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Running head: Amphibians conservation status in southern Italy

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 endangered 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 between 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* occupancy in its natural habitats, which was related to a negative trend of populations. Overall, our results provide an encouraging framework for the conservation of amphibian populations in this area, but highlight the low coverage of endangered amphibian populations in protected areas, claiming for a reassessment of conservation policies and spatial conservation planning for the Aspromonte region.

Keywords: Apennine yellow-bellied toad, amphibian decline, 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, Collins 2010; Stuart et al. 2004, 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 at threat categories of the International Union for Conservation of Nature European Red List (Temple and Cox 2009, Sindaco 2016). Despite the extinction rate in amphibians exceeding that of any other group of vertebrates (Stuart et al. 2004, McCallum 2007), the conservation community has 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 (*Bombina pachypus*), the Italian stream frog (*Rana italica*), the fire salamander (*Salamandra salamandra gigliolii*) and the spectacled salamander (*Salamandrina terdigitata* and *S. perspicillata*) (Barbieri et al. 2004, Lanza et al. 2007, Andreone et al. 2009). Despite the widespread decline of amphibians in Italy (Barbieri et al. 2004, Lanza et al. 2007, Andreone et al. 2009), monitoring schemes aimed at establishing demographic trends and conservation statuses are scattered and mostly limited to a few populations. 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 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). 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 to characterize the distribution and conservation status of the endangered amphibians inhabiting the Aspromonte Massif, a mountain region in the southernmost part of the Italian Peninsula (Tab. 1). The Aspromonte Massif is an extended and heterogeneous mountain region surrounded by the sea and characterized by a high diversity of habitats spanning from dry grasslands and shrublands to oak, pine, and beech forests, and including also 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 2010), including amphibians (Iannella 2018, Canestrelli et al. 2006a). 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 endangered amphibians, suggest 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 endangered amphibians in southern Italy. To achieve this, we apply a fine-scale field survey to map the current presence of endangered amphibians in the Aspromonte region. We will focus on four amphibian species that are endemic to the Italian Peninsula and 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).

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 (Figure 1) and characterized by a high diversity of habitats and peculiar 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. In the hilly and coastal strips, most humid habitats show dependence from 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, the average summer temperatures are high, exceeding 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. (2021) identified two distinct macro-bioclimates in Aspromonte (then divided them 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. 2009). 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. 2009, 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 (SAC) and two are Special Protection Areas (SPA).

Field survey

The identification of potential presence sites for all the investigated species was obtained by consulting the available literature (Stoch 2000-2005, 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. We applied standard methodologies, such as the visual encounter survey (VES) and calling survey (CS), as reported by Heyer et al. (1994) and Sindaco (2016). We examined adults, subadults, larvae, and eggs. Each site was visited three times. The field survey involved both species natural habitats and artificial environments (Table S2). Investigation of *B. pachypus* covered a large part of the Aspromonte Massif (UTM grids = 19.1822 km^2) whereas investigations of the other three species were almost exclusively in the ANP and adjacent territories (UTM grids = 18, 913 km). 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, for these two species, we added abundance estimates of populations using visual counts. 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 (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 average nearest neighbor is a method used to describe the spatial structure of occurrences (Pommerening 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: $\alpha = 1/k$ 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;

α <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 21 UTM cells (Figure 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 SAC 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 SAC (Table S6). The distribution of species occurrences are summarized in Table 2 and Figure 2.

Fire salamander

Salamandra salamandra gigliolii was observed at 63 sites, mostly within the ANP (Figure 2A, Table S1). This species is mainly distributed along the western side of the mountain massif. The altitude and habitat distributions are summarized in Figure 3 and Table S2-S5. The altitudinal distribution spanned from 718 to 1.774 m asl, with most observations occurring between 1300 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-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 order 1 (38%) and order 2 (35%). This species was found in seven SAC (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 (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 (Figure 2B, Table S1). The altitude and habitat distributions are summarized in Figure 4 and Table S2-S5. The altitudinal distribution was from 122–1.573 m asl, with most observations being below 700 m asl. The species mainly occupies 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 (Table S6).

ANN analysis (Table 2) showed a dispersed distribution pattern (NNR: 1.284; z-score: 2.828; p = 0.004). The Manly Index (Table S8) suggests a positive selection for altitudes of 100–200 m asl, 700–800 m, and 1500 m above sea level, as well as positive selection for slopes below 15° on cool and humid sides (i.e., W, NW, and E exposure). Interestingly, despite the large 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.

