Exploring the different facets of plant endemism in the South-Eastern Carpathians: a manifold approach for the determination of biotic elements, centres and areas of endemism

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In the European Alpine System, the Carpathian Mountains are recognized as one of the major centres of diversity and endemism. In the present study, we aimed to explain the spatial structure of plant endemism in its South-Eastern subunit by the complementary use of diversity indices, parsimony analysis of endemicity (PAE), biotic element analysis (BEA), and barrier analysis. We analyzed the available information on 111 plant taxa confined to the South-Eastern Carpathians, mapped using two different sets of operational geographical units (OGUs): 71 geomorphological units and 64 quadrats. Our results showed that centres of endemics diversity largely corresponded to the areas of endemism and biotic elements. PAE consensus cladogram outlined four major areas of endemism (with three nested ones): (1) Danubian; (2) western part of the Southern Carpathians; (3) eastern part of the Southern Carpathians; and (4) Pocutico-Marmarossian. Out of the seven identified biotic elements, five were spatially clustered and overlapped the major areas of endemism, with one notable exception: the calcareous massifs from the Eastern Carpathians, not identified through PAE. Conversely, the latter outlined a nested area of endemism (Cozia – Buila-Vânturarița), omitted by BEA. Barrier analysis identified three major breaks in the distribution of endemics: (1) south of the Retezat – Țarcu – Godeanu mountain group; (2) north of the Piatra Craiului – Bucegi – Ciucaș mountain group; and (3) north of the Rodna massif. The results obtained in here using different methods are generally spatially convergent, indicating highly structured patterns of endemism in the South-Eastern Carpathians. These patterns mostly follow the present-day distribution of alpine habitats and calcareous bedrock, which might have acted as isolating factors through insularity. Interestingly, three of the spatial clusters of OGUs obtained from the endemics distribution analyses (the Eastern Carpathians, as well as eastern and western parts of the Southern Carpathians) largely also correspond to the mid-Miocene

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[§]To the memory of my friend and colleague Alexandru Ioan Tatu (1991–2013), a young and passionate researcher of the Carpathian Mountains' nature.

archipelago configuration of landmasses in this part of the Carpathians. This might suggest the existence of older migration barriers that emerged throughout the Neogene Period. Differences in the spatial patterns outlined by PAE and BEA could stem from partial sympatry of endemics caused by post-speciation processes such as dispersal or extinction. Additionally, sympatric distribution of taxa with disjunct populations may be caused by the absence of divergence among segregated populations, such as the patterns of relict distributions seen in alpine plants. Finally, the complementary use of these methods may prove to be an efficient approach for better understanding the geographical structure of endemism and provide a starting point for further testing of hypotheses on evolutionary processes. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **00**, 000–000.

INTRODUCTION

Biogeographical patterns are shaped by processes that develop over a bidimensional scale, defined by time and space (Kruckeberg & Rabinowitz, 1985; Cracraft, 1994; Levin, 2000; Morrone, 2009; Noguera-Urbano, 2016). Among them, speciation, dispersal, and extinction are considered to be fundamental in driving any region's biotic component distribution and diversification in both space and time (Morrone, 2009; Wiens, 2011; Linder & Bouchenak-Khelladi, 2015). These processes are a result of both historical (developed at long time scales) and ecological factors (considered in recent periods) that have acted on the geographical ranges of species (Brown, Stevens & Kaufman, 1996).

