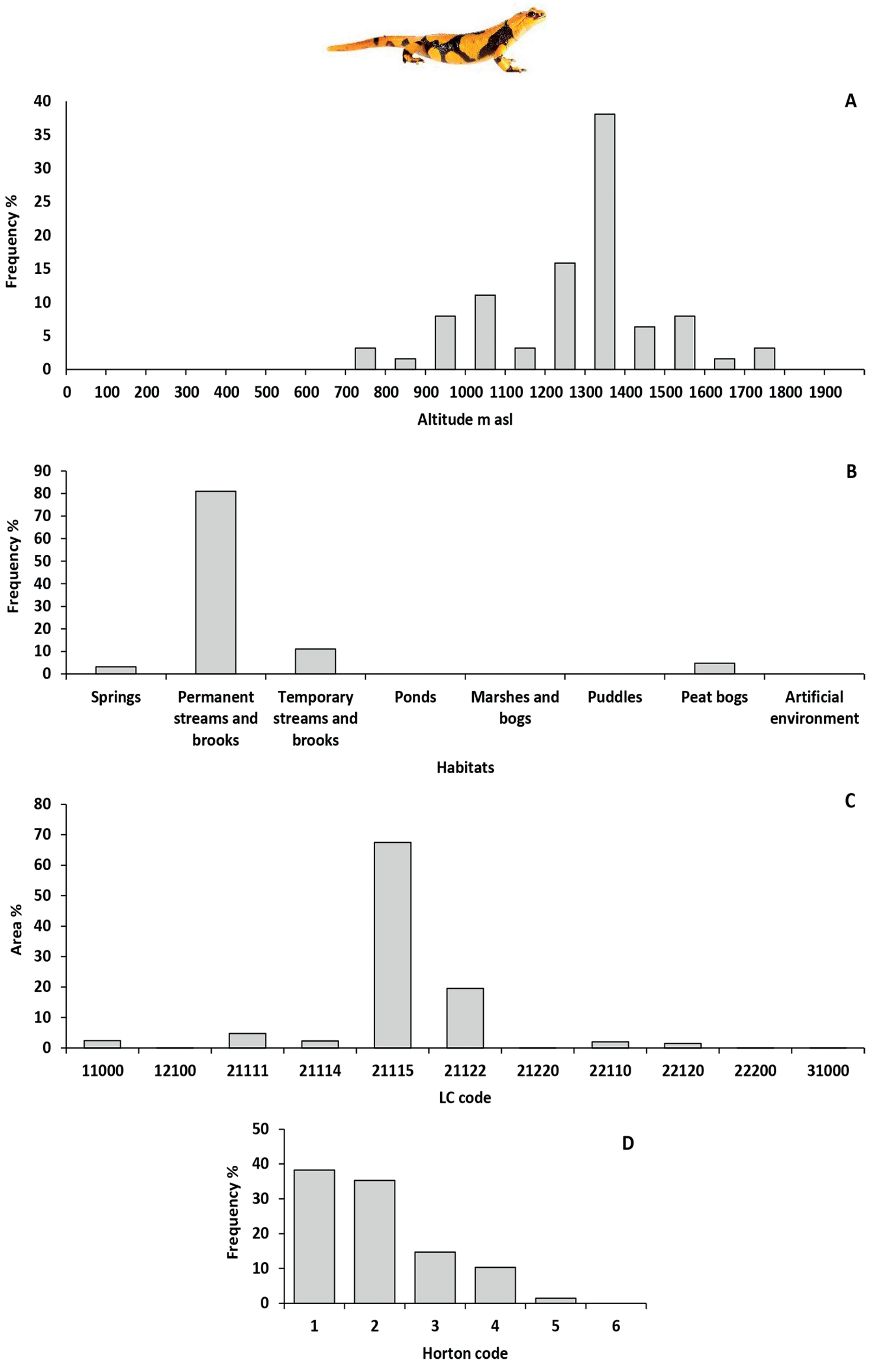

Figure 3. Frequency distribution of the *Salamandra salamandra gigliolii* occurrences for **A** altitude, **B** habitats, **C** land cover, and **D** Horton categories.

Mediterranean mountain pine forests (LC code 21122, 19.54%). Permanent streams and brooks represented the most frequented aquatic habitats (81%), with a few interesting observations in peat bogs (5%). The most frequent river courses belonged to Horton's orders 1 (38%) and 2 (35%). This species was found in seven SACs (Suppl. material 2: Table S6).

ANN analysis (Table 2) showed a random distribution pattern (NNR: 0.986; z-score: -0.197; p-value: 0.843). The Manly Index (Suppl. material 2: Table S7) suggests a species positive selection for altitude range between 1,200 and 1,500 m asl, slope classes of 0–15° and 15–30°, fresh sides affected by the Tyrrhenian humid currents (i.e., N, NW, and W exposure), beech forest and mountain pine forest habitats (LC codes 21115 and 21122, respectively).

Spectacled salamander

Salamandrina terdigitata was observed at 29 sites, most of which were within the ANP (Fig. 2B, Suppl. material 1: Table S1). The altitude and habitat distributions are summarized in Fig. 4 and Suppl. material 2: Tables S2–S5. The altitudinal distribution was from 122 to 1,573 m asl, with most observations below 700 m asl. The species mainly lives in forest environments, such as oak forests and broad-leaved woods (LC code 21111; 25.52%), beech woods (LC code 21115; 16.78%), and olive groves (LC code 21132; 13.96%). The species was mainly found in permanent streams and brooks (61%) and temporary streams and brooks (32%). The most frequent river course was Horton's order 4 (29%) and 3 (26%). This species was found at four SAC sites (Suppl. material 2: Table S6).

ANN analysis (Table 2) showed a dispersed distribution pattern (NNR: 1.284; z-score: 2.828; p = 0.004). The Manly Index (Suppl. material 2: Table S8) suggests a positive selection for altitudes of 100–200 m asl, 700–800 m, and 1,500 m above sea level, as well as a positive selection for slopes below 15° on cool and humid sides (i.e., W, NW, and E exposure). Interestingly, despite the extensive use of oak forests (LC code 21111) and beech forests (LC code 21115), the Manley index showed species positive selection for less available habitats, that is, orchards (LC code 21131) and chestnut (LC code 21114).

