

# 1 An automated approach for systematic detection of Key 2 Biodiversity Areas

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## 14 Abstract

15 The new Key Biodiversity Areas (KBA) standard is an important method for identifying regions of  
16 the planet hosting unique biodiversity. KBAs are identified through the implementation of  
17 threshold-based criteria that can be applied to any target species and region. Efficient methods to  
18 rapidly assess the existence of potential KBAs in different areas of the planet are still missing,  
19 although they are needed to accelerate the KBA identification process for large numbers of  
20 species globally. We developed a methodology to scan geographical regions and detect potential  
21 KBAs under multiple criteria. We tested the methodology on 59 species of reptiles and amphibians  
22 in Italy through the application of selected KBA criteria. Potential KBAs were identified for multiple  
23 species under most criteria, covering 1.4% to 12% of the study area, depending on analytical  
24 settings. Unit size used to identify KBAs played an important role in shaping the distribution of  
25 potential KBAs, also affecting the degree of overlap between areas triggered by different criteria.  
26 New potential KBAs identified in this study are only partially nested within current KBAs in Italy  
27 (previously identified for birds) and within the national protected areas.

28

## 29 Introduction

30 Global change is profoundly affecting Earth’s biodiversity (IPBES, 2019). As species decline and  
31 their extinction risk continues to grow globally (Hoffmann et al., 2010), there is an urgent need to

32 identify sites that are important for biodiversity persistence. Currently, there are several methods  
33 to identify important sites for biodiversity, some of them being target based and others threshold  
34 based (Smith et al., 2018). The most recent Key Biodiversity Areas (KBA) standard was developed  
35 to identify sites that can contribute to the global persistence of biodiversity (IUCN, 2016).

36

37 The KBA approach has been included by the Convention on Biological Diversity as a valuable  
38 resource to inform protected areas expansion for the achievement of the Aichi Biodiversity  
39 Targets (CBD, 2022). The Important Bird Areas (IBA), which are now part of the KBA network, have  
40 been used by the European Union to design protected areas under its first legislation on the  
41 environment, the Birds Directive (Directive 2009/147/EC on the conservation of wild birds). KBAs  
42 have been included by the United Nations in the Sustainable Development Goals Report (UN  
43 DESA, 2021) to assess the efficiency of protected areas in covering sites that can ensure the  
44 persistence of biodiversity. The KBA approach is therefore already being used extensively in  
45 conservation policy.

46

47 Globally, 79% of currently recognized KBAs have been identified based on the presence of bird  
48 species (keybiodiversityareas.org, April 11th 2022), revealing an important gap of knowledge on the  
49 distribution of KBAs for non-avian taxa. In order to reduce the taxonomic bias of KBAs, it would be  
50 particularly useful to rely on methodologies that can rapidly assess KBAs within large areas for high  
51 numbers of species. Potential KBAs have been identified for different taxa, including vertebrates,  
52 invertebrates, and plant species (Ambal et al., 2012; Yahi et al., 2012; Plumtree et al., 2019) using  
53 different approaches depending on the geographic scale of the analysis, as well as on the target taxa  
54 and data availability. However, such methods do not allow simultaneous KBA assessments for large  
55 numbers of species, resulting in a significantly slow process of mapping KBAs worldwide.

56

57 We developed a methodology that scans a geographic region to detect potential KBAs for target  
58 species based on their habitat distribution. We applied our approach to Area of Habitat (AOH)  
59 maps. The AOH maps provide information on the spatial distribution of available habitat for the  
60 species within its distribution range and altitude limits (Rondinini et al., 2011; Lumbierres et al.,  
61 2022). AOH represents a useful tool for large-scale assessments of the geographic distribution of

62 species' populations for conservation purposes (Brooks et al., 2019), and is considered a valid  
63 proxy of population size by the Global Standard for the Identification of Key Biodiversity Areas  
64 (IUCN, 2016). The methodology can be used to efficiently apply KBA criteria which rely on  
65 population size and distribution of the examined taxa.

66

67 We tested the method on Italian reptiles and amphibians, for which KBAs have not been identified  
68 so far. Italy is an important center of diversity and endemism of European herpetofauna (Sindaco  
69 et al., 2006; Cox & Temple 2009; Sillero et al, 2014). Seven amphibians and two reptiles occurring  
70 in Italy are listed as threatened by the Red List of the International Union for Conservation of  
71 Nature (IUCN, 2021). The geographical distribution as well as habitat availability for many reptiles  
72 and amphibian species in Italy is well known (IUCN, 2020; Nania et al., 2022). We assessed the  
73 effects of KBA criteria, grid size, and species attributes (such as distribution extent, degree of  
74 endemism and IUCN Red List status) on the total area of potential KBAs identified. We then  
75 compared the distribution of new potential KBAs with the current KBA and protected area  
76 networks of Italy.

77

## 78 **Methods**

### 79 ***Species distribution maps***

80 We estimated the AOH of 59 species of amphibians and reptiles within their geographic range in  
81 Italy. For 55 species, we used AOH maps from Nania et al. (2022), These maps were built using the  
82 ranges downloaded from the IUCN Red List of Threatened Species (IUCN 2021). For four species  
83 endemic to Italy we built new AOH maps based on ranges that were obtained from specific Italian  
84 datasets because of their higher accuracy. In particular:

85     • For three species endemic to Italy, the range was obtained from the Atlas of  
86       Amphibians and Reptiles of Italy, curated by the Societas Herpetologica Italica (SHI).  
87       The maps are available upon request from SHI;  
88     • For one species microendemic to Italy (*Podarcis raffonei*), we used the range from  
89       Ficetola et al. (2021). Since the range is very small (5000 m<sup>2</sup>), we did not calculate an  
90       AOH and considered the whole range suitable.

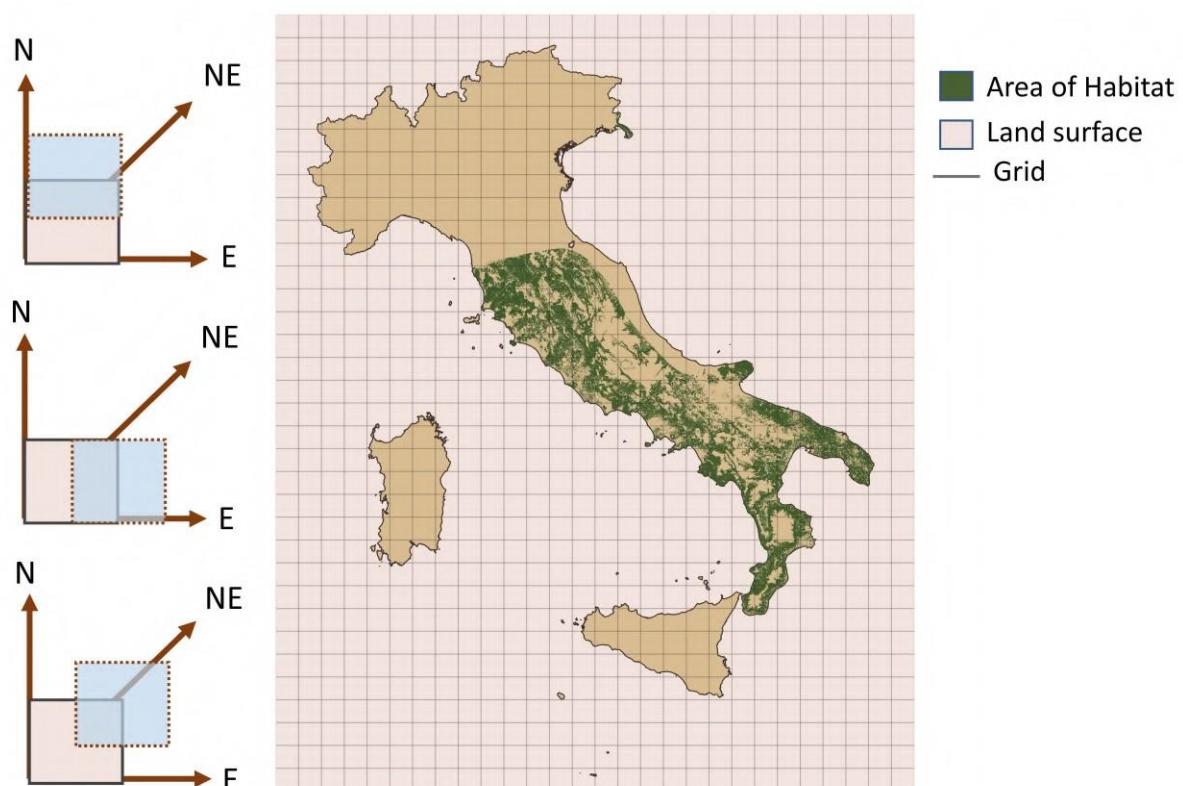
91 These four AOH maps were developed following the procedure shown in Nania et al. (2022).  
92 Species were mapped to their habitat using Copernicus Global Land Service Land Cover (CGLS-  
93 LC100) 2019 classes as a habitat surrogate. We compiled species-habitat associations and altitude  
94 limits based on monographs on the biology of target species (Sindaco et al., 2006; Lanza et al.,  
95 2007; Corti et al., 2011). We then reclassified a base map combining land cover and altitude  
96 information to produce area of habitat maps for the target species. A complete description of how  
97 the additional AOH maps were produced is included in Appendix S1.

