

# The distribution of amphibians in the Matese Massif of southern Italy

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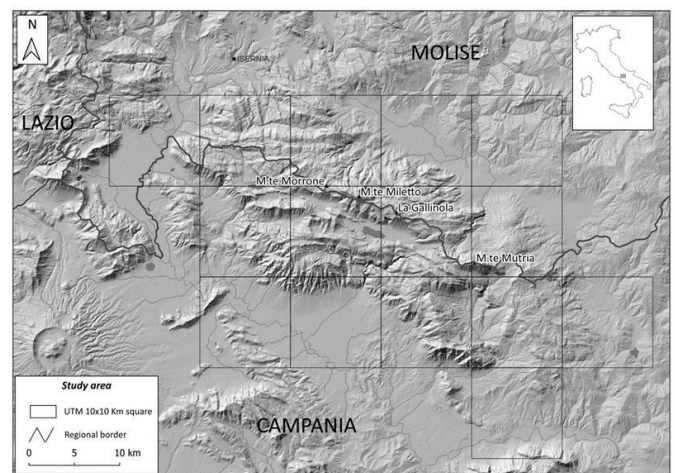
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The Matese Massif is a wide mountainous area that is located between the Campania and Molise regions of southern Italy. The only study of the local herpetological fauna, although relating specifically to the Campania side of the massif, is that of Guarino et al. (2002) in the protected area of the Matese Regional Park (Province of Caserta), where a total of six amphibian species were found. The present work presents the results of original investigations undertaken from 2008 to 2019 on the presence and distribution of the amphibian species in the Matese Massif, which we have reported alongside data taken from previous studies. The purpose of the research was to acquire better information on the presence and distribution of amphibians in support of the conservation of local populations.

## MATERIALS & METHODS

### Study area

The study area corresponds entirely to the Matese Massif (Fig. 1), including both the Campania side and the Molise side. The massif spreads over an area of about 50,000 hectares and extends, with an east/west orientation, between the Volturno River and the upper valley of the Tammaro River, and culminates with the Monte Miletto (2050 m a.s.l.). The massif is characterised by a predominance of limestone soils and karst phenomena. There are some lake basins, among which Lake Matese stands out, lying in a karst depression about 8 km long and about 2 km wide, at the foot of the Monte Miletto. Some of the main protected natural reserves of the Molise and Campania regions fall within the Matese area. On the Molise side there are the WWF Guardiaregia-Campochiaro Regional Nature Reserve, and the Callora Torrent Nature Reserve, both falling within a special conservation zone (ZSC IT 7222287) called 'La Gallinola-Monte Miletto-Monti del Matese'. On the Campania side there is the Matese Regional Park, which extends for 33,327 ha. The Campania side is administratively assigned to the provinces of Caserta and Benevento, while the Molise side is assigned to the provinces of Isernia and Campobasso. On both sides of the Matese Massif there is a mosaic of habitats:



**Figure 1.** The survey grid of 15 UTM 10 x 10 km squares superimposed on the Matese Massif. At the top right there is a map of the Italian peninsula showing the position of the study area.

plant formations typical of the Mediterranean scrub, semi-desert stony ground on the south-western side, wood formations consisting of beech forests, calcicolous and rocky grassy formations of the sunny slopes, up to the mountain and alpine calcareous scree.

On both sides of the massif the guide species for the phytoclimatic type are: *Fagus sylvatica*, *Taxus baccata*, *Ilex aequilifolium*, *Acer lobelii*, *Ostrya carpinifolia*, *Sorbus aria*, *Arum lucanum*, *Linum capitatum* and *Geranium cinereum*.

### Survey methodology

The current study is based mostly on records from field surveys performed over a period of 12 years (2008–2019) although also included were records from previous study obtained by screening the scientific literature and records from the herpetological database "Progetto Atlante degli Anfibi e dei Rettili del Molise" (Capula et al., 2008; 2010; 2018).