The number of observed individuals at each site during each visit spanned from 1 to approximately 1000: 1 to 7 adult individuals were observed at 10 sites; up to 50 individuals (larvae and/or adults) were observed at 15 sites; more than 50 individuals were observed at four sites, of which, at one we counted approximately 100 individuals (mainly larvae) and at another approximately 1000 larvae (see Suppl. material 1: Table S1).

Apennine yellow-bellied toad

Bombina pachypus was observed at 83 sites, most of which were outside the ANP (Fig. 2C, Suppl. material 1: Table S1). The altitude and habitat distributions are summarized in Fig. 5. The altitudinal distribution spanned from 51–1,613 m asl, with most observations occurring between 400–600 m asl (49%). The species mainly occupy

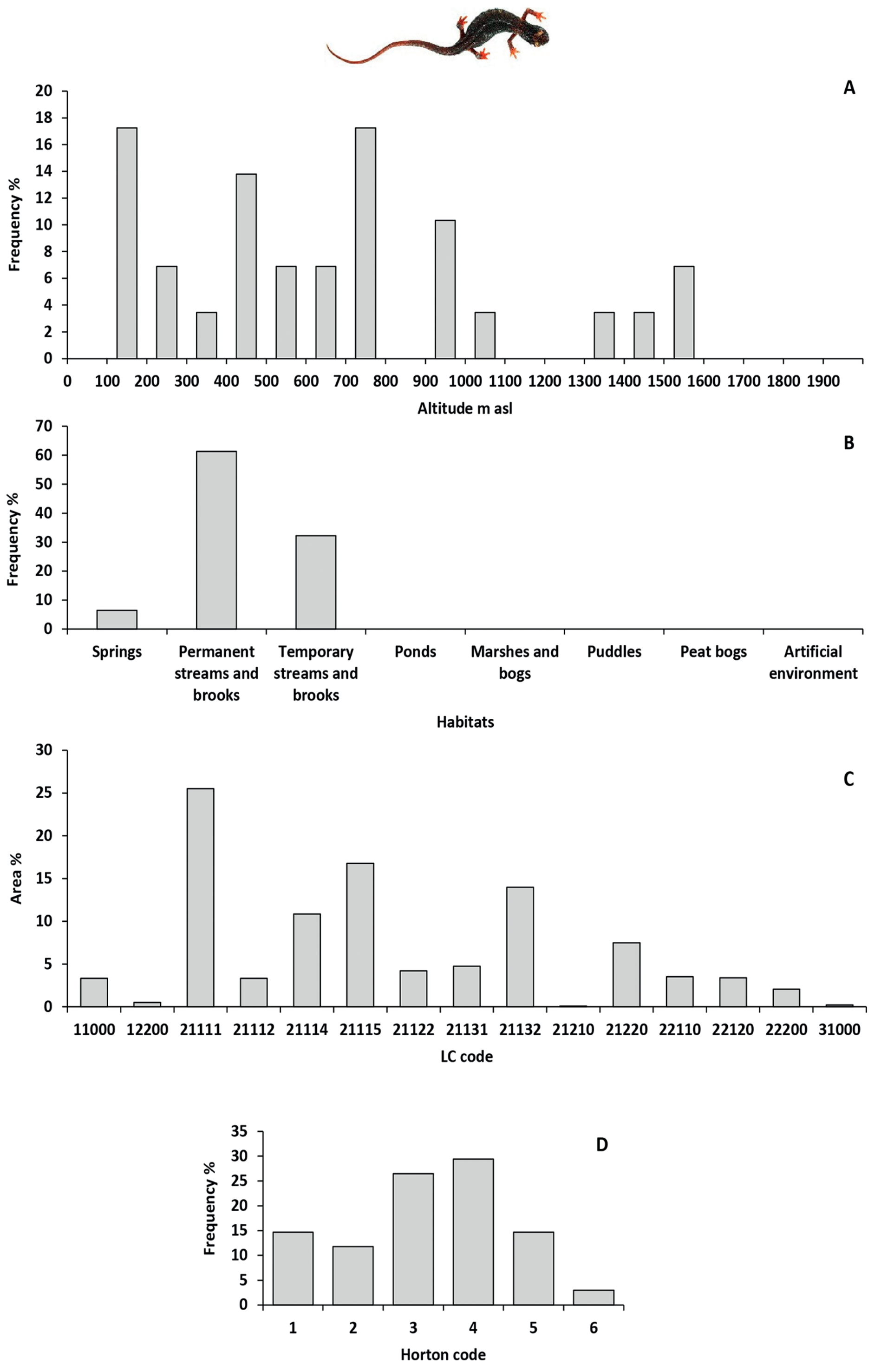

Figure 4. Frequency distribution of the *Salamandrina terdigitata* occurrences for **A** altitude, **B** habitats, **C** land cover, and **D** Horton categories.

open environments, such as pastures (LC code 22110–27.66%) and shrublands (LC code 21220–14.87%), but also oak woods and evergreen broad-leaved trees (LC code 2111–15.21%). Artificial environments were the most frequent aquatic habitat (64%). The species was found in four SACs (Suppl. material 2: Table S6).

ANN analysis (Table 2) showed a strongly aggregated distribution pattern (NNR: 0.753; z-score: -4.328; p-value < 0.001). The Manly Index (Suppl. material 2: Table S9) showed a general positive selection for altitudes between 400–1,100 m asl, slopes from 0° to 45°, for the sunniest sides (i.e., SE, W, SW, S, and NW exposures), as well as for pastures (LC code 22110), artificial abiotic surfaces (LC code 11000), and grassy soils (LC code 22200).

The number of observed individuals spanned from 1 to 120 adult individuals: up to 50 individuals were counted at 71 sites and more than 50 were observed at 12 sites, of which 5 showed more than 100 individuals. It is worth noting that *B. pachypus* was not found at 14 sites of historical presence, which have been checked at least three times each year.