Apennine yellow-bellied toad

Bombina pachypus was observed at 83 sites, most of which were outside the ANP (Figure 2C, Table S1). The altitude and habitat distributions are summarized in Figure 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 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 at seven SAC (Table S6).

ANN analysis (Table 2) showed a strongly aggregated distribution pattern (NNR: 0.753; z-score: -4.328; p-value: 0.000). The Manly Index (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.

Italian stream frog

Rana italica was observed at 161 sites, most of which were within the ANP (Figure 2D, Table S1). The altitude and habitat distributions are summarized in Figure 6. The altitudinal distribution spans 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 SAC (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 (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 updates the information on distribution and conservation status of four endangered 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.

Overall, we found that these four species were widespread in the study area. The inspection of the distribution maps (Figure 2) outlined *R. italica* as the most abundant species and *S. terdigitata* as the least abundant species. *S. terdigitata* also showed the 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. salamandra* 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 (Figure 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 were localized to the eastern side of the ANP. Furthermore, inspection of the *B. pachypus* distribution (Figure 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 (Figure 5).

The habitat use of the four species in the Aspromonte Massif (Figure 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. We also 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), is the highest record for *B. pachypus*. We also reported a wider range of habitats for *S. terdigitata*, suggesting a wider ecological tolerance 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 that of previous surveys, rather than an 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 watercourses (6% of the observations), with the majority of observations (53) occurring in artificial habitats: irrigated tanks (35), drinking troughs (15), and artificial ponds (3). Thus, despite 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 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 (Figure 2C), makes clear the need for rapid and effective conservation actions for *B. pachypus* in the Aspromonte region.

Conclusions

This study provides further evidence on 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; Bisconti et al., 2018b; Iannella 2018; Zampiglia et al. 2019; Mattoccia et al. 2011). 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 located 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 during 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.

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Competing interests

The Authors have declared no competing interests

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Figures and tables

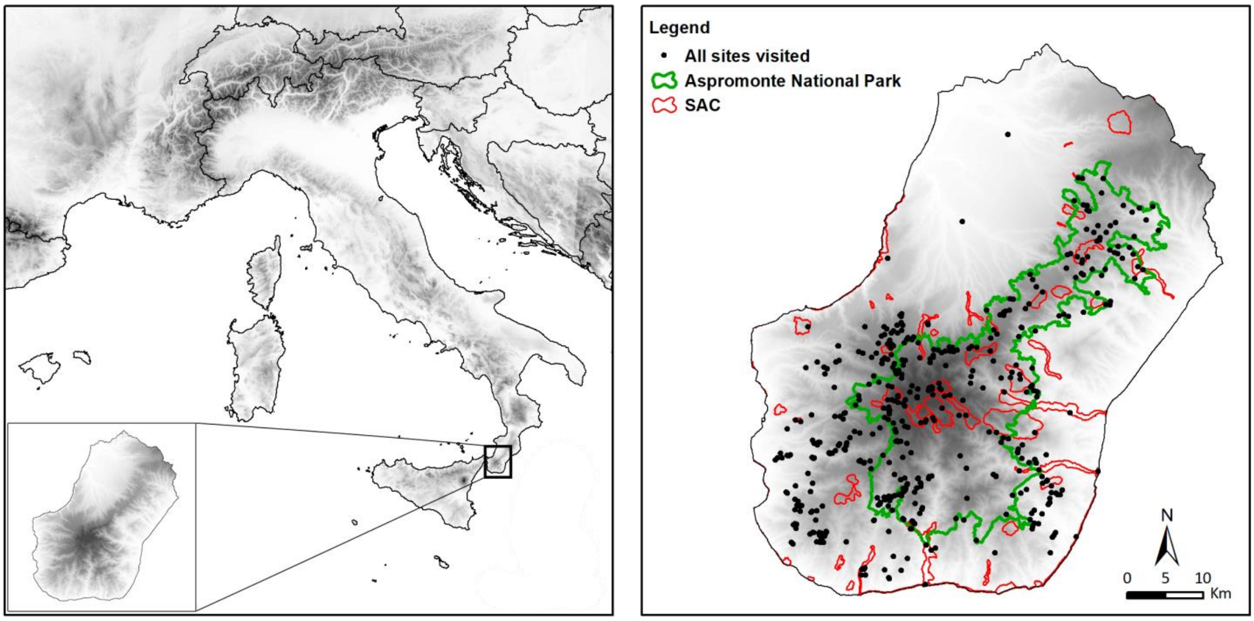

Figure 1 – Geographic location of the study area. Location of the Aspromonte massif within the Italian peninsula (**left**), and the distribution of the sites investigated during the field survey (**right**); black dots indicate occurrences, green line indicates the ANP boundary, red lines indicate SAC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