Among all biological entities, the highest level of historical and ecological imprint is found in the distribution of endemics (Casazza *et al.*, 2008). They comprise spatially restricted species that are confined to one or few geographical units (Hobohm, 2014). Endemism characterizes all the biogeographical regions, developing at different spatial scales (Kruckeberg & Rabinowitz, 1985; Anderson, 1994) and across all major taxonomical groups (Crisp *et al.*, 2001; Marques & Peña Cantero, 2010; Szumik *et al.*, 2012; Fang *et al.*, 2013; Hoffmeister & Ferrari, 2016). Nevertheless, endemic species richness (SR) is distributed heterogeneously across Earth, being hierarchically organized and historically structured (Cracraft, 1994; Crother & Murray, 2013). Although it is known that islands harbour a high number of endemics (Kier *et al.*, 2009), similar high values of endemism have been observed on continents, in areas where the insular distribution of specific natural habitats and more prominent influence of historical processes led to areal limitations (García-Barros- *et al.*, 2002; Sklenář, Hedberg & Cleef, 2014). Such areas are mostly found in the mountain regions, which are known to act as refugia ('species museums') and diversification zones ('cradles of evolution'), especially for plant species (López-Pujol *et al.*, 2011b; Bitencourt & Rapini, 2013).

Studies on patterns of endemism have been a focus of either historical biogeography (Posadas, Crisci & Katinas, 2006) or ecological biogeography (Monge-Najera, 2008) and their results found application in conservation biology (Cavieres *et al.*, 2002; Huang, Qiao & Lei, 2010; Ladle & Whittaker, 2011). In this tradition, one prerequisite for any causal explanation on the current spatial patterns of endemics is to delimit the primary areas of distribution. Accordingly, several approaches have been proposed in the conceptual framework of biogeographical analyses (Posadas *et al.*, 2006; Morrone, 2014a). Among them, areas of endemism (AoE), centres of endemism (CoE), and biotic elements (BE) have been frequently employed in characterizing the spatial structure of endemism. These are defined by either high endemic SR (Laffan & Crisp, 2003) or by a congruent distribution of range-restricted taxa (spatial homology) (Morrone, 2001; Hausdorf, 2002; Szumik *et al.*, 2002).

In the recent literature, the terms were often indistinctly or ambiguously used when referring to either CoE or AoE. Although the drivers causing the emergence of both types of biogeographical units may indicate similar ecological or historical legacies, we emphasize that CoE should be differentiated from AoE because they relate to different methods of identification and are based on different assumptions (Linder, 2001). AoE can be defined as the 'congruent distributional limits of two or more species' (Platnick, 1991) and implies a method for testing this congruency (Linder, 2001; Morrone, 2009). This concept is based on the assumption that two sympatric taxa share a common biogeographical history (Morrone, 1994). By contrast, a CoE is an area including more endemics than its surroundings, regardless of the degree of distributional congruence among co-occurring taxa, therefore indicating a closer meaning to 'hotspots of endemism' (Laffan & Crisp, 2003). CoE were often used to infer historical explanations for the distribution of endemics (Crisp *et al.*, 2001; Jetz, Rahbek & Colwell, 2004; López-Pujol *et al.*, 2011a).

However, AoE are considered to be the fundamental units in historical biogeography, and their delimitation is required in any subsequent causal analysis on area relationships (Crisci, Katinas & Posadas, 2003).

The identification of AoE is in general not easily achieved. This is mainly because the primary distribution limits of overlapping ranges are masked by post-speciation processes such as dispersal or extinction (Szumik *et al.*, 2002). Several methods have been proposed for identifying AoE, and their advantages and applicability have been thoroughly reviewed (Linder, 2001; Szumik *et al.*, 2002; Crisci *et al.*, 2003; Moline & Linder, 2006; Giokas & Sfenthourakis, 2008; Carine *et al.*, 2009; Casazza & Minuto, 2009; Casagranda, Taher & Szumik, 2012; Escalante, 2015; Oliveira, Brescovit & Santos, 2015). Among them, parsimony analysis of endemicity (PAE) was the most frequently employed (Morrone, 2014b) and hereby our method of choice.