Italian stream frog

Rana italica was observed at 161 sites, most of which were within the ANP (Fig. 2D, Suppl. material 1: Table S1). The altitude and habitat distributions are summarized in Fig. 6. The altitudinal distribution spanned from 118 to 1,822 m asl, with a peak of observations around 1,300 m asl (12%). The species has been frequently observed in beech forests (code LC 21115, 32.23%) and oak and evergreen broad-leaved tree forests (code LC 21111, 22.91%). The species has been mostly observed in permanent and temporary streams and river courses of 1–4 Horton's code. These species were observed in 14 SACs (Suppl. material 2: Table S6).

ANN analysis (Table 2) showed a strongly aggregated distribution pattern (NNR: 0.74; z-score: -6.092; p-value: 0.000). Inspection of the Manly Index (Suppl. material 2: Table S10) suggests a positive selection at an altitude of 100 m asl and from 1,200–1,500 m asl. Positive selection was also observed for slopes between 0–30° for wetter and cooler sides (i.e., N, NE, E, and NW exposure), deciduous oak forests (LC code 21112), and oro-Mediterranean and mountain pine forests (LC code 21122) (S7).

Discussion

Results from this survey update the information on distribution and conservation status of four threatened amphibian species inhabiting the Italian Peninsula. We focused on the southernmost portion of the Italian Peninsula, as it is an acknowledged hotspot of biodiversity within the Mediterranean region and one of the most unexplored regions of Europe in terms of amphibian ecology and conservation. We collected data on a large number of new and previously unknown breeding sites for the four investigated species, which depicts an encouraging frame on the conservation status of the populations in this area.

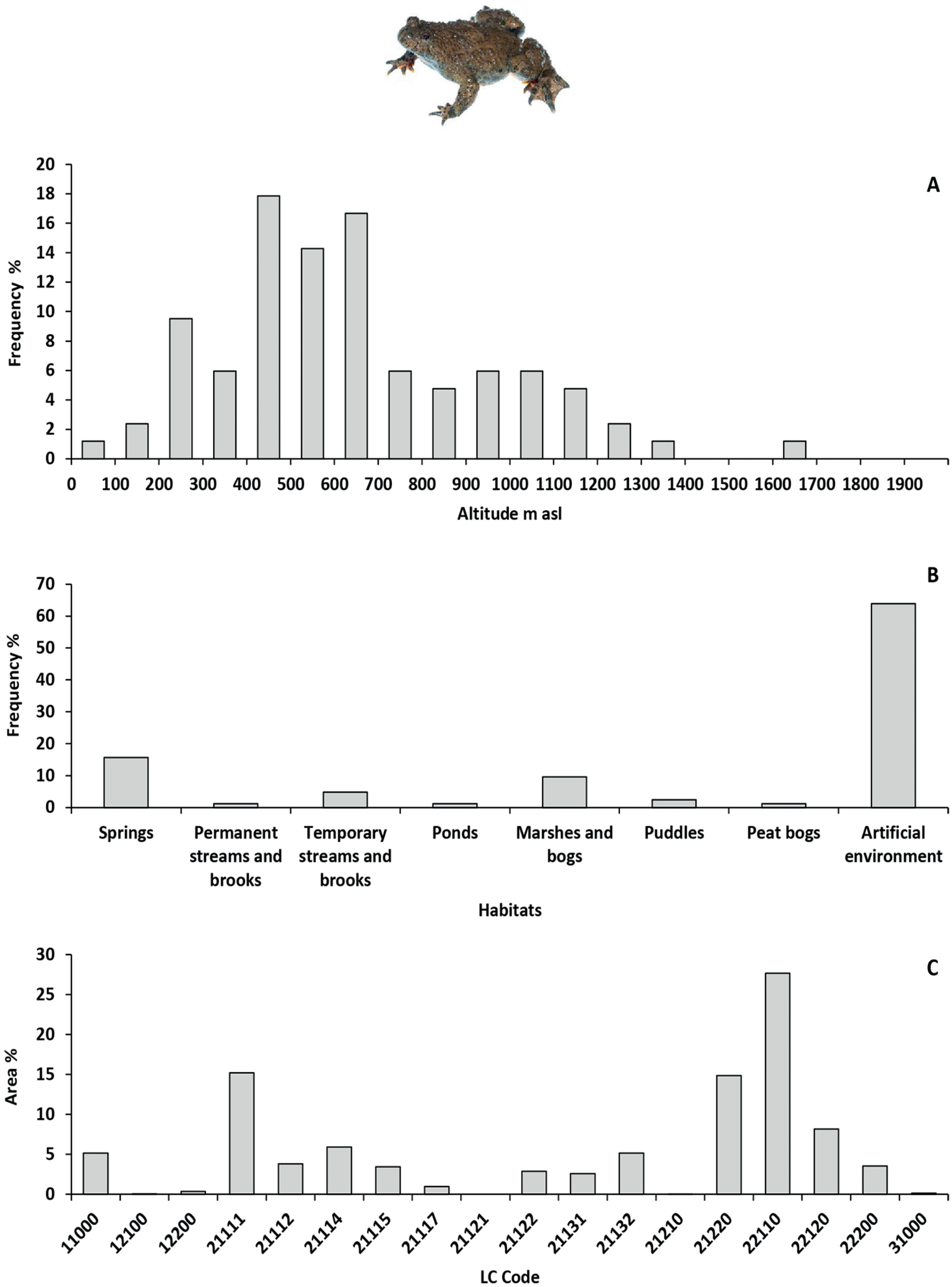

Figure 5. Frequency distribution of the *Bombina pachypus* occurrences for **A** altitude, **B** habitats, **C** land cover, and **D** Horton categories.