98

99 ***Systematic identification of KBAs***

100 To systematically identify candidate KBAs under five selected criteria (A1, B1, B2, B3, E), we  
101 produced four grids of squared cells of different size (10x10, 20x20, 30x30 and 40x40 km) in a  
102 Lambert-Azimuthal equal area projection (ETRS89, EPSG:3035) in GRASS GIS version 7.8.5 (GRASS  
103 2020). The grids were built on the country's land surface, covering the Italian peninsula and its  
104 islands. Cell size was selected based on the current mean and median area of reptiles and  
105 amphibians KBAs worldwide, according to the Key Biodiversity Areas Secretariat database  
106 (<https://www.keybiodiversityareas.org/>). Currently, the global mean size of KBAs triggered by  
107 reptile or amphibian species is 1400 km<sup>2</sup> (SD = 16910), while the median is 240 km<sup>2</sup>. Thus, by  
108 selecting 40x40 km as the largest cell size, we ensured that it could potentially include a KBA with  
109 a maximum size of 1600 km<sup>2</sup>, which is slightly above the current average KBA size for reptiles and  
110 amphibians worldwide. The smallest cell size can detect KBAs with a maximum size of 100 km<sup>2</sup>.  
111 The four grid resolutions allowed us to test for sensitivity of the KBA criteria to the cell size, and  
112 therefore to the dimension of the unit used to detect KBAs. To avoid missing potential KBAs due  
113 to the fixed position of the grid on the country's surface, for each resolution we replicated the grid  
114 in three additional positions. Specifically, the grid was moved to half the size of the grid cell along  
115 two cardinal directions (North and East) and one ordinal direction (North-east), as illustrated in  
116 Figure 1. Each of the 16 grids (four resolutions by four positions) was used as a basis for assessing  
117 the existence of potential KBAs following the procedure described below. The GRASS and R code  
118 used for the analysis is available in Appendix S2.

119



120 **Figure 1:** An explanatory scheme showing how the moving window approach works. Here we  
121 show the 40x40 km grid positioned on top of the AOH map. During the surface scanning, the grid is  
122 moved to three additional positions (left).

123

124 Potential KBAs for target species are detected through the application of several threshold- based  
125 criteria, which rely on the distribution of important populations of one or more species. The main  
126 five KBA criteria refer to “Threatened biodiversity” (criterion A), “Biogeographically restricted  
127 biodiversity” (criterion B), “Ecological integrity” (criterion C), “Biological processes” (criterion D)  
128 and “Irreplaceability through quantitative analysis” (criterion E) (IUCN 2016). We tested selected  
129 criteria/subcriteria that can be informed by the AOH as detailed below.

130 Criterion A1 refers to globally threatened species. According to the guidelines for KBA  
131 identification (IUCN, 2020), a site qualifies as a KBA under criterion A1 if it hosts a minimum  
132 threshold percentage of the global population of a species. The percentage threshold depends on  
133 the threatened status of the species. For Critically endangered (CR) and endangered (EN) species,  
134 the threshold is  $\geq 0.5\%$  of the global population. For a species assessed as vulnerable (VU), the  
135 threshold is  $\geq 1\%$ . Species for which criterion A1 was applied are indicated in Table 1. For each

136 species, we calculated the proportion of AOH inside each grid cell, and the cell was considered a  
137 potential KBA if the percentage exceeded the relevant threshold. Criterion B1 refers to  
138 geographically restricted species, which are defined as any species whose population is so  
139 concentrated that at least 10% of the global population is found within a site (IUCN, 2020). For  
140 each species, we calculated the proportion of AOH inside each grid cell, and the cell was  
141 considered a potential KBA if the percentage exceeded 10% of the total AOH of the species.

142

Species	Global AOH	Italian AOH	IUCN RL status	A1	Irreplaceability
<i>Bombina pachypus</i>		X	EN	X	X
<i>Bombina variegata</i>	X		LC		
<i>Bufo bufo</i>	X		LC		
<i>Bufo viridis</i>	X		LC		
<i>Discoglossus sardus</i>	X		LC		X
<i>Euproctis platycephalus</i>	X		EN	X	X
<i>Hyla arborea</i>	X		LC		
<i>Hyla intermedia</i>	X		LC		X
<i>Hyla sarda</i>	X		LC		X
<i>Ichthyosaura alpestris</i>	X		LC		
<i>Lissotriton italicus</i>	X		LC		X
<i>Lissotriton vulgaris</i>	X		LC		
<i>Pelobates fuscus</i>	X		LC		
<i>Pelophylax ridibundus</i>	X		LC		
<i>Rana dalmatina</i>	X		LC		X
<i>Rana italica</i>	X		LC		X
<i>Rana latastei</i>		X	VU	X	X
<i>Rana temporaria</i>	X		LC		
<i>Salamandra atra</i>	X		LC		X
<i>Salamandra lanzai</i>		X	VU	X	X
<i>Salamandra salamandra</i>	X		LC		
<i>Salamandrina perspicillata</i>	X		LC		X
<i>Salamandrina terdigitata</i>	X		LC		X
<i>Triturus carnifex</i>	X		LC		X
<i>Algyroides fitzingeri</i>	X		LC		X
<i>Algyroides nigropunctatus</i>	X		LC		
<i>Anguis fragilis</i>	X		LC		X
<i>Archaeolacerta bedriagae</i>	X		LC		
<i>Chalcides chalcides</i>	X		LC		X
<i>Coronella austriaca</i>	X		LC		
<i>Coronella girondica</i>	X		LC		
<i>Elaphe quatuorlineata</i>	X		NT		X
<i>Emys trinacris</i>	X		LC		X
<i>Euleptes europaea</i>	X		NT		X
<i>Hemidactylus turcicus</i>	X		LC		
<i>Hierophis viridiflavus</i>	X		LC		X
<i>Lacerta bilineata</i>	X		LC		X
<i>Malpolon monspessulanus</i>	X		LC		
<i>Mediodactylus kotschyi</i>	X		LC		
<i>Natrix maura</i>	X		LC		

<i>Natrix tessellata</i>	X		LC		
<i>Podarcis filfolensis</i>	X		LC		
<i>Podarcis melisellensis</i>	X		LC		
<i>Podarcis muralis</i>	X		LC		X
<i>Podarcis raffonei</i>	X		CR	X	X
<i>Podarcis tiliguerta</i>	X		LC		X
<i>Podarcis waglerianus</i>	X		LC		X
<i>Testudo marginata</i>	X		LC		
<i>Vipera ammodytes</i>	X		LC		
<i>Vipera aspis</i>	X		LC		X
<i>Vipera ursinii</i>	X		VU	X	X
<i>Vipera walser</i>	X		DD	X	X
<i>Zamenis lineatus</i>	X		DD		X
<i>Zamenis situla</i>	X		LC		
<i>Zootoca vivipara</i>	X		LC		

143

144 **Table 1.** List of the species, type of AOH maps, IUCN status, detailing which species were tested for  
145 criteria A1 and for Irreplaceability (criterion E).

146

147 Criterion B2 refers to co-occurring geographically restricted species, which are defined as species  
148 with a global range size  $\leq 10000 \text{ km}^2$  that co-occur within a site. A site triggers a potential KBA  
149 under criterion B2 if it holds  $\geq 1\%$  of the global population of at least 2 geographically restricted  
150 species (IUCN, 2020). First, we identified geographically restricted species which have a global  
151 range  $\leq 10000 \text{ km}^2$ . For each of these species, we calculated the proportion of AOH inside each grid  
152 cell and retained the cells containing more than 10% of the total AOH. Finally, we overlapped  
153 these cells across species, and we considered potential KBAs those that were retained for at least  
154 2 species.