As a complementary viewpoint to the area relationship approach used for delimiting the AoE, Hausdorf (2002) proposed a taxon approach by applying biotic element analysis (BEA). Biotic elements are defined as 'groups of taxa whose ranges are significantly more similar to each other than to those of taxa of other such groups' (Hausdorf & Hennig, 2003). In other words, BEA implies neither exclusivity, nor homopatry with respect to identifying spatial congruency (the case of PAE; Escalante, 2015) but, instead, only similarity in ranges. Although BEA allows for the detection of cases where post-speciation dispersal or extinction events have occurred, it can also include potential area plesiomorphies (Cracraft, 1991). Moreover, we emphasize that biotic elements may be operationally closer to the concept of 'chorotypes' *sensu* Baroni Urbani, Ruffo & Vigna (1978), who used this concept in reference to groups of taxa with similar distributions (Fattorini, 2015).

CoE, AoE and biotic elements have been used as concurrent approaches in biogeography for characterizing the distribution patterns of endemics, with few exceptions (Casazza & Minuto, 2009). Nonetheless, given their subtle differences in addressing species range analysis, we consider their complementary and not alternative usage more suitable for a complete view over the distribution patterns of endemics in any given region. CoE and AoE imply individuality of biotas (common biogeographical history of the taxa), whereas biotic elements may include the temporal dimension (may be subsets of biotas) (Morrone, 2014a). All of these approaches allow us to explore the evolutionary and geographical/temporal (current) relationship of taxa, respectively.

The unique and highly diverse flora of the Carpathians consolidates their position as one of the major centres of mountain plant diversity and endemism (Pawłowski, 1970; Hendrych, 1982; Ozenda, 1995; Coldea, 2003), among the other European Alpine System mountains (Ozenda, 1985) (Fig. 1A): the Alps (Aeschimann, Rasolofo & Theurillat, 2011), the Pyrenees (Favarger, 1972) or the Northern Balkan Peninsula mountains (Petrova & Vladimirov, 2010; Tomović *et al.*, 2014). Although plant endemism has been explored by means of quantitative biogeographical methods in the Alps (Tribsch, 2004; Casazza & Minuto, 2009) or Pyrenees (García-Barros- *et al.*, 2002), similar approaches have been less frequent in the Carpathians. Quantitative studies were recently developed for the Western Carpathians concerning CoE in relation to the environmental factors and species traits (Mráz *et al.*, 2016). In the South-Eastern Carpathians, hierarchical clustering was previously used by Negrean & Oltean (1989) to characterize plant endemism, whereas Coldea *et al.* (2009) evaluated rarity and endemism in the alpine-subalpine flora through multivariate analyses. Studies on endemism in the South-Eastern Carpathians were mainly focused on (i) the overview of taxonomic or chorologic validity (Morariu & Beldie, 1976; Heltmann, 1985; Piękoś-Mirkowa & Mirek, 2003; Tasenkevich, 2003; Chorney, 2006, 2011; Hurdu *et al.*, 2012b; Novikoff & Hurdu, 2015); (ii) assessments of diversity (Piękoś-Mirkowa & Mirek, 2009; Hurdu *et al.*, 2012a; Tasenkevich, 2013); or (iii) phytogeographical regionalization (Georgescu & Doniţă, 1965) at a national scale, with few comprehensive studies synthesizing knowledge across all the Carpathian range (Pawłowski, 1970; Tasenkevich, 2005, 2014; Kliment, Turis & Janišová, 2016).

Based on the theoretical framework and assumptions described above, the main scope of the present study is to characterize the spatial structure of plant endemism in the South-Eastern Carpathians as a model for the complementary use of several widely applied quantitative biogeography methods. Consequently, our approach focuses on exploring the following aspects of plant endemism: (1) delimiting the centres of endemism considered as major hotspots of endemism; (2) identifying the AoE regarded as primary historical units; (3) determining the biotic elements as groups of significantly co-occurring endemic taxa (chorotypes); and (4) identifying the break zones in the distribution of endemics regarded as putative barriers that limited their dispersal.