Data on amphibians in the field were collected by visual observation, and no individual was caught during the

**Table 1.** Amphibian species occurring in the study area, number of UTM 10 x 10 km squares in which the species were found, their altitudinal range, and number of records. Records marked p.s. = previous study, c.s. = current study, all other records come from both sources.

Species	No. of UTM squares	% study area occupied by the species	Altitudinal range (min–max m a.s.l.)	No. of records
<i>Salamandra salamandra</i> (Linnaeus, 1758)	4	27	864–1259	41
<i>Salamandrina perspicillata</i> (Savi, 1821)	2	13	456–1128	76
<i>Salamandrina terdigitata</i> (Bonnaterre, 1789) (p.s.)	2	13	403–832	5
<i>Salamandrina</i> sp. (c.s.)	6	40	427–959	18
<i>Lissotriton italicus</i> (Peracca, 1898)	5	33	340–1400	50
<i>Lissotriton vulgaris</i> (Linnaeus, 1758)	4	27	105–1400	16
<i>Triturus carnifex</i> (Laurenti, 1768)	8	53	790–1330	27
<i>Bombina pachypus</i> (Bonaparte, 1838)	5	33	552–1269	49
<i>Bufo bufo</i> (Linnaeus, 1758)	10	67	140–1459	47
<i>Bufo balearicus</i> (Boettger, 1880)	1	7	172	1
<i>Hyla intermedia</i> Boulenger, 1882	4	27	187–1076	11
<i>Pelophylax bergeri</i> (Günther, in Engelmann, Fritzsche, Günther & Obst, 1986) / <i>P. kl. hispanicus</i> (Bonaparte, 1839)	9	60	94–1330	44
<i>Rana dalmatina</i> Fitzinger, in Bonaparte, 1838	3	20	450–1105	20
<i>Rana italica</i> Dubois, 1987	10	67	235–1341	105

research. Surveys were mainly undertaken during spring and summer months (March–October), according to the methodologies reported by Maio et al. (2000). These surveys each lasted one day (for a total of 103 research days) and were undertaken mainly during daylight hours by travelling along predetermined transects that were based on cartographic and vegetational characteristics. During field investigations, data relating to distribution, frequency and type of activity (reproduction, feeding) of the observed species were collected, and the macro-environmental characteristics of the observation sites were recorded. Particular attention was paid to the collection of data relating to the species included in the Habitat Directive 92/43/EEC, and to the threatened species included in the Italian and European Community red lists (Rondinini et al., 2013; IUCN, 2022). During the 12 years of monitoring, we covered 93.3 % of the study area, i.e. 14 out of the 15 10 x 10 km grid cells of the Matese Massif (Fig. 1).

The records were collected from 26 municipalities that are listed in Table S1 (see Supplementary Material). Both the original records from the field and those from previous study were screened, validated and entered as records in a database prepared using Microsoft EXCEL software. Each record was geo-referenced using WGS 84/UTM33N. The database was projected to the same co-ordinate system (WGS84) and transformed into a 10 x 10 km grid. We aggregated the occurrence records to the Universal Transverse Mercator (UTM) grid system at a spatial resolution of 100 km<sup>2</sup> (UTM 10 x 10 km). We mapped the species occurrence by assigning each species to the corresponding UTM 10 x 10 km square following Sillero et al. (2005).

Species were identified according to their morphology and coloration following Lanza et al. (2007). The nomenclature adopted for the species in this paper is that proposed by Di Nicola et al. (2019).

## RESULTS & DISCUSSION

Overall, a total of 510 records were collected, 170 from field investigations and 340 from an analysis of the literature (Table 1). This gave a total of 14 autochthonous species of amphibians (Caudata n=6, Anura n=8) that are listed in Table 1. The 14 species listed correspond to the total number of amphibian species occurring in much wider regions of southern Italy, i.e. Campania (13.670 km<sup>2</sup>; Guarino et al., 2012) and Molise (4.438 km<sup>2</sup>; Capula et al., 2018). To date, the Matese Massif has the greatest number of recorded amphibian species of any mountainous area of central or southern Italy (Table 2). The large number of species probably reflects the mosaic of natural habitats, the optimal biological and chemical conditions of local waterbodies, and the absence of threats such as water pollution.