155

156 Criterion B3 refers to geographically restricted assemblages. The KBA identification guidelines  
157 state that: “A site qualifies as potential KBA under criterion B3 if it holds  $\geq 5$  species within a  
158 taxonomic group or 10% of the species restricted to the ecoregion, whichever is larger” (IUCN  
159 2020). According to the guidelines, a species can be considered ecoregion-restricted if at least 95%  
160 of its global population is confined to a single ecoregion. In order to test criterion B3, we used  
161 WWF Palaearctic Terrestrial Ecoregion map (Olson et al., 2001), which is freely available at the  
162 WWF portal (<https://www.worldwildlife.org/publications/terrestrial-ecoregions-of-the-world>). Of  
163 these maps, we retained only the portion of ecoregions which fell within the administrative  
164 boundaries of Italy. We counted a total of 10 ecoregions, listed in Appendix S1. First, we calculated  
165 the percentage of global population of the species, based on the AOH maps that was confined to

166 each of the 10 ecoregions. Only species for which at least 95% of the global population is  
167 restricted to a single ecoregion were considered candidate species potentially able to trigger  
168 criterion B3. All raster grids of ecoregion-restricted species were filtered according to criterion B3  
169 thresholds: only cells hosting  $\geq 0.5\%$  of the global population of the species were retained. Finally,  
170 for each resolution, we considered potential KBAs under B3 only areas which matched the B3  
171 population threshold for at least 5 ecoregion-restricted species.

172

173 Criterion E refers to the irreplaceability of a site, as measured through quantitative analysis. A  
174 site may trigger a potential KBA under criterion E if it has a level of irreplaceability of at least 0.9  
175 on a 0-1 scale (IUCN, 2016). Irreplaceability is the likelihood that the conservation of a given site is  
176 needed to achieve a set of conservation targets (Ferrier et al., 2000). We used Marxan's selection  
177 frequency as a proxy to measure irreplaceability (Ball et al., 2009). Given a set of conservation  
178 goals, Marxan identifies through a stochastic process a set of spatial planning solutions to achieve  
179 the goals. The selection frequency reflects the number of times a specific site has been selected  
180 across the set of solutions identified by repeated Marxan runs. According to the KBA guidelines,  
181 the target must be set according to the number of mature individuals hosted in a site. Global  
182 population size can be inferred through the AOH. If the global range of a species is less than 1,000  
183 km<sup>2</sup>, the whole population should become the target to conserve (IUCN, 2020). The target can be  
184 set at 1000 km<sup>2</sup> if this is the largest value among the other possible values listed in the KBA  
185 guidelines (IUCN, 2020). However, since we aimed at identifying KBAs at the national scale, we  
186 scaled the targets for species with a global range  $\geq 1,000$  km<sup>2</sup> according to the percentage of the  
187 global range which is actually found within the boundaries of Italy. Moreover, we did not include  
188 in the irreplaceability analysis all species in our dataset for which less than 10% of their global  
189 range falls within Italy. In doing so, we avoided potential errors due to marginal fractions of the  
190 global range of widely distributed species. All species included in the irreplaceability analysis are  
191 reported in Table 1.

192

193 Marxan requires a set of planning units to find optimal solutions to meet the conservation  
194 targets. We used grid cells as the planning units and overlaid them with AOH maps to quantify the  
195 amount of AOH in each cell. Marxan also allows to assign a cost to each planning unit, to be  
196 minimized when computing the solutions. We calculated the cost of each planning unit based on

197 the percentage of land within the planning unit. A cost value between 0 (all water) and 1 (all land)  
198 was assigned to the planning units. The cost assigned to planning units falling along the coastline  
199 reflects the percentage of land area within the planning unit. Marxan analyses were run separately  
200 for amphibian and reptile species and repeated for the 16 sets of planning units. The number of  
201 runs for each analysis was set to 1000. For each cell resolution, we identified as potential KBAs all  
202 cells with selection frequency  $\geq 900$ .

203

204 ***Sensitivity and overlap analysis***

205 We tested the response of KBA criteria in relation to the different cell sizes used to detect KBAs.  
206 This was done by calculating the total AOH found within the cells identified as potential KBAs for  
207 each species at a specific cell resolution, regardless of the position of the grid on the Italian  
208 peninsula. The sensitivity analysis was performed for criteria A1 and B1, as they could be triggered  
209 by single species. A measure of the degree of overlap between KBAs identified using different  
210 criteria was obtained by summing the total AOH identified as KBA for all species under each  
211 criterion. Subsequently, we examined the percentage of potential Key Biodiversity Areas identified  
212 under each criterion, which was nested in the area of other tested criteria. We followed the same  
213 procedure for KBAs identified using each of the four cell resolutions. Criterion E was excluded from  
214 the analysis, as the method for measuring irreplaceability does not allow to link the KBA to a single  
215 species, and therefore to the AOH which triggered criterion E.

216 Finally, we calculated the total area identified as potential KBA for all criteria, using each of the  
217 four cell resolutions. Thus, for each of the implemented cell resolutions, we produced a map of  
218 the total AOH area identified as KBA, regardless of the species or the criteria which triggered it.  
219 Subsequently, we measured the percentage of potential KBA falling within the current KBA  
220 network, according to the World Database of Key Biodiversity Areas  
221 (<https://www.keybiodiversityareas.org/>). Additionally, we measured the percentage of potential  
222 KBA which falls within the Natura 2000 network (EEA, 2021) and the national protected area  
223 network of Italy (NNB, 2021).

224

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226

227 **Results**

228 **Potential KBAs under main criteria A and B**

229 Potential KBAs were detected for multiple species under different criteria (Figure 2 and 3).

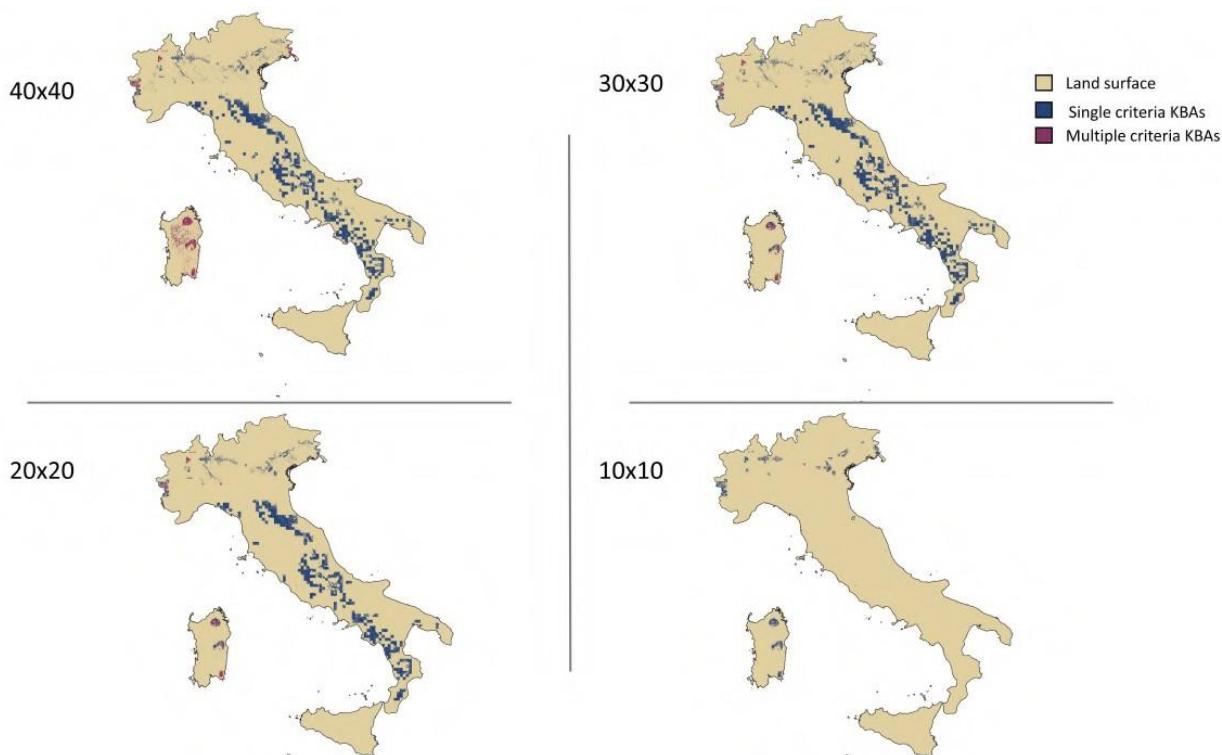
230 Criterion A1 was applied to 4 species of amphibians and 3 species of reptiles, because they hold a

231 'Threatened' status in the IUCN Red List (IUCN, 2021). The total extent of potential KBAs identified

232 for the target species decreased with cell size (Figure 2). While the majority of potential KBAs were

233 identified under a single criterion, some areas triggered multiple KBA criteria regardless of the

234 resolution of the grid cell units (Figure 2).