MATERIAL AND METHODS

STUDY AREA

By contrast to the Alps, which cover a comparable area but feature higher elevations and extensive alpine habitats, the Carpathians are significantly lower, appearing as a more fragmented mountain
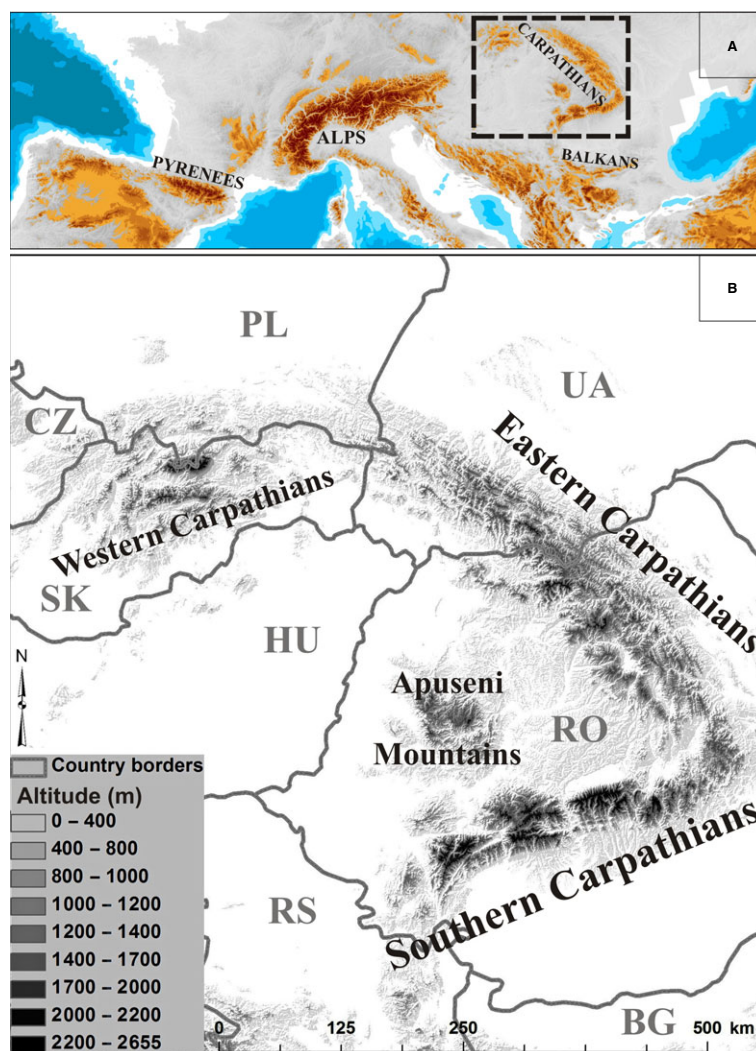
Figure 1. Study area. A, geographical position of the Carpathian Mountains in the European Alpine System. B, subunits of the Carpathians. CZ, Czech Republic; SK, Slovakia; PL, Poland; HU, Hungary; UA, Ukraine; RO, Romania; RS, Republic of Serbia).

range, with an insular-type distribution of both alpine zone and geological structure (Mihăilescu, 1963). Two important historical events might have driven plant endemism in the South-Eastern Carpathians throughout the Neogene and Quaternary Periods. First, repeated marine transgressions and regressions of the Paratethys (Harzhauser & Piller, 2007), coupled with the landmass uplifts (Popov *et al.*, 2004), led to an archipelago configuration of Carpathian palaeo-islands during the Miocene and Pliocene. Second, because of the relatively isolated action of glacial processes during the Pleistocene, expressed mainly in the highest zones of the Western (the High Tatras), Southern (e.g. the Făgăraş, Parâng or Retezat Mountains) or Eastern Carpathians (e.g. the Rodna Mountains), many areas

remained unglaciated, acting as important refugia for many cold-adapted or even temperate plants (Schmitt, 2009; Ronikier, 2011).