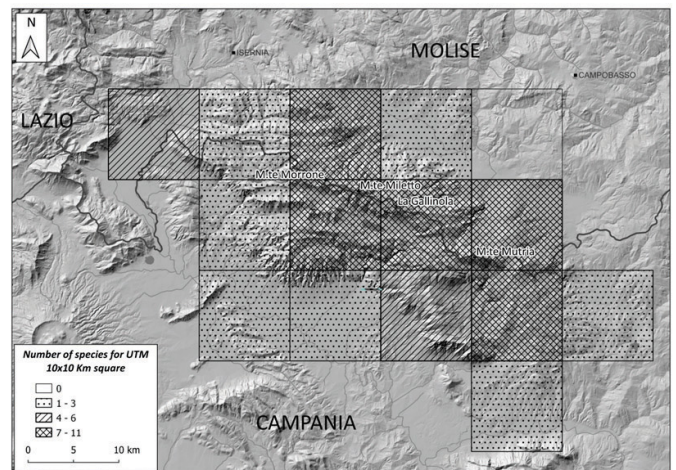
As to the species richness, the analysis shows that in five out of 15 UTM 10 x 10 km squares, that is one third of the area investigated, the number of recorded species ranges from 7 to 11, and in two UTM squares (13.3 % of the investigated area) this number ranges from 4 to 6 (Fig. 2). In one UTM 10 x 10 km square no amphibian species were recorded. Two localities (Guardiaregia and Campitello di Sepino) can be considered as very important hotspots for amphibians within the study area as well as in southern Italy. In Guardiaregia, nine species have been recorded - *Salamandra salamandra*, *Salamandrina perspicillata*, *Lissotriton italicus*, *Bombina pachypus*, *Bufo bufo*, *Hyla intermedia*, *Rana italica* and *Pelophylax bergeri* / *P. kl. hispanicus*. In Campitello di Sepino, six species were found - *Salamandra salamandra*, *Salamandrina perspicillata*, *L. italicus*, *Lissotriton vulgaris*, *Triturus carnifex*, and *R. italica*.

The distribution maps of the species recorded in the study area can be found in the Supplementary Material (Figs. S1

**Table 2.** Number of amphibian species recorded in some mountainous areas of central and southern Italy

Mountainous area	No. of species	Reference
Sibillini Mounts (Sibillini National Park, Central Italy)	13	Fiacchini, 2013
Majella Massif (Majella National Park, Central Italy)	13	Scalera et al., 2006
Simbruini Mountains (Central Italy)	9	Crucitti et al., 2010
Preneestini Mountains (Central Italy)	10	Bologna et al., 2001
Lepini Mountains (Central Italy)	11	Corsetti & Capula, 1992
Lucretili Mountains (Central Italy)	9	Carpaneto, 2000
Aurunci Mounts (Central Italy)	9	Romano et al., 2007
Ausoni Mountains (Central Italy)	8	Corsetti & Romano, 2007
Vesuvio (Vesuvius National Park, Southern Italy)	8	Maio et al., 2000
Alburni Massif (Southern Italy)	11	Caputo et al., 1985
Matese Massif (Southern Italy)	14	Current study