Overall, we found that these four species were widespread in the study area. The inspection of the distribution maps (Fig. 2) outlined *R. italica* as the most abundant species and *S. terdigitata* as the least abundant species. *S. terdigitata* also showed the

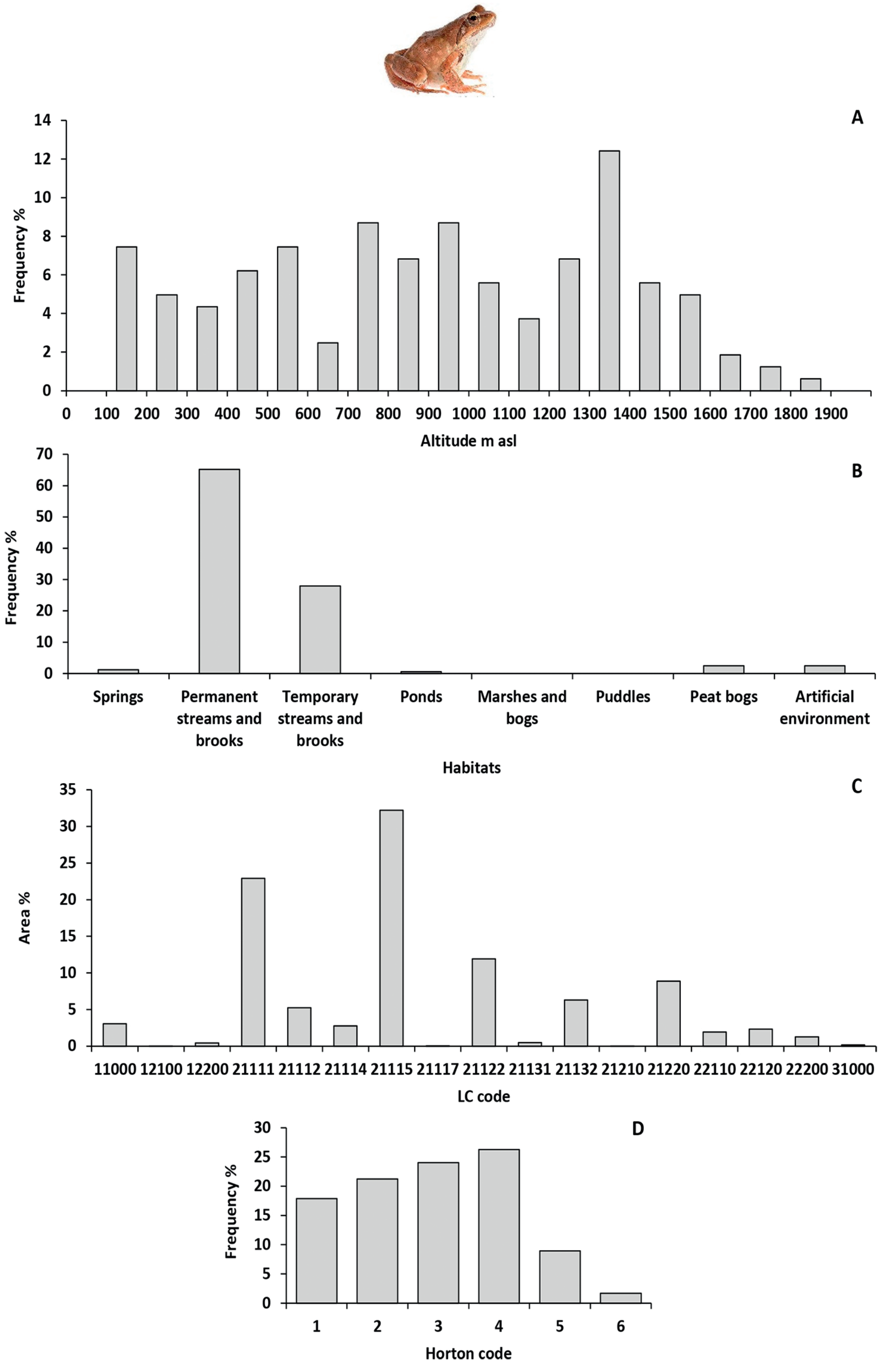

Figure 6. Frequency distribution of the *Rana italica* occurrences for **A** altitude, **B** habitats, **C** land cover, and **D** Horton categories.

most fragmented distribution, as supported by ANN analysis with a highly dispersed distribution pattern. These distribution patterns are in line with those shown by these species on the Italian Peninsula in previous studies (Lanza et al. 2007). By contrast, *S. s. gigliolii* and *B. pachypus* were more widespread in the Aspromonte Massif than in the northern regions of the Italian Peninsula (Lanza et al. 2007), despite their non-uniform distributions in the study area. Indeed, *S. s. gigliolii* was mainly distributed on the western side of the mountain massif, with only a few observations on the eastern side. This distribution reflects the needs of the species for cooler microclimate conditions, which are more common on the western and northwestern mountain sides in this region. Interestingly, the distribution map of *S. s. gigliolii* occurrences (Fig. 2A) highlighted strong contiguity of populations, as supported by ANN analysis, which excluded either clustered or dispersed patterns of distribution. Conversely, *B. pachypus* was more abundant in the southwestern part of the Aspromonte region, despite many observations having been localized to the eastern side of the ANP. Furthermore, inspection of the *B. pachypus* distribution (Fig. 2C) showed a strongly clustered distribution, as confirmed by ANN analysis. This pattern of distribution could reflect the patchy availability of the artificial environments colonized by *B. pachypus*, that is, irrigation tanks, drinking troughs, and artificial ponds, which currently constitute the species-preferred habitats (Fig. 5).

The habitat use of the four species in the Aspromonte Massif (Figs 3–6) agrees with available information on species ecology, although there were some relevant differences. *S. s. gigliolii* showed a marked preference for a higher altitudinal range in Aspromonte (1,200–1,300 m asl) compared to the rest of the peninsula (where the species shows a preference for altitudes around 800–1,000 m) (Lanza et al. 2007). This difference is even more marked in *R. italica*, which is commonly more frequent below 800 m asl (Lanza et al. 2007) but showed a peak frequency distribution around 1,300 m asl in Aspromonte. In contrast, *B. pachypus* showed a wider distribution at lower altitudes than the rest of the peninsula. Moreover, we found a population at 1,613 m asl, which, along with observations reported in the Pollino Massif (around 1,600 m asl: Barbieri et al. 2004; Talarico et al. 2004; Romano et al. 2012), is the highest record for *B. pachypus*. We also reported a wider range of habitats for *S. terdigitata*, suggesting a wider ecological niche of populations in Aspromonte than in the northernmost populations. Finally, our results emphasize the importance of preserving forests, which comprised the most frequented habitats of *S. terdigitata*, *S. s. gigliolii*, and *R. italica*.