The Carpathian Mountain Range (CMR) is traditionally divided into two major subunits, as a result of a high geomorphological differentiation (Mihăilescu, 1963): (1) Western Carpathians and (2) South-Eastern Carpathians. This cleavage has also been outlined by the existence of a major break in the distribution of both endemic taxa (Pawłowski, 1970) and alleles in the genetic structure of subalpine or alpine plants (Ronikier, 2011). The specific delimitation of the two subunits has been an issue of debate, with several alternatives proposed based on phytogeographical arguments (Zemanek, 1991). For the purpose of the present study, we adhere to the

general view, which places the geographical border on the Łupków Pass (Pawłowski, 1970; Kliment *et al.*, 2016; Mráz *et al.*, 2016).

The South-Eastern Carpathians extend over a large latitudinal gradient, from Poland and Slovakia through Ukraine, Romania to North-Eastern Serbia. They are further subdivided into three distinct units: the Eastern Carpathians, the Southern Carpathians, and the Apuseni Mountains (Fig. 1B). The first two are clearly differentiated by their main axis of folding, the Eastern Carpathians extending from north-west to south-east, whereas the Southern range is oriented on a longitudinal east–west direction. However, their accurate delimitation is not straightforward. From a geographical perspective, one boundary was proposed along the Prahova Valley (Mihăilescu, 1963; Pop, 2006), although other geographers placed the limit even more to the west (Badea, Băcăuan & Posea, 1983). In terms of altitude, the Southern Carpathians possess an extensive alpine environment, mostly developed in the Făgăraş, Bucegi, Retezat, and Parâng massifs, where altitudes exceeding 2300 m are frequent. By contrast, the Eastern Carpathians seldom rise above 2000 m (in the Rodna, Chornohora or Călimani Mountains). Lastly, the Apuseni Mountains appear as an isolated unit north of the Mureş Valley, characterized both by lower elevations (up to 1849 m in Curcubăta Mare) and the absence of true alpine zone (Fig. 1B).

In a recent study by Kliment *et al.* (2016), the intra-Carpathian basin (Transylvanian Basin) is included as part of the CMR (*sensu* Kondracki, 1989). This approach has important implications concerning the meaning of plant endemism and its evolution in the CMR. We admit that clear advantages ensue from a holistic spatial approach in biogeography, which is possible here by including the Transylvanian Basin in the spatial context of a Carpathian Region. However, the clear differences in both geomorphological and vegetation aspects do not justify its rank as a true mountainous region. Therefore, we did not include this geographical view in the present study, which discusses the endemism phenomenon strictly related to the concept of orobiom (Ozenda, 2002).

SELECTION OF TAXA

Evaluation of endemism implies solving two main aspects prior to the selection of taxa: the spatial meaning of endemism (Anderson, 1994; Peterson & Watson, 1998) and the recognition of taxa as distinct evolutive units (Riddle & Hafner, 1999; Kelt & Brown, 2000).

First, in terms of spatial extent, Carpathian endemics are traditionally classified into three main categories (Pawłowski, 1970; Hurdu *et al.*, 2012b; Kliment *et al.*, 2016; Mráz *et al.*, 2016): (1) pan-Carpathian endemics occurring in both major subunits of the CMR; (2) taxa confined to the Western subunit and termed Western Carpathian endemics; and (3) South-Eastern Carpathian endemics including elements distributed exclusively in this subunit, irrespective to their distribution range inside the region. Additionally, taxa mainly distributed in the CMR, although with few occurrences outside their limits, were considered Carpathian subendemics (Pawłowski, 1970; Hurdu *et al.*, 2012b; Kliment *et al.*, 2016; Mráz *et al.*, 2016).

Second, the choice of operational taxonomic units previously centred only on their morphological differentiation when assigning the taxonomic status of endemics (Pawłowski, 1970; Morariu & Beldie, 1976; Heltmann, 1985). Only recently have molecular studies focusing on genetic divergence of taxa provided more insights for delimiting cryptic lineages with low morphological differentiation (Kuzmanović *et al.*, 2013; Ronikier & Zalewska-Gałosz, 2014; Surina *et al.*, 2014).