– Fig. S14). The most widespread species in the 15 UTM 10 x 10 km squares of the study were *R. italica* (10 squares), *B. bufo* (10 squares), *P. bergeri* / *P. kl. hispanicus* (9 squares), and *T. carnifex* (8 squares) which were found in more than 67 % of the monitored localities (Table 1). Species with relatively narrow ranges were *R. dalmatina* (3 squares), *S. perspicillata* (2 squares), *S. terdigitata* (2 squares), and *Bufoles balearicus* (1 square). *Bombina pachypus*, though recorded in the past in a number of sites (> 40 literature records), now appears to be very rare and extremely localised. The current rarity of the latter species seems to be real and not due to lack of field investigations, since its range is currently shrinking fast throughout the Italian Peninsula (Bulgarini et al., 1998; Stagni et al., 2004; Rondini et al., 2013; Di Nicola et al., 2019) and it is considered as Endangered in the Red List of the Italian threatened species (Rondini et al., 2013). The altitudinal range for Caudata (6 species) was 105–1400 m a.s.l., and the Anura (8 species) was very similar at 94–1459 m a.s.l. (Table 1); *L. vulgaris* and *B. bufo* were respectively Caudata and Anura with the widest altitudinal ranges. The Matese Massif is extremely interesting from the herpetological perspective as it is the only Italian geographic area in which the two species of the genus *Salamandrina* (*S. perspicillata* and *S. terdigitata*) - which is strictly endemic to the Italian Peninsula - are sympatric. *Salamandrina perspicillata* is present on both sides of the Matese Massif, and would be the only *Salamandrina* species occurring in the Molise region according to Romano et al. (2009). *Salamandrina terdigitata* has been reported with certainty in some localities sited on the Campania

**Figure 2.** Number of amphibian species in the Matese Massif recorded for each of the UTM 10 x 10 km squares investigated**Figure 3.** Adult *Salamandrina perspicillata* from the Molise side of the Matese Massif

side (province of Benevento, Romano et al., 2009)(Fig. S3). Extremely interesting are the populations of *S. perspicillata* occurring in some localities of the Molise side (see Romano et al., 2009 for the list of localities), as they are characterised by a genome in which alleles of *S. terdigitata* are present (Fig. 3). This could be the evidence of past hybridisation and introgression between the two species (Hauswaldt et al., 2011; Mattoccia et al., 2011). However, it should be noted that the taxonomic distinction between *S. perspicillata* and *S. terdigitata* is based on mitochondrial (Mattoccia et al., 2005; Nascetti et al., 2005) and nuclear analyses (Nascetti et al., 2005), while no morphological traits allow a clear and easy distinction between the two species in the field (Angelini et al., 2007). Although some small and hardly noticeable differences in size and coloration could allow a tentative distinction between adults of the two species (Romano et al., 2009), neither young individuals nor larvae can be identified exclusively on the basis of morphological characters. Since our observations were based on field investigations it did not allow the unambiguous taxonomic attribution to one or the other species. In the present contribution the original data

**Table 3.** Phenology of 11 amphibian species in the Matese Massif, \* = adults; E = eggs; L= larvae. The months indicated are those in which there were field investigations.

	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
<i>Salamandra salamandra</i>		*	*	*L	L			*
<i>Salamandrina perspicillata</i>	*	*	*E	*L	L			*
<i>Lissotriton italicus</i>	*	*E	*E	*L	L	L	L	
<i>Lissotriton vulgaris</i>	*	*	*E	*L	L	L		
<i>Triturus carnifex</i>	*	*	*E	*L	*L	L		
<i>Bombina pachypus</i>			*	*E	*L	*L	*L	
<i>Bufo bufo</i>	*E	*EL	*L	*L	L	L	*	*
<i>Hyla intermedia</i>	*	*E	*EL	*L	*L			*
<i>Pelophylax bergeri</i> / <i>P. kl. hispanicus</i>	*	*E	*E	*L	*L	*L	*	*
<i>Rana italica</i>	*	*EL	*EL	*L	*L	*L	*	*

relating to specimens (adults, young, larvae) observed in the wild on the Campania side, where both species are known to occur, were treated as *Salamandrina* sp. (Table 1; Fig. S4).