The comparison of our data with those of previously published studies (Tripepi and Sperone 2007) highlights a general increase in species presence, especially for *B. pachypus* and *S. terdigitata*. This increase can be attributed to the wider geographic coverage of this survey, compared to previous surveys, rather than a real increase in species distribution. It is worth noting that the unavailability of exact locations from previous surveys does not allow for direct comparison and inferences on population trends. However, we visited 26 known historical sites for *B. pachypus* (see Zampiglia et al. 2019) and in 14 of them (54%), the presence of the species was not confirmed. A negative trend was also reported by local people (shepherds, farmers, woodcutters, and walkers), who confirmed that *B. pachypus* is less widespread now compared to the early 1990s when it was ubiquitous and abundant in hilly and mountain streams and ponds, as well as in artificial aquatic environments used for agriculture and local pastoralism. Our data suggest a change in species habitat suitability, as we only observed *B. pachypus* in five natural waterbodies (6% of the observations), with the majority of observations (53) occurring in artificial habitats: irrigated tanks (35), drinking troughs (15), and artificial ponds (3). Interestingly, the importance of artificial habitats, especially drinking troughs, in preserving amphibian populations has been recently raised for other Italian species in different regions (see Buono et al. 2019). This pattern necessitates the possible integration of amphibian conservation with rural activities in a sustainable development scenario. Finally, although our data outline a good conservation status of *B. pachypus* populations in Aspromonte compared to that in central and northern Italy, we report a negative trend in population that has to be accounted for by conservation stakeholders. This evidence, coupled with the expected reduction in habitat availability in the next 50 years due to climate change (Zampiglia et al. 2019) and the scarcity of populations enclosed in protected areas (Fig. 2C), makes clear the need for rapid and effective conservation actions for *B. pachypus* in the Aspromonte region.

Conclusions

This study provides further evidence of the vital role of the Aspromonte Massif in biodiversity conservation. In this area, all investigated species were found to be more widespread and relatively more abundant than in the central and northern parts of the Italian Peninsula (see, e.g. Vanni and Nistri 2006). The better conservation status of the populations in this area can be attributed to two main reasons. First, during the Anthropocene, human activity has been less intensive in this region than in the rest of Italy. Because of this, Aspromonte contains several patches of ancient forests which act as “biodiversity tanks” in the face of anthropogenic habitat degradation. Second, Aspromonte harbors a hotspot of genetic diversity for all four investigated species (Canestrelli et al. 2006a, 2006b, 2008; Mattoccia et al. 2011; Bisconti et al. 2018b; Iannella et al. 2018; Zampiglia et al. 2019). Because genetic diversity provides populations with the potential to adapt to environmental changes, hotspots of genetic diversity represent invaluable resources for species to adapt to global changes (Hampe and Petit 2005; Zachos and Habel 2011). Therefore, preserving populations within the Aspromonte region preserves populations with the highest potential to survive extinction threats that can be used for effective, genetically informed, translocation, and repopulation programs. Nevertheless, even though most of the populations were within the ANP boundaries, our data showed only a few occurrences within the Natura 2000 network. In particular, almost all observed populations of *B. pachypus*, one of the most endangered vertebrates in Italy, were outside protected areas. These results highlight the need for rapid implementation of new distribution data for these species in future conservation policies concerning the Aspromonte region. In this respect, it is worth mentioning that Aspromonte underwent several destructive fires over the last 10 years, many of which were considered human-induced. In particular, during the summer of 2021, after our field survey was complete, a large fire swept through 17.733 ha of the Aspromonte Massif, destroying 3,200 ha of forest, of which approximately 25 ha were ancient forests, with severe impacts on biodiversity. This fire event involved 34 of 337 sites in this study (10%), of which 18 were *B. pachypus* breeding sites (20% of *B. pachypus* breeding sites in this study). This event stresses the need to implement management methods that reduce the spread of fires and, thus, their impact on biodiversity, avoiding to set the hotspot on fire.

Acknowledgements

This paper is dedicated to the memory of Sergio Tralongo, who supported this research but suddenly passed away before seeing the results published. We thank Mauro Zampiglia, Paolo Nucera, Chiara Martino, Eugenio Potitò, Manuela Policastrese, and Emanuele Rosace for their help in the field survey activity. We also thank Antonio Barca, Francesco Manti, Giuseppe Bruzzaniti, Francesco d'Aleo and Valentina Laface for their advice and suggestions on potential breeding sites in the study area.

This study was funded by the Aspromonte National Park and The Italian Ministry of Education, University and Research.

References

Andreone F, Corti C, Sindaco R, Romano A, Giachi F, Vanni S, Delfino G (2009) *Bombina pachypus*. (Errata Version Published in 2016) The IUCN Red List of Threatened Species 2009: e.T54450A86629977. [Accessed on: 2022-02-01]

APAT (2004) Gli habitat secondo la nomenclatura EUNIS: manuale di classificazione per la realtà italiana. APAT, Rapporti 39/2004.