235

236

237 **Figure 2:** Potential Key Biodiversity Areas identified using all four grid cell resolutions.

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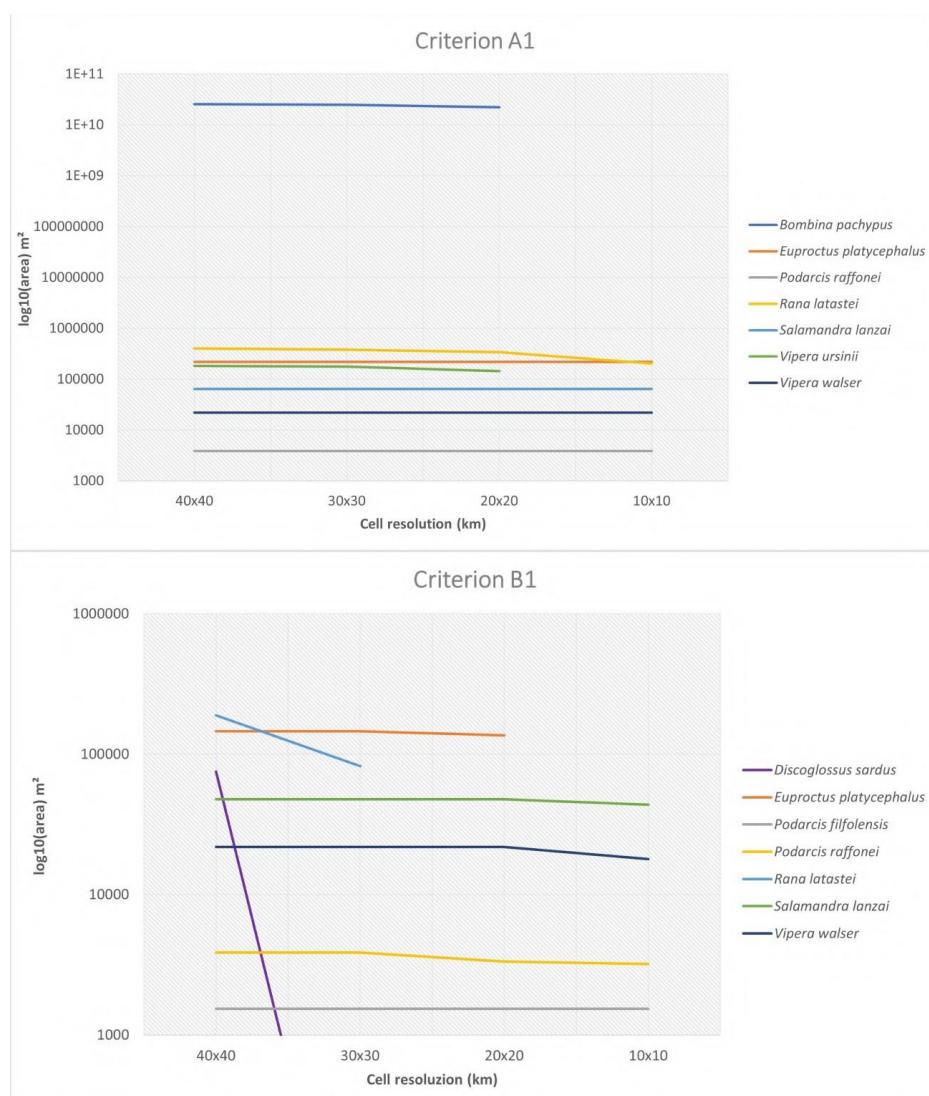
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248

249 **Figure 3:** Extent of KBAs under criterion A1 and B1 identified for each species using different grid  
250 cell resolutions.

251

252 Potential Key Biodiversity Areas under criterion A1 were detected for seven species using 40x40,  
253 30x30 and 20x20 km grid cells, while only five species triggered potential KBAs under criterion A1  
254 using the 10x10 km grid cells, as it is shown in Figure 3. Total area of potential KBA under criterion  
255 A1 for *Bombina pachypus* and *Vipera ursinii* dropped to zero when using a 10x10 km cell size.  
256 Potential KBAs for *Rana latastei* show a slight decrease of extent with decreasing cell size, with the  
257 maximum decrease rate observed when using 10x10 km cell size (Figure 3). For the remaining four  
258 species, the potential KBA extent did not vary significantly according to the cell resolution.

259

260 We detected potential KBAs under criterion B1 for six species with a grid cell size of 40x40 km.  
261 With a resolution of 30x30 km, the number of species which could trigger KBAs under criterion B1

262 was reduced to four, while only for three species we could trigger B1 using 10x10 km grid  
263 resolution. The potential KBA extent of *Discoglossus sardus* and *Rana latastei* responded to the  
264 variation of cell size by quickly dropping to zero when using smaller cell sizes. *Euproctus*  
265 *platycephalus* showed a slightly decreased KBA extent using 30x30-20x20 km cells, with potential  
266 KBA extent dropping to zero when using 10x10 km cells. The remaining three species showed very  
267 little to no variation of potential KBA extent using different cell sizes (Figure 3). Criterion B2 could  
268 be triggered only by two species of amphibians, *Discoglossus sardus* and *Euproctus platycephalus*,  
269 using 40x40 km grid cell size. The total potential KBA area under B2 was concentrated in the island  
270 of Sardinia (Figure 2). Concerning criterion B3, we could not identify any area which could satisfy  
271 all requirements of B3 and thus trigger a potential KBA under this criterion.

272

273 Percentage of overlap between potential KBAs identified under different criteria is shown in  
274 Table 2. Regardless of the implemented cell size, the percentage of potential KBAs detected under  
275 criterion A1 which was nested within potential KBAs for B1 or B2 remained below 8%. Criterion B1  
276 was nested within potential KBAs for A1 from 30% to 62% of its extent depending on the  
277 implemented cell size. Criterion B2 could be triggered only using 40x40 km cells and showed 27%  
278 and 71% overlap with potential KBAs identified under criteria A1 and B2 respectively. The  
279 percentage of potential KBAs under criterion A1 nested within potential KBAs for B1 was 7.6%  
280 using 10x10 km cells and 3.7% using 40x40 km cells. The same trend was observed for the  
281 percentage of potential KBAs for B1 which are nested within potential KBAs for A1 (63% with  
282 10x10 km cells, 31% with 40x40 km cells).

283

	<b>A1</b>	<b>B1</b>	<b>B2</b>
<b>40x40</b>			
<b>A1</b>	100	3.7	1.86
<b>B1</b>	30.67	100	39.6
<b>B2</b>	27.47	70.78	100
<b>30x30</b>			
<b>A1</b>	100	3.05	/
<b>B1</b>	39.75	100	/
<b>20x20</b>			
<b>A1</b>	100	3.32	/
<b>B1</b>	56.56	100	/
<b>10x10</b>			
<b>A1</b>	100	7.62	/
<b>B1</b>	62.58	100	/

284

285 **Table 2:** For each cell resolution, we report the percentage (%) of cells identified by each criterion  
286 (left column) that are also identified by other criteria.

287

288 ***Overlap with current KBA, Protected Area, and Natura 2000 networks in Italy***

289 The percentage overlap of the potential KBAs we identified with the existing KBA, Protected Area,  
290 and Natura 2000 networks in Italy are shown in Table 3. The new potential KBAs detected with  
291 10x10 km cells in this study showed an overlap of 18% with the current KBA network, while using  
292 larger cells the percentage of overlap remained stable around 25%. The lowest percentage of  
293 overlap between the new potential KBAs and the Italian national protected areas network was  
294 observed when using 10x10 km cells. For potential KBAs identified with larger cells the percentage  
295 of overlap was 22% to 24%. The Natura 2000 network enclosed from 33% to 35% of the new  
296 potential KBAs identified in this study with 20x20 to 40x40 km cells, respectively, with the lowest  
297 percentage of overlap observed with KBAs identified through a 10x10 km cell (28%). The mean  
298 percentage of new potential KBAs not encompassed by the current KBA network using the four  
299 different cell sizes was equal to 70%, while the mean percentage not included in national  
300 designated protected areas was 81%. The Natura 2000 network currently excludes a mean of 68%  
301 new KBAs using the four cell resolutions.