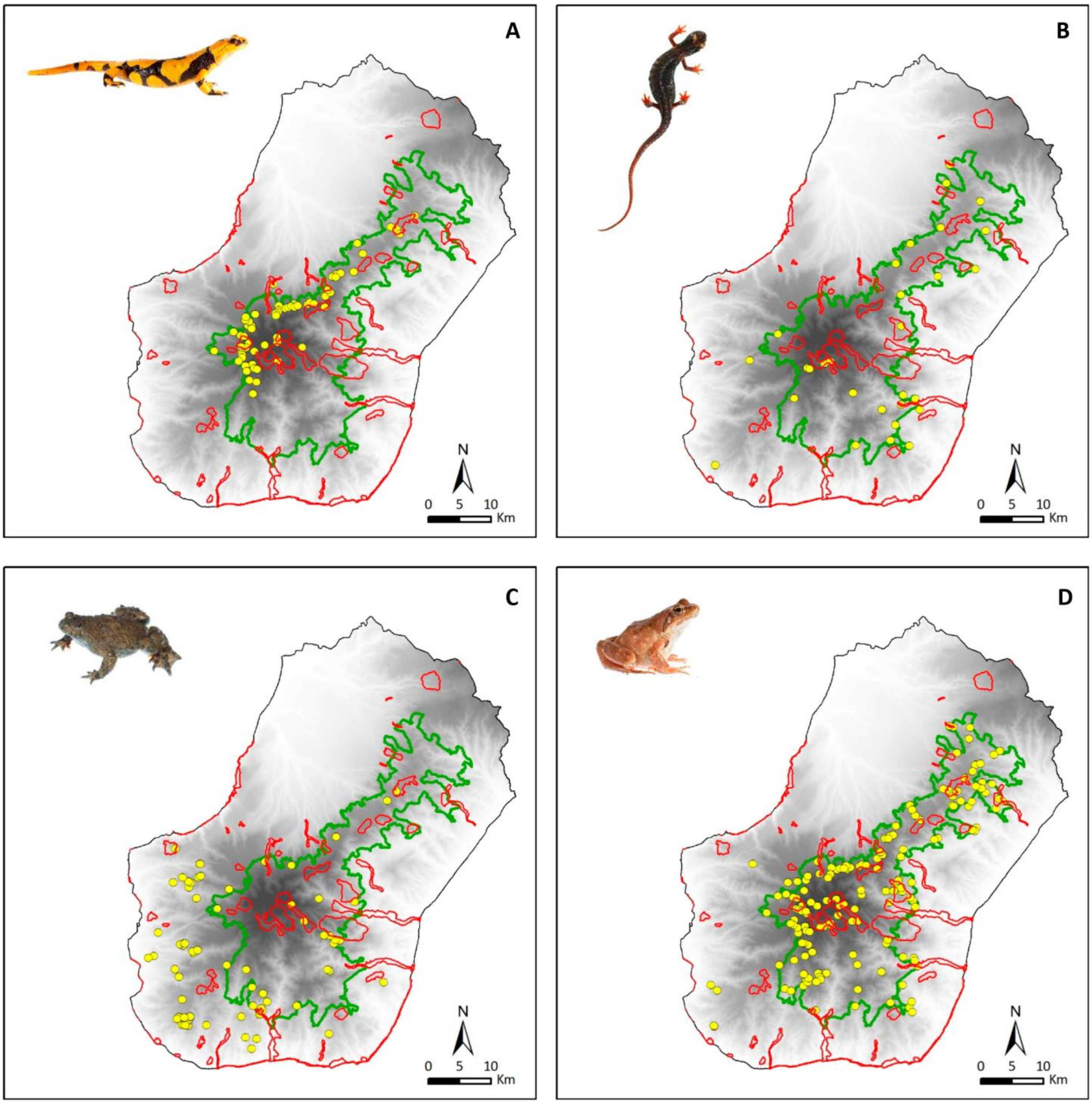

Figure 2 - Location of occurrences for each species investigated in this study. Yellow dots indicate species occurrences, green line indicates the ANP boundary, red lines indicate SAC boundaries. A: *Salamandra salamandra gigliolii*; B: *Salamandrina terdigitata*; C: *Bombina pachypus*; D: *Rana italica*.