Barbieri F, Bernini F, Guarino FM, Venchi A (2004) Distribution and conservation status of *Bombina variegata* in Italy (Amphibia, Bombinatoridae). The Italian Journal of Zoology 71(sup002): 83–90. https://doi.org/10.1080/11250003.2004.9525565

Bisconti R, Aloise G, Siclari A, Fava V, Provenzano M, Arduino P, Chiocchio A, Nascetti G, Canestrelli D (2018a) Forest Dormouse (*Dryomys nitedula*) populations in southern Italy belong to a deeply divergent evolutionary lineage: implications for taxonomy and conservation. Hystrix, the Italian Journal of Mammalogy 29(1): 75–79. https://doi:10.4404/hystrix-00023-2017

Bisconti R, Porretta D, Arduino P, Nascetti G, Canestrelli D (2018b) Hybridization and extensive mitochondrial introgression among fire salamanders in peninsular Italy. Scientific Reports 8(1): 13187. https://doi.org/10.1038/s41598-018-31535-x

Blaustein AR, Kiesecker JM (2002) Complexity in conservation: Lessons from the global decline of amphibian populations. Ecology Letters 5(4): 597–608. https://doi.org/10.1046/j.1461-0248.2002.00352.x

Blaustein AR, Han BA, Relyea RA, Johnson PTJ, Buck JC, Gervasi SS, Kats LB (2011) The complexity of amphibian population declines: Understanding the role of cofactors in driving amphibian losses. Annals of the New York Academy of Sciences 1223(1): 108–119. https://doi.org/10.1111/j.1749-6632.2010.05909.x

Borger L, Franconi N, De Michele G, Gantz A, Meschi F, Manica A, Lovari S, Coulson T (2006) Effects of sampling regime on the mean and variance of home range size estimates. Journal of Animal Ecology 75(6): 1393–1405. https://doi.org/10.1111/j.1365-2656.2006.01164.x

Brandmayr P, Mazzei A, Aloise G, Sperone E, Tripepi S, Mazzei A, Storino P (2017) Protocolli di monitoraggio delle Rete Natura 2000 in Calabria. Specie animali. Azione D1 Progetto "LIFEPAN 2013 Life 13 NAT/IT/001075".

Brullo S, Scelsi F, Spampinato G (2001) La Vegetazione dell'Aspromonte. Studio fitosociologico. Laruffa editore. Reggio Calabria.

Buono V, Bissattini AM, Vignoli L (2019) Can a cow save a newt? The role of cattle drinking troughs in amphibian conservation. Aquatic Conservation: Marine and Freshwater Ecosystems 29(6): 964–975. https://doi.org/10.1002/aqc.3126.

Burt WH (1943) Territoriality e home-range concepts as applied to mammals. Journal of Mammalogy 24(3): 346–352. https://doi.org/10.2307/1374834

Canessa S, Oneto F, Ottonello D, Arillo A, Salvidio S (2013) Land abandonment may reduce disturbance and affect the breeding sites of an Endangered amphibian in northern Italy. Oryx 47(2): 280–287. https://doi.org/10.1017/S0030605311001542

Canestrelli D, Cimmaruta R, Costantini V, Nascetti G (2006a) Genetic diversity and phylogeography of the Apennine yellow-bellied toad *Bombina pachypus*, with implications for conservation. Molecular Ecology 15(12): 3741–3754. https://doi.org/10.1111/j.1365-294X.2006.03055.x

Canestrelli D, Zangari F, Nascetti G (2006b) Genetic evidence for two distinct species within the Italian endemic Salamandrina terdigitata Bonnaterre, 1789 (Amphibia: Urodela: Salamandridae). The Herpetological Journal 16: 221–227.

Canestrelli D, Cimmaruta R, Nascetti G (2008) Population genetic structure and diversity of the Apennine endemic stream frog, *Rana italica* - insights on the Pleistocene evolutionary history of the Italian peninsular biota. Molecular Ecology 17(17): 3856–3872. https://doi.org/10.1111/j.1365-294X.2008.03870.x

Canestrelli D, Aloise G, Cecchetti S, Nascetti G (2010) Birth of a hotspot of intraspecific genetic diversity: Notes from the underground. Molecular Ecology 19(24): 5432–5451. https://doi.org/10.1111/j.1365-294X.2010.04900.x

Canestrelli D, Sacco F, Nascetti G (2012) On glacial refugia, genetic diversity, and microevolutionary processes: Deep phylogeographical structure in the endemic newt *Lissotriton italicus*. Biological Journal of the Linnean Society. Linnean Society of London 105(1): 42–55. https://doi.org/10.1111/j.1095-8312.2011.01767.x

Canestrelli D, Zampiglia M, Nascetti G (2013) Widespread occurrence of *Batrachochytrium dendrobatidis* in contemporary and historical samples of the endangered *Bombina pachypus* along the Italian peninsula. PLoS ONE 8(5): e63349. https://doi.org/10.1371/journal.pone.0063349

Canestrelli D, Bisconti R, Sacco F, Nascetti G (2014) What triggers the rising of an intraspecific biodiversity hotspot? Hints from the agile frog. Scientific Reports 4(1): 1–9. https://doi.org/10.1038/srep05042

Catenazzi A (2015) State of the World's Amphibians. Annual Review of Environment and Resources 40(1): 91–119. https://doi.org/10.1146/annurev-environ-102014-021358

Chiocchio A, Colangelo P, Aloise G, Amori G, Bertolino S, Bisconti R, Castiglia R, Canestrelli D (2019) Population genetic structure of the bank vole Myodes glareolus within its glacial refugium in peninsular Italy. Zoological Systematics and Evolutionary Research 2019(4): 1–11. https://doi.org/10.1111/jzs.12289

Chiocchio A, Arntzen J, Martínez-Solano I, de Vries W, Bisconti R, Pezzarossa A, Maiorano L, Canestrelli D (2021) Reconstructing hotspots of genetic diversity from glacial refugia and subsequent dispersal in Italian common toads (*Bufo bufo*). Scientific Reports 11(1): 1–14. https://doi.org/10.1038/s41598-020-79046-y

Clark PJ, Evans FC (1954) Distance to Nearest Neighbor as a Measure of Spatial Relationships in Populations. Ecology 35(4): 445–453. https://doi.org/10.2307/1931034

Colacino M, Conte M, Piervitali E (1997) Elementi di climatologia della Calabria. IFA-CNR.