	<b>current KBAs</b>	<b>National PA</b>	<b>N2000</b>
<b>40x40</b>	24.35	21.86	33.37
<b>30x30</b>	24.48	22.91	33.99
<b>20x20</b>	25.28	24.02	35.21
<b>10x10</b>	18.04	8.28	28.59

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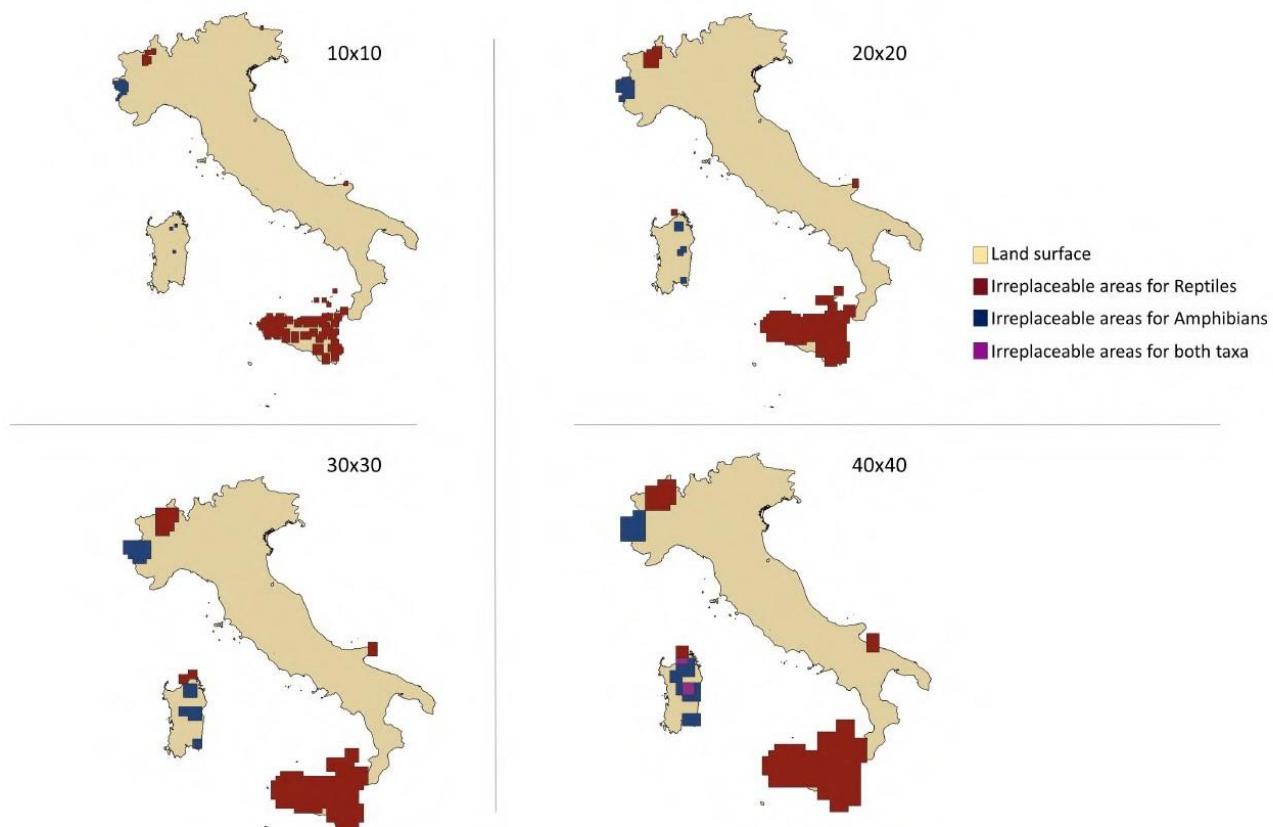
303 **Table 3:** Percentage (%) overlap between candidate KBAs and the current KBA, Protected area and  
304 Natura 2000 networks.

305

306 ***Potential KBAs under criterion E (Irreplaceability)***

307 Irreplaceable areas for amphibians are reported on the western Italian alps and in the island of  
308 Sardinia (Figure 4). In Sardinia, the extent of irreplaceable area increased when larger planning  
309 units were implemented. A single planning unit in the north of the island was irreplaceable using  
310 10x10 cells. When using larger planning units, the irreplaceable area extended towards the central  
311 and southern parts of the island. Irreplaceability maps for reptiles showed a high density of  
312 irreplaceable planning units in Sicily along with the Eolian islands, as well as in a small area of  
313 North-western Italian alps independently of the size of the planning unit (Figure 4). A few small  
314 areas in the South-eastern part of the country and in Sardinia were reported as irreplaceable, but

315 these areas were not the same across different planning unit sizes. For all identified irreplaceable  
316 areas we provided a list of species of which area of habitat is found within them (Appendix S1).



317  
318 **Figure 4:** Extent of irreplaceable areas triggered by criterion E for reptiles (red) and amphibians  
319 (blue), purple sites are irreplaceable for both taxa.

320

321

## 322 **Discussion**

### 323 ***Systematic detection of KBAs***

324 Using a set of grids with different cell sizes that can move along the land surface and scan the  
325 distribution of species' habitat availability, allowed us to detect multiple sets of potential KBAs.  
326 The number of output potential KBA maps can be high, depending on the number of cell  
327 resolutions and grid positions implemented to detect KBAs under different criteria. However,  
328 maps of potential KBAs identified using the same cell size can be combined to obtain a single map  
329 of KBAs, identified under single or multiple criteria. The resulting map accounts for all identified  
330 KBAs, independently of which specific cell and which grid configuration detected it. The extent of  
331 potential KBAs identified for criteria A1 and B1, as well as the number of species that were able to  
332 trigger them was affected by the size of the cell. For species with a relatively wide distribution

333 range such as *Bombina pachypus* for criterion A1 and *Euproctis platycephalus* for B1, potential  
334 KBAs can only be identified using larger cell sizes, as the total KBA extent drops to zero using  
335 10x10 km cells (Figure 3). The opposite is true for microendemics species such as *Vipera walser*,  
336 *Podarcis raffonei* and *Salamandra lanzai*, for which the extent of potential KBA is not affected by  
337 the size of the grid cells and remains constant. Thus, the selection of cell size is particularly  
338 relevant when testing KBA criteria for species which do not have a very narrow distribution range.  
339 Testing different sizes of grid cells provides information on the response of single or multiple  
340 species to the KBA criteria in the process of defining new potential KBAs.

341

342 We could identify potential KBAs under criterion B2 for 2 species only when using the largest cells  
343 (40x40 km), and no potential KBAs under criterion B3 were detected regardless of the  
344 implemented cell size. While A1 and B1 criteria can be triggered by a single species, both B2 and  
345 B3 criteria depend on the population distribution of more than one species, as they respectively  
346 refer to geographically co-occurring species and species assemblage (IUCN, 2020). In the Global  
347 Standards for the Identification of Key Biodiversity Areas, an assemblage is defined as a set of  
348 species within a taxonomic group for which 95% of the distribution range is confined to an  
349 ecoregion or bioregion for at least one life-history stage, or multiple species which share their  
350 most important habitats (IUCN, 2020). Despite being the richest Italian region for endemic species  
351 of amphibians (Sindaco et al., 2006), our approach could not detect potential KBAs under criterion  
352 B3 in the island of Sardinia, as well as in the rest of the peninsula using all four cell resolutions  
353 implemented in this study. The minimum cell size able to detect KBAs under criteria B2 and B3 is,  
354 as expected, linked to density and proximity of different species populations within a specific area.  
355 Our method was developed to efficiently detect potential KBAs for a set of species under multiple  
356 criteria, but not intended to delineate and propose new KBAs, as this process requires different  
357 approaches (IUCN, 2020). The purpose of use of this method is to support rapid KBA assessments  
358 in different regions of the planet, regardless of their geographic extent. The assessment should be  
359 followed by a more accurate evaluation of the identified potential KBA sites to define the true  
360 boundaries of a KBA.

361

362 **Extent, overlap and nestedness of KBAs**

363 Of all the potential KBAs identified using single criteria, the largest potential KBA network was  
364 identified using criterion A1, for which the extent of triggered sites covers, on average using all  
365 four cell sizes, 24226 km<sup>2</sup> of land surface. A large portion of the total area in central Italy for A1  
366 was triggered by *B. pachypus*, an endemic species of toad (Canestrelli et al., 2006). However, the  
367 species has been facing population decline over its distribution range in the Apennines during the  
368 last two decades (Stagni et al., 2010; Mori & Giovani, 2012; Talarico et al., 2020). Thus, many of  
369 the identified potential KBAs for criterion A1 may include sites where the species is not present  
370 anymore or close to local extinction.