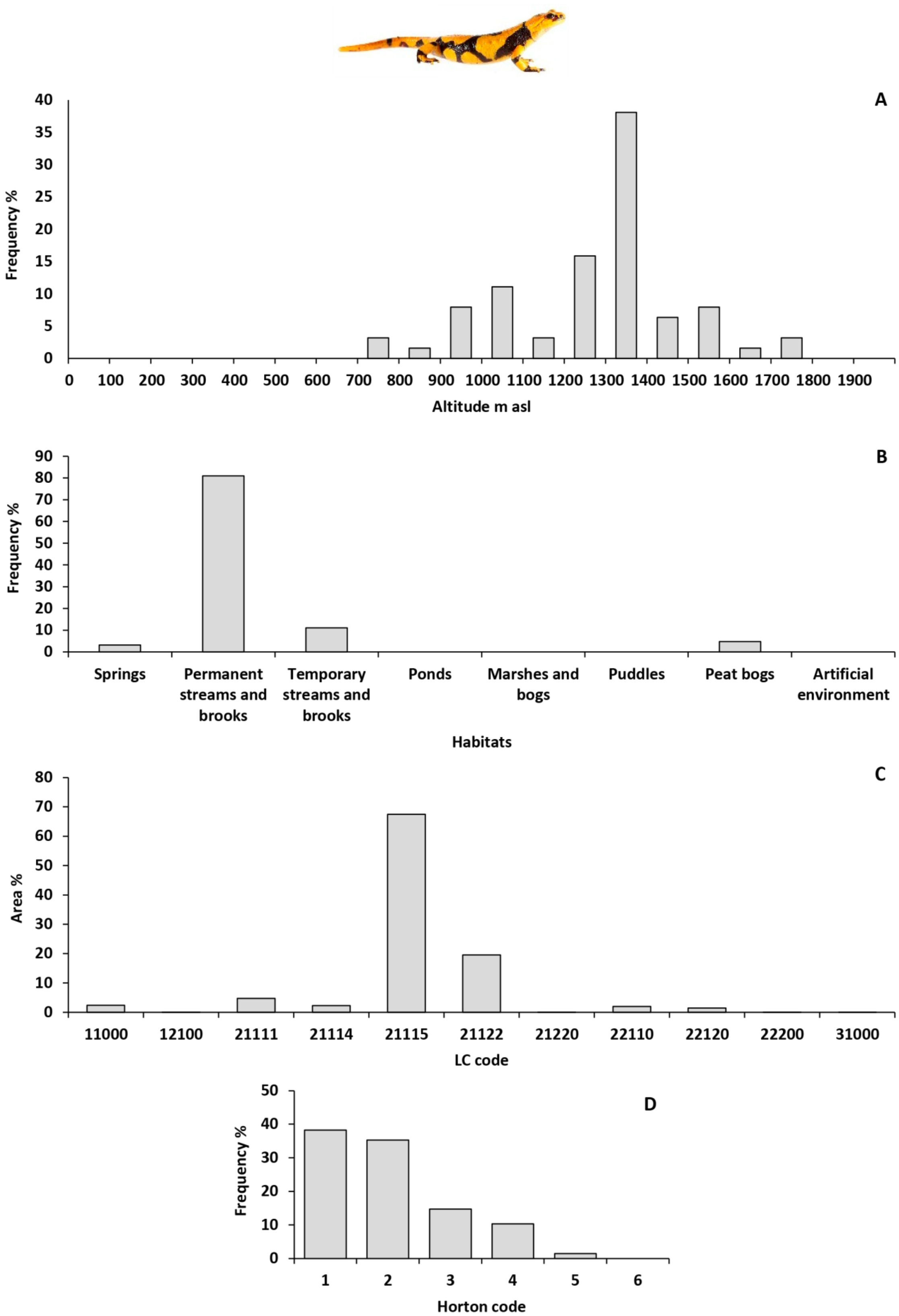

Figure 3. Frequency distribution of the *Salamandra salamandra gigliolii* occurrences for (A) altitude, (B) habitats, (C) land cover, and (D) Horton categories.