Therefore, there were many views on the geographical and taxonomic validation of endemics, at both national (Morariu & Beldie, 1976; Stoyko & Tasenkevich, 1991; Kricsfalusy & Budnikov, 2000; Malynovskiy *et al.*, 2002; Piękoś-Mirkowa & Mirek, 2003; Tasenkevich, 2003; Chorney, 2006, 2011; Hurdu, 2012; Hurdu *et al.*, 2012a,b; Novikoff & Hurdu, 2015) and regional (Pawłowski, 1970; Heltmann, 1985; Negrean & Oltean, 1989; Tasenkevich, 2014; Kliment *et al.*, 2016) levels. The differences in view were generated mostly by the chorological data availability, the author's accepted taxonomic concept and the strictness of criteria regarding the range limits applied in the selection of endemics. As a result of these differences among authors, although biodiversity evaluation was targeted by their surveys, they were mostly prolific for generating a 'diversity of endemic taxa lists'.

Several syntheses on the Carpathian endemics (Pawłowski, 1970; Morariu & Beldie, 1976; Stoyko, 1977; Heltmann, 1985; Negrean & Oltean, 1989; Stoyko & Tasenkevich, 1991; Kricsfalusy & Budnikov, 2000; Malynovskiy *et al.*, 2002; Piękoś-Mirkowa & Mirek, 2003; Tasenkevich, 2003; Chorney, 2006, 2011; Hurdu, 2012; Hurdu *et al.*, 2012a,b; Novikoff & Hurdu, 2015; Kliment *et al.*, 2016) were used for generating our final list of accepted taxa. Our selection process centred on several criteria, in accordance with our aims: (1) the taxa should be confined to the area of interest to increase the strength of range restriction signal; (2) their taxonomic status should be clear to avoid any uncertainties generated by 'Linnean' (taxonomic) shortfalls; and (3) good

distribution information over the whole study area was required for reducing inherent 'Wallacean' (chorological) shortfalls (Whittaker *et al.*, 2005). In accordance to the first criterion, taxa with few occurrences beyond the CMR designated limits, defined as sub-endemics in the recent literature (Hurdu *et al.*, 2012b; Novikoff & Hurdu, 2015; Kliment *et al.*, 2016), were not considered in the analyses. Additionally, we excluded the pan-Carpathian endemics, with their distribution range spanning over to the Western Carpathians and thus becoming a special case of sub-endemics in reference to the spatial extent of the present study. Moreover, based on our accepted view of the South-Eastern Carpathians (see above), taxa considered as Carpathian endemics by Kliment *et al.* (2016) but occurring in the Transylvanian Basin were not included in these analyses. Finally, by applying the second and third criteria, critical taxa, together with several mostly apomictic groups (e.g. *Hieracium*, *Alchemilla*, *Rubus*), were also removed from the list. The poor level of information concerning both their taxonomic status and their distribution could have led to false area relationships by inducing biogeographical noise in the analyses. In the end, 111 endemic plant species or subspecies were selected by applying these criteria (see Supporting information, Appendix S1).

DISTRIBUTION DATA AND SELECTION OF OPERATIONAL GEOGRAPHICAL UNITS (OGUs)

Choosing an appropriate system of spatial units is of major importance in any quantitative biogeographical inference (Crovello, 1981). The choice of units depends on the aims of the study and methods employed. Although natural units (e.g. ecoregions, geomorphological units) are recommended for analyzing the relationship between previously identified AoE (Cracraft, 1991; Escalante, Morrone & Rodríguez-Tapia, 2013), the search for AoE is more practical by using quadrats of comparable size (Morrone, 2014b). Because endemism is spatial scale-dependent, the size of units can influence the resulting structure of pattern analysis (Morrone & Escalante, 2002; Casagranda, Roig-Juñent & Szumik, 2009). This is valid especially when analyzing pattern similarity. In the identification of AoE, mapping the ranges of species based on very small geographical units will generate highly discontinuous distributions and reduce the chances of successfully identifying overlapping ranges (Szumik *et al.*, 2002). Similarly, increasing the grain size too much will lead to excessive sympatry and unrealistic AoE characterized by a high number of synendemic taxa, therefore obscuring much of the biogeographical signal (Szumik *et al.*, 2002).