371

372 Systematic conservation planning practices have been compared to the KBA identification  
373 process, as the two approaches share broad similarities in the way they aim at identifying  
374 important sites for biodiversity (Smith et al., 2018). In the context of conservation planning, using  
375 smaller planning units to achieve specific conservation targets was proven to be more efficient  
376 than using larger planning units (Pressey & Logan, 1998). The use of smaller planning units tends  
377 to maximize spatial and cost efficiency leading to easier compromises, and therefore provides  
378 better solutions to design conservation networks (Pressey & Logan, 1998; Hamel et al., 2012;  
379 Cheok et al., 2016). The percentage of overlap between potential KBAs detected using different  
380 criteria showed a tendency to decrease when using larger cells (Table 2). Thus, keeping the cell  
381 unit small may limit the KBA detection to areas which are most relevant for the conservation of  
382 one or several species under multiple criteria.

383

384 The overlap scores between the total network of potential KBAs (A1, B1 and B2) and current  
385 KBAs, national protected areas and Natura 2000 sites indicate that, regardless of the implemented  
386 cell size, a high percentage of the identified potential KBAs is currently located outside of the three  
387 different networks of important biodiversity areas (Table 3). In particular, ≥75% is excluded from  
388 the current KBA network, ≥76% is excluded from national designated protected areas and ≥65% is  
389 not located within the Natura 2000 network. We stress the importance of considering the  
390 identified sites as potential KBAs and not as proposed KBAs, as the presence of the species in  
391 those sites must be confirmed with a minimum threshold of mature individuals (IUCN, 2020).  
392 Therefore, the effective potential KBA extent of a site may be reduced when undergoing the  
393 proposal process. However, the current KBA network in Italy proves to be insufficient at capturing

394 potential KBAs for non-avian taxa. As new potential KBAs will be detected for other species, this  
395 evidence is likely to become stronger. Thus, rapid assessments of potential KBAs for high numbers  
396 of species will play an important role in bridging the gap of knowledge on the true distribution of  
397 KBAs globally. Methods to systematically apply KBA criteria such as the one presented in this  
398 study, can significantly accelerate the KBA mapping process.

399

400 ***Irreplaceable sites***

401 Areas with a high value of irreplaceability for both amphibians and reptiles were detected mainly,  
402 but not exclusively, where endemic species are known to occur (Tessa et al., 2007; Ficetola et al.,  
403 2021; Ficetola et al., 2020; Salvi et al., 2017). This suggests that the endemic status of a species is  
404 an important factor affecting the identification of KBAs under criterion E. Areas hosting endemic  
405 and microendemic species are more likely to be identified as irreplaceable. Using larger cells as  
406 planning units for our irreplaceability analysis implied the identification of larger irreplaceable  
407 sites which most likely do not represent important sites for the species which triggered criterion E.  
408 A large cell unit would capture a sufficient percentage of the global habitat distribution of a  
409 species to trigger criterion E. However, it also tends to capture a large amount of area where the  
410 species does not occur, simply because the whole planning unit will result as irreplaceable and  
411 thus, equally contributing to the achievement of the conservation targets. This circumstance is  
412 particularly evident in the areas hosting microendemic species, such as *Salamandra lanzai*,  
413 *Podarcis raffonei* and *Vipera walser*. The implementation of criterion E identified important areas  
414 of biodiversity which were not detected by criteria A1, B1, B2 and B3. Thus, underlying the  
415 important role of Criterion E as a support to the other KBA criteria, as previously suggested (Di  
416 Marco et al., 2016; Smith et al., 2018). For instance, a large extent of irreplaceable sites was  
417 identified within the island of Sicily, although these sites did not trigger KBAs under other criteria.  
418 This suggests that an integration of KBA criteria is needed to perform large scale assessments of  
419 potential KBAs.

420

421

422

423

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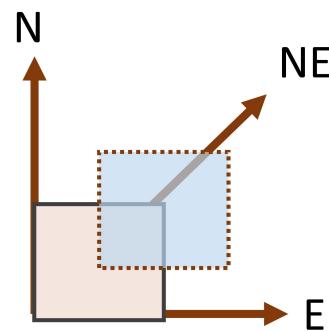
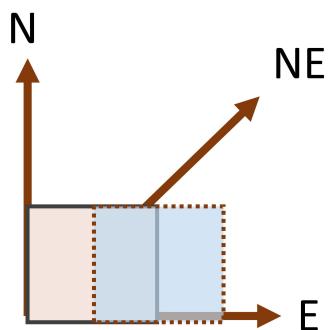
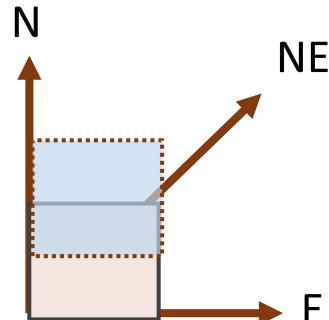
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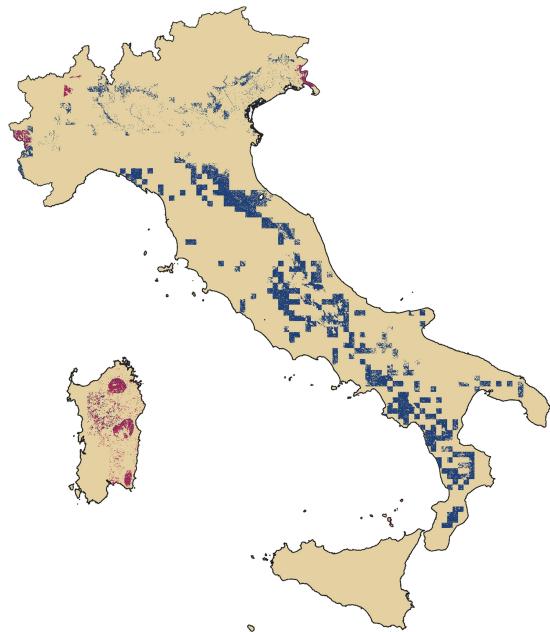
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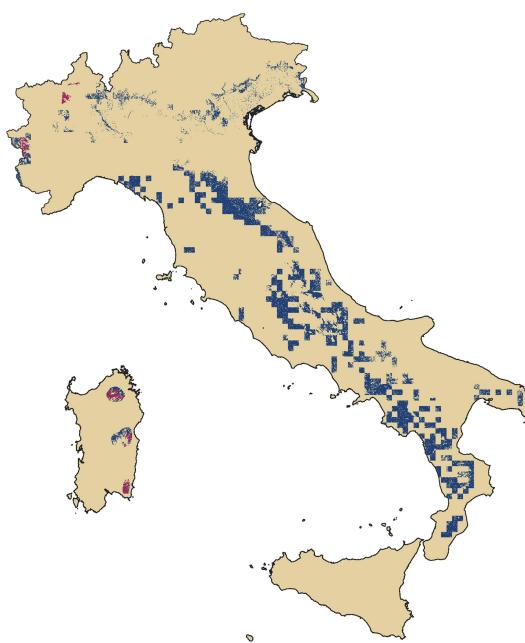