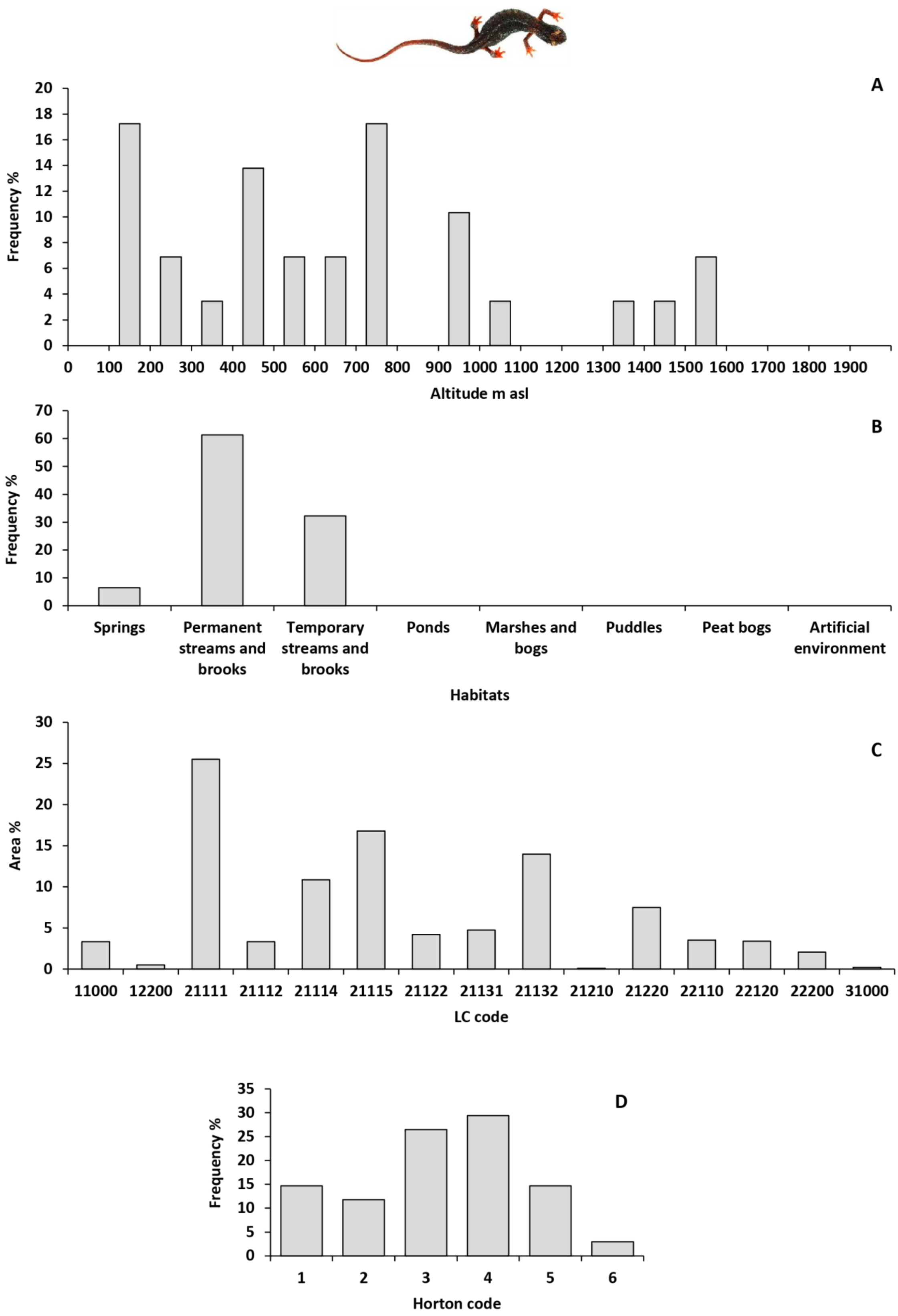

Figure 4 - Frequency distribution of the *Salamandrina terdigitata* occurrences for (A) altitude, (B) habitats, (C) land cover, and (D) Horton categories.