Collins JP (2010) Amphibian decline and extinction: What we know and what we need to learn. Diseases of Aquatic Organisms 92(2–3): 93–99. https://doi.org/10.3354/dao02307

Collins JP, Storfer A (2003) Global amphibian declines: Sorting the hypotheses. Diversity & Distributions 9(2): 89–98. https://doi.org/10.1046/j.1472-4642.2003.00012.x

Costa A, Dondero L, Allaria G, Morales Sanchez BN, Rosa G, Salvidio S, Grasselli E (2021) Modelling the amphibian chytrid fungus spread by connectivity analysis: Towards a national monitoring network in Italy. Biodiversity and Conservation 30(10): 2807–2825. https://doi.org/10.1007/s10531-021-02224-5

De Fioravante P, Strollo A, Assennato F, Marinosci I, Congedo L, Munafò M (2022) High Resolution Land Cover Integrating Copernicus Products: A 2012–2020 Map of Italy. Land 11(1): 35. https://doi.org/10.3390/land11010035

Desbiez AL, Bodmer RE, Santos R (2009) Wildlife habitat selection and sustainable resources management in a Neotropical wetland. International Journal of Biodiversity and Conservation 1: 011–020.

European Commission (1991) CORINE Biotopes manual, habitats of the European Community. A method to identify and describe consistently sites of major importance for nature conservation. EUR 12587/3. Office for Official publications of the European Communities, Luxembourg.

Fagotti A, Rossi R, Canestrelli D, La Porta G, Paracucchi R, Lucentini L, Simoncelli F, Di Rosa I (2019) Longitudinal study of Amphibiocystidium sp. infection in a natural population of the Italian stream frogs (*Rana italica*). Parasitology 146(07): 903–910. https://doi.org/10.1017/S0031182019000076

Grant E, Miller D, Schmidt B, Adams MJ, Amburgey SM, Chambert T, Cruickshank SS, Fisher RN, Green DM, Hossack BR, Johnson PTJ, Joseph MB, Rittenhouse TAG, Ryan ME, Waddle JH, Walls SC, Bailey LL, Fellers GM, Gorman TA, Ray AM, Pilliod DS, Price SJ, Saenz D, Sadinski W, Muths E (2016) Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. Scientific Reports 6(1): 25625. https://doi.org/10.1038/srep25625

Grasselli E, Bianchi G, Dondero L, Machiano V, Carafa M, Perrone M, Salvidio S (2019) First screening for *Batrachochytrium salamandrivorans* (Bsal) in wild and captive salamanders from Italy. Salamandra 55(2): 124–126.

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: The rear edge matters. Ecology Letters 8(5): 461–467. https://doi.org/10.1111/j.1461-0248.2005.00739.x

Heyer WR, Rand AS, Cruz CAG, Peixoto O, Nelson CE (1990) Frogs of Boraceia. Arquivos de Zoologia 31(4): 231–410.

Horton RA (1945) Erosional development of Streams and their Drainage Basins: Hydrophisical approach to Quantitative Morphology. Geological Society of America Bulletin 56(3): 275–370. https://doi.org/10.1130/0016-7606(1945)56[275:EDOSAT]2.0.CO;2

Iannella M, D'Alessandro P, Biondi M (2018) Evidences for a shared history for spectacled salamanders, haplotypes and climate. Scientific Reports 8(1): 1–11. https://doi.org/10.1038/s41598-018-34854-1

Italica S (2011) Assessing the status of amphibian breeding sites in Italy: A national survey. Acta Herpetologica 6(1): 119–126. https://doi.org/10.13128/Acta_Herpetol-9584

Lanza B, Andreone F, Bologna MA, Corti C, Razzetti E (2007) Amphibia – Fauna d'Italia, Vol. XLII. edn. Calderini, Bologna.

Manly BFJ, Miller P, Cook L (1972) Analysis of a selective predation experiment. American Naturalist 106(952): 719–736. https://doi.org/10.1086/282808

Manly BFJ, McDonald LL, Thomas DL, McDonald TL, Erickson WP (2002) Resource selection by animals - Statistical design and analysis for field studies. 2nd edn., London, Kluwer Boston, Massachusetts, 222 pp.

Mattoccia M, Marta S, Romano A, Sbordoni V (2011) Phylogeography of an Italian endemic salamander (genus *Salamandrina*): *Glacial refugia*, postglacial expansions, and secondary contact. Biological Journal of the Linnean Society. Linnean Society of London 104(4): 903–922. https://doi.org/10.1111/j.1095-8312.2011.01747.x

McCallum ML (2007) Amphibian decline or extinction? Current declines dwarf background extinction rate. Journal of Herpetology 41(3): 483–491. https://doi.org/10.1670/0022-1511(2007)41[483:ADOECD]2.0.CO;2

Mohr C (1947) Table of equivalent populations of North American small mammals. American Midland Naturalist 37(1): 223–249. https://doi.org/10.2307/2421652

Phillott AD, Speare R, Hines HB, Skerratt LF, Meyer E, McDonald KR, Cashins SD, Mendez D, Berger L (2010) Minimising exposure of amphibians to pathogens during field studies. Diseases of Aquatic Organisms 92(2–3): 175–185. https://doi.org/10.3354/dao02162

Piovesan G, Baliva M, Calcagnile L, D'Elia M, Dorado-Liñán I, Palli J, Siclari A, Quarta G (2020) Radiocarbon dating of Aspromonte sessile oaks reveals the oldest dated temperate flowering tree in the world. Ecology 101(12): e03179. https://doi.org/10.1002/ecy.3179

Plăiaşu R, Băncilă R, Samoilă C, Hartel T, Cogălniceanu D (2012) Waterbody availability and use by amphibian communities in a rural landscape. The Herpetological Journal 22: 13–21.

Pommerening A, Szmyt J, Zhang G (2020) A new nearest-neighbour index for monitoring spatial size diversity: The hyperbolic tangent index. Ecological Modelling 435: 109232. https://doi.org/10.1016/j.ecolmodel.2020.109232

Puschendorf R, Bolaños F, Chaves G (2006) The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. Biological Conservation 132(1): 136–142. https://doi.org/10.1016/j.biocon.2006.03.010

Ragni B (2002) Atlante dei mammiferi dell'Umbria. Petruzzi Editore, Città di Castello (PG), 223 pp.

Romano A, Bartolomei R, Conte A, Fulco E (2012) Amphibians in Southern Apennine: Distribution, ecology and conservation notes in the "Appennino Lucano, Val d'Agri e Lagonegrese" National Park (Southern Italy). Acta Herpetologica 7(2): 203–219. https://doi.org/10.13128/Acta_Herpetol-10503

Rondinini C, Battistoni A, Peronace V, Teofili C (2013) Lista Rossa IUCN dei Vertebrati Italiani. Comitato Italiano IUCN, Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma.