**Area of Habitat**  
**Land surface**  
**Grid**

40x40



30x30



Land surface  
Single criteria KBAs  
Multiple criteria KBAs

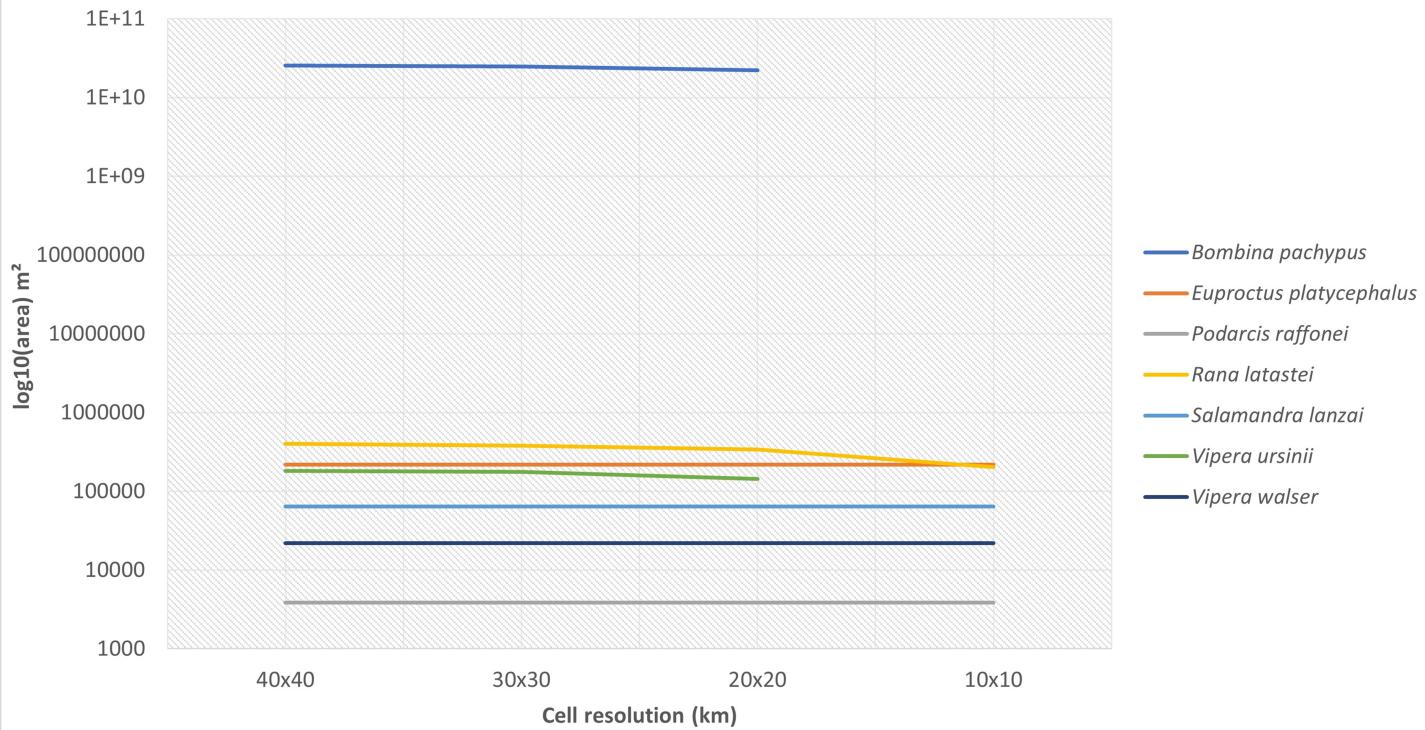
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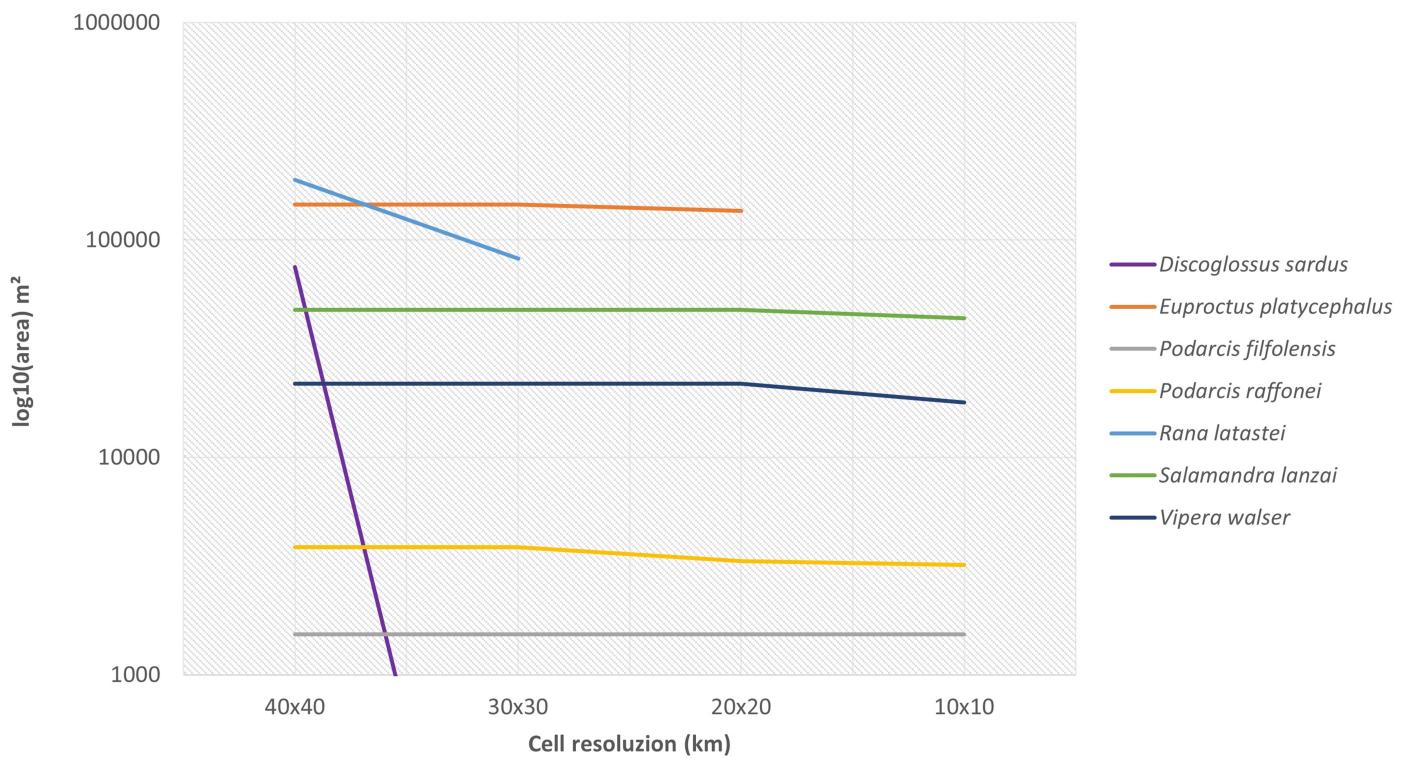
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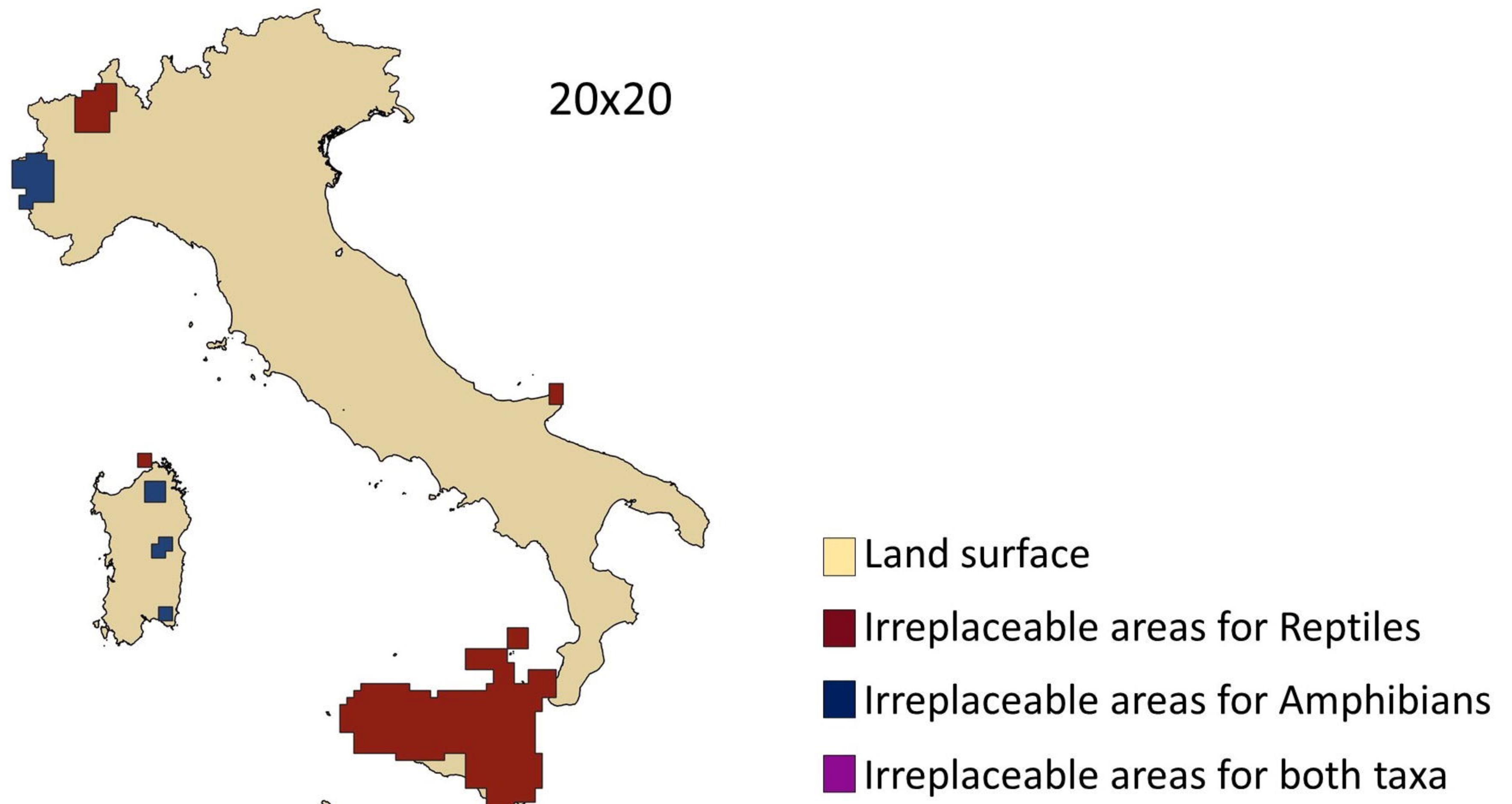
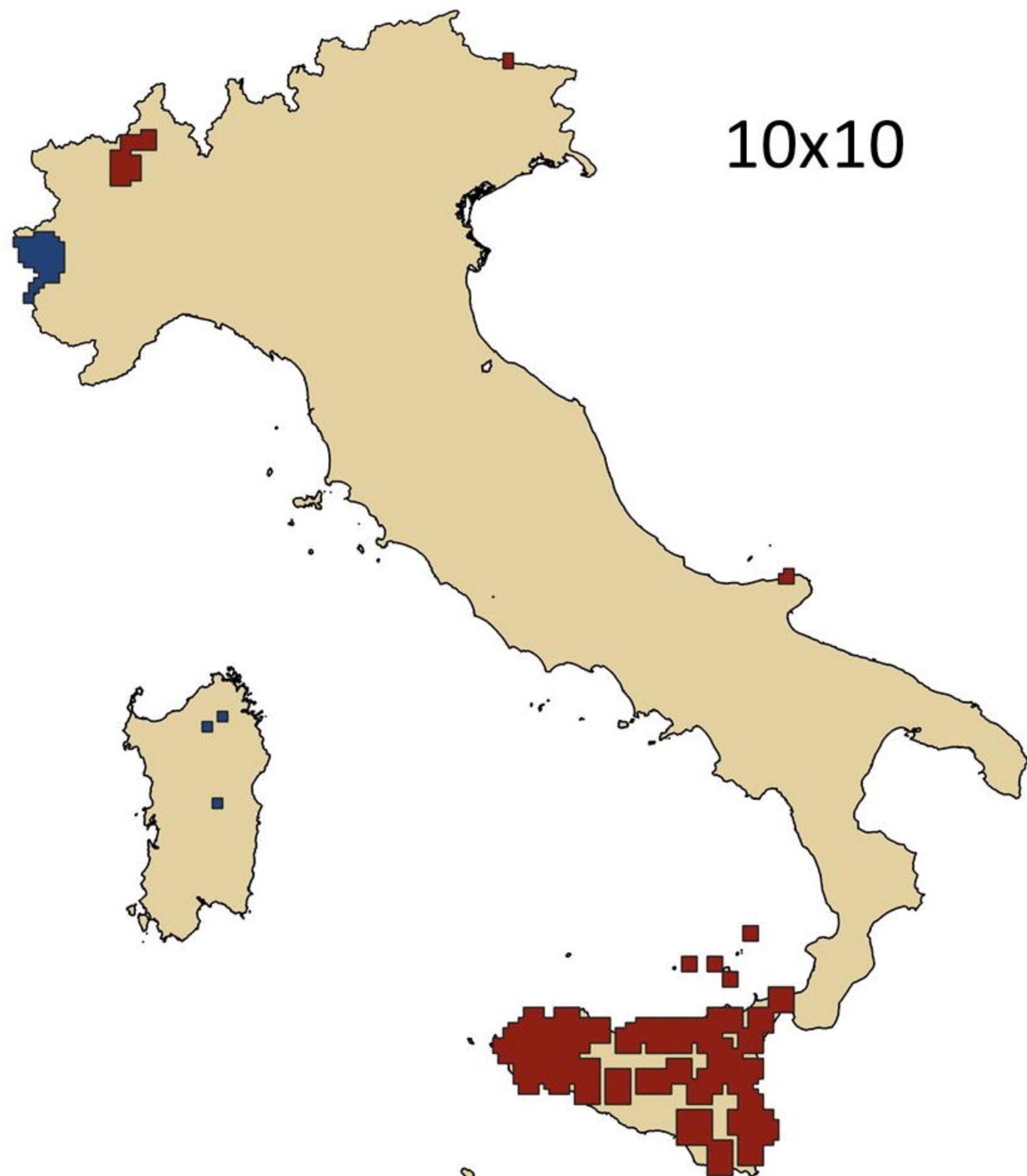