As in other regions of central and southern Italy, green frogs are represented in the Matese Massif by a sinklepton, that is, by two closely related taxa from the genetic point of view, one of which is the parent species, *Pelophylax bergeri*, and the other is the hybridogenetic hybrid or klepton hybrid, *Pelophylax* kl. *hispanicus* (Dubois & Ohler, 1994; Günther & Plötner, 1994; Capula et al., 2007; Dubey & Dufresnes, 2017). The genetic makeup of the Italian hybridogenetic hybrids has recently been analysed and a new endemic lineage of eastern-Mediterranean origin as one parental ancestor of *P. kl. hispanicus* identified. Apparently, this ancestor “is nowadays extinct in the wild but its germline subsists through its hybridogenetic descendant (*P. kl. hispanicus*), which can thus be considered as a semi living fossil” (Dubey & Dufresnes, 2017). *Pelophylax bergeri* and *P. kl. hispanicus* are morphologically and chromatically very similar (Capula et al., 2007) and in Molise and Campania, as in the rest of central and southern Italy, they usually coexist in the same habitat (Capula et al., 2007; Di Nicola et al., 2019). As the two taxa can only be distinguished with certainty by genetic and/or molecular analyses, all records relating to the presence of green frogs in the study area were processed in the database as *P. bergeri* / *P. kl. hispanicus* (Table 1, Fig. S14).

The most commonly observed reproductive habitats by the three newt species (*L. italicus*, *L. vulgaris*, *T. carnifex*) and *B. pachypus* were drinking troughs and small ponds located in small valleys or pastures along the border of dry grassland and woods (Fig. 4). For reproduction *Salamandra salamandra*, *Salamandrina perspicillata*, *S. terdigitata* and



**Figure 4.** Drinking trough in which adults and larvae of *Lissotriton italicus*, *Bufo bufo*, *Hyla intermedia* and *Pelophylax bergeri* / *P. kl. hispanicus* were observed

*R. italica* mainly frequented streams and water springs located inside or at the edge of mixed oak woods and beech woods. *Rana dalmatina* was only found in localities characterised by riparian hygrophilous woods close to ponds and swamps. *B. bufo*, *H. intermedia* and the taxa of the genus *Pelophylax* were eurytopic species and reproduced in several wet habitats, i.e. swamps, ponds, streams, small lakes and drinking troughs, either in woods or pastures. According to the available records, the phenology of 11 amphibian species is summarised in Table 3.

The observation of uncommon levels of species richness, with 7 to 11 species in certain UTM 10 x 10 km squares, suggests that the Matese Massif area is important for the conservation of amphibian species as well as their natural wet and freshwater habitats, and thus it should be continuously monitored and strictly protected by local authorities. Within this area intensive agriculture and pastoral activities and the increase in the extension of land used for agricultural purposes should be avoided as much as possible. It has been mentioned that drinking troughs are important for the reproductive biology of the three species of newts as well for *B. pachypus* and, occasionally, for other amphibian species (*B. bufo*, *H. intermedia*, *P. bergeri* / *P. kl. hispanicus*). These small water bodies are widespread, but they are under threat due to 1) periodic clearing and emptying by farmers and shepherds, and to 2) introduction of fish by humans, causing the rapid disappearance of eggs, larvae and adults of amphibians as well as the aquatic invertebrate fauna (Scoccianti, 2001; Peria et al., 2016). Streams and fresh water springs are extremely important for conservation of local populations of some species that tolerate only a narrow range of environmental conditions (*Salamandra salamandra*, *Salamandrina perspicillata*, *S. terdigitata* and *R. italica*) and they should be strictly protected from water pollution, drying up of springs, introduction of fish and alien species of vertebrates and invertebrates (Scoccianti, 2001; Capula & Contini, 2009; Peria et al., 2016). It should be noted that the species considered in the present study are monitored and strictly protected inside the WWF Guardiaregia-Campochiaro

Regional Nature Reserve, and the Callora Torrent Nature Reserve (Province of Campobasso, Molise side), and within the Matese Regional Park (Province of Caserta, Campania side). Moreover, all species of amphibians are formally protected by the regional law of Molise (Regional Law 26/1996), and some species (*S. perspicillata*, *S. terdigitata*, *L. italicus*, *T. carnifex*, *B. pachypus*, *R. dalmatina* and *R. italica*) are also included in the Appendices of the European Council Directive 92/43/EEC (Habitat Directive on the conservation of natural habitats and wild fauna and flora) as deserving high conservation priority.

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