Ruffo S, Stoch F [Eds] (2005) Checklist e distribuzione della fauna italiana. Memorie del Museo Civico di Storia Naturale di Verona, 2. serie, Sezione Scienze della Vita 16.

Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, Acevedo AA, Burrowes PA, Carvalho T, Catenazzi A, De la Riva I, Fisher MC, Flechas SV, Foster CN, Frías-Álvarez P, Garner TWJ, Gratwicke B, Guayasamin JM, Hirschfeld M, Kolby JE, Kosch TA, La Marca E, Lindenmayer DB, Lips KR, Longo AV, Maneyro R, McDonald CA, Mendelson J 3rd, Palacios-Rodriguez P, Parra-Olea G, Richards-Zawacki CL, Rödel MO, Rovito SM, Soto-Azat C, Toledo LF, Voyles J, Weldon C, Whitfield SM, Wilkinson M, Zamudio KR, Canessa S (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363(6434): 1459–1463. https://doi.org/10.1126/science.aav0379

Sindaco R (2016) In: Stoch F, Genovesi P (Eds) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida, 141/2016.

Sindaco R, Doria G, Razzetti E, Bernini F (2006) Atlante degli Anfibi e Rettili d'Italia. Atlas of Amphibians and Reptiles in Italy. SHI, Edizioni Polistampa, Firenze.

Spampinato G, Cameriere P, Caridi D, Crisafulli A (2008) Carta della biodiversità vegetale del Parco Nazionale dell'Aspromonte. (Italia Meridionale). Quaderni di Botanica Ambientale e Applicata 19(3–36): 8–12.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306(5702): 1783–1786. https://doi.org/10.1126/science.1103538

Talarico E, Sperone E, Tripepi S (2004) Amphibians of the Pollino National Park: Distribution and notes on conservation. The Italian Journal of Zoology 71(sup2): 203–208. https://doi.org/10.1080/11250000409356636

Temi Srl, Agristudio Srl (2018) La Rete Natura 2000 nel Parco Nazionale dell'Aspromonte. Analisi dello stato attuale e stesura delle Misure di conservazione dei Siti della Rete Natura 2000 ricompresi interamente o parzialmente nel Parco Nazionale dell'Aspromonte.

Temple HJ, Cox NA (2009) European Red List of Amphibians. Office for Official Publications of the European Communities, Luxembourg.

Todisco V, Gratton P, Cesaroni D, Sbordoni V (2010) Phylogeography of *Parnassius apollo*: Hints on taxonomy and conservation of a vulnerable glacial butterfly invader. Biological

Journal of the Linnean Society. Linnean Society of London 101(1): 169–183. https://doi.org/10.1111/j.1095-8312.2010.01476.x
Tripepi S, Sperone E (2007) Monitoraggio degli ambienti acquatici del Parco Nazionale dell'Aspromonte ai fini della salvaguardia degli anfibi. Relazione finale.
Tripepi S, Carelli B, Giardinazzo E, Sperone E, Serroni P (1999) Distribuzione degli Anfibi nella provincia di Reggio Calabria: dati preliminari. In: Tripepi S (Ed.) Atti II° Convegno Nazionale "Societas Herpetologica Italica" (Praia a Mare, 6–10 ottobre 1998), Rivista di Idrobiologia, 38, 1/2/3, 141–149.
Vanni S, Nistri A (2006) Atlante degli Anfibi e dei Rettili della Toscana. Regione Toscana, Università degli Studi di Firenze, Museo di Storia Naturale, Sezione Zoologica "La Specola", Firenze, 379 pp.
Zachos FE, Habel JC (2011) [Eds]: Biodiversity hotspots – distribution and protection of conservationpriority areas. Springer, Berlin, 546 pp. https://doi.org/10.1007/978-3-642-20992-5
Zampiglia M, Canestrelli D, Chiocchio A, Nascetti G (2013) Geographic distribution of the chytrid pathogen *Batrachochytrium dendrobatidis* among mountain amphibians along the Italian peninsula. Diseases of Aquatic Organisms 107(1): 61–68. https://doi.org/10.3354/dao02655
Zampiglia M, Bisconti R, Maiorano L, Aloise G, Siclari A, Pellegrino F, Martino G, Pezzarossa A, Chiocchio A, Martino C, Nascetti G, Canestrelli D (2019) Drilling down hotspots of intraspecific diversity to bring them into on-ground conservation of threatened species. Frontiers in Ecology and Evolution 7: 205. https://doi.org/10.3389/fevo.2019.00205

Supplementary material 1

Table S1

Authors: Giuseppe Martino, Andrea Chiocchio, Antonino Siclari, Daniele Canestrelli

Data type: Occurrences.

Explanation note: List of the 337 species occurrences.

Link: https://doi.org/10.3897/natureconservation.50.86002.suppl1

Supplementary material 2

Tables S1–S10

Authors: Giuseppe Martino, Andrea Chiocchio, Antonino Siclari, Daniele Canestrelli

Data type: Docx file

Explanation note: **Table S2.** Habitat distribution for each studied species (RF - Relative Frequency). The correspondence to the habitats of Annex 1 of the Habitats Directive is specified (HD). **Table S3.** Land cover distribution in study area and for each studied species. **Table S4.** Altitude distribution for each studied species (RF - Relative Frequency). **Table S5.** Horton order distribution for each studied species (RF - Relative Frequency). **Table S6.** SAC (Special Area of Conservation - Habitat Directive) with presence of species studied. **Table S7.** Manly index of *Salamandra salamandra gigliolii* for environmental variables considered. **Table S8.** Manly index of *Salamandrina terdigitata* for environmental variables considered. **Table S9.** Manly index of *Bombina pachypus* for environmental variables considered. **Table S10.** Manly index of *Rana italica* for environmental variables considered.

Link: https://doi.org/10.3897/natureconservation.50.86002.suppl2