## Criterion A1

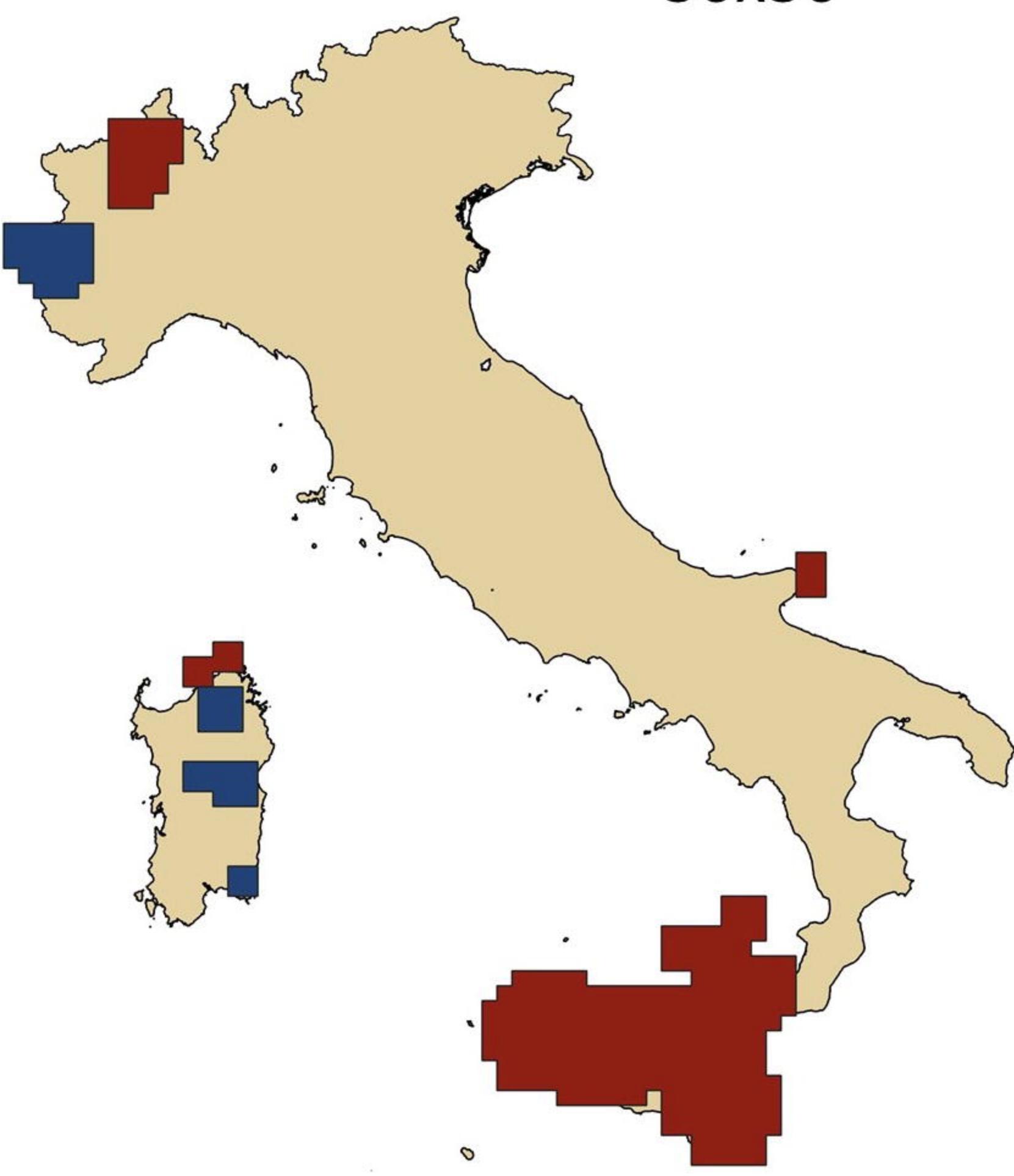


## Criterion B1





30x30



40x40