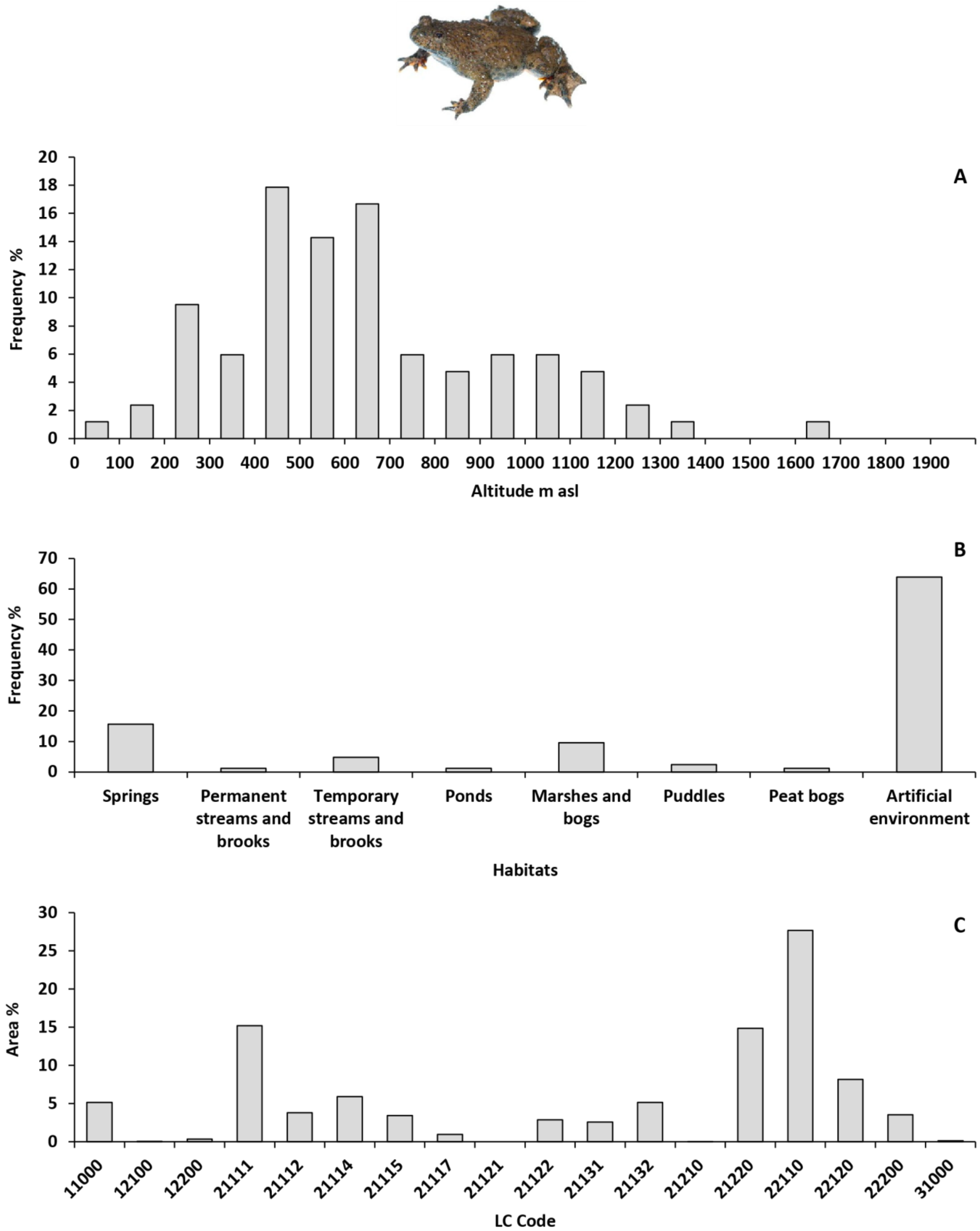

Figure 5 - Frequency distribution of the *Bombina pachypus* occurrences for (A) altitude, (B) habitats, (C) land cover, and (D) Horton categories.

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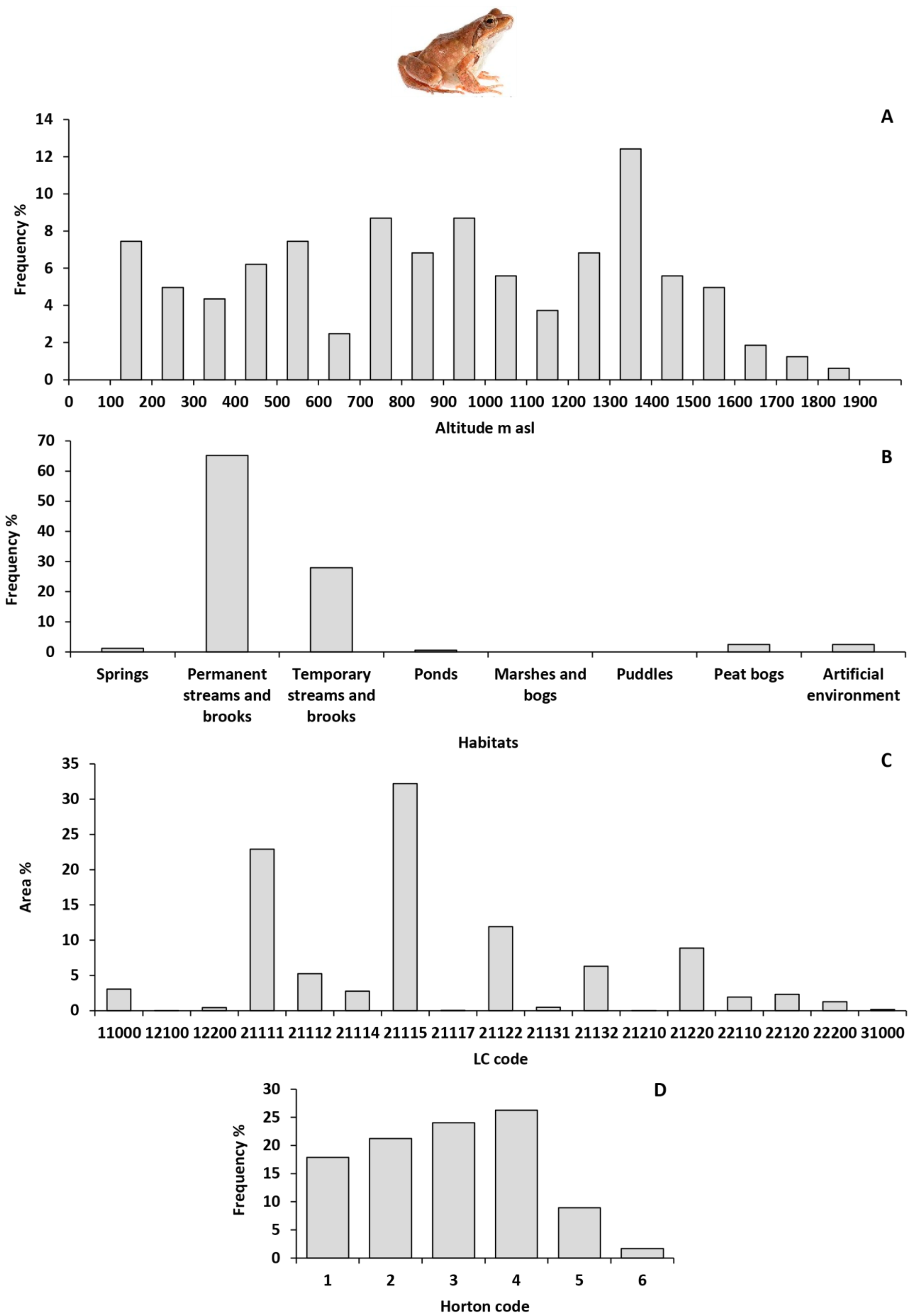

Figure 6 - Frequency distribution of the *Rana italica* occurrences for (A) altitude, (B) habitats, (C) land cover, and (D) Horton categories.

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			IV	Yes[3,5]

643 **Table 1** - Conservation status of the taxa investigated in this study. [1]Costa et al., 2021; [2]Grasselli et
644 al., 2019; [3]Zampiglia et al., 2013; [4]Canestrelli et al., 2013; [5]Zampiglia et al., 2019; [5]Fagotti et al.,
645 2019.

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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

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

650