Distribution of endemics was assessed based on current knowledge resulting from field surveys, herbarium collections and, most importantly, floristic syntheses at a national level. We used two different sets of OGUs for building presence/absence matrices of the distribution of endemics. First, to assess the natural distribution of diversity and identify putative dispersal barriers reflected by relief and geological traits, we registered the occurrences of endemics based on the operational geomorphological units (nOGUs) (see Supporting information, Appendix S2). These were delimited according to studies by Pop (2006), Mihǎilescu (1963), and Chopyk (1977), with a few modifications being made to keep a comparable area between nOGUs, at the same time as taking into account the geological uniformity and natural topography. Large intra-mountainous depressions and hilly areas were not considered for thr distribution of endemics because we aimed to analyze only the floristic links between well-differentiated mountain units. Second, we used an artificial grid system for identifying AoE, which is an approach recommended by Morrone (2014b). The quadrat size (24′ latitude and 40′ longitude, or approximately 45 × 50 km) was eight-fold the quadrat size applied for mapping the distribution of Central European flora (Niklfeld, 1971). Despite using a geographical coordinate system, the difference in the area of quadrats along latitude was not large (< 11%).

RICHNESS AND RARITY PATTERN ANALYSIS

Laffan & Crisp (2003) described centres of endemism (CoE) as hotspots of richness in range-restricted taxa. It is accepted that endemism is highly indicative for assigning priorities in conservation of important plant areas (Zhao *et al.*, 2016). In this context, we analyzed the diversity patterns by employing two different measures: species richness (SR) and weighted endemism (WE) (Crisp *et al.*, 2001; Kier *et al.*, 2009; Mráz *et al.*, 2016). Species richness is a simple count of taxa within each OGU; thus, it cannot provide any information on their range size. For this purpose, we calculated new values for each taxon by weighting them by the inverse of their range. This approach results in higher values (with a maximum of 1 for single-OGU occurring endemics) for rare taxa instead of equal values for both rare and widespread taxa. As a consequence, areas with high richness in widespread taxa will have comparable values with poorer areas that have more range-restricted taxa. Finally, SR and WE were mapped in ARCGIS, version 9.3.1 (ESRI, 1999-2009) for spatial visualization of distribution patterns. Finally, we considered areas where high endemism (either SR or WE) was spatially clustered as CoE.

IDENTIFYING AREAS OF ENDEMISM

We used PAE to identify the AoE in the South-Eastern Carpathians. This approach involves applying a phylogenetic algorithm to a species × area matrix to obtain an area cladogram (Morrone, 1994, 2014b; Escalante, 2015). Similar to phylogenetic inferences, where a clade is diagnosed by synapomorphies (Felsenstein, 2004), one area is considered as a candidate AoE when it is diagnosed by at least two geographical autapomorphies (autendemic taxa) or synapomorphies (synendemic taxa) (Rosen, 1992). In this case, taxa are treated as characters in the area cladogram, whereas a congruent distribution of two or more taxa (also named geographical apomorphies) may indicate a shared evolutionary history in the distribution area. Several other approaches have been proposed, including the extensively used endemicity analysis (EA) (Szumik *et al.*, 2002). Although EA was shown to outperform PAE in the number of identified AoE (Escalante, Szumik & Morrone, 2009; Casagranda *et al.*, 2012), it was also shown to not recover all the cases of homopatry in a strict comparison with PAE (Escalante, 2015) and also to overestimate the number (redundancy) and boundaries (spurious areas) of putative AoE (Casagranda *et al.*, 2012; Sandoval & Ferro, 2014).

We based our searches on a presence/absence matrix of 64 quadrats × 111 endemic taxa (see Supporting information, Appendix S1). A hypothetical area (A0) with all taxa absent was added for rooting the tree (Morrone, 1994). We chose a greater quadrat size to decrease the OGU/species ratio, which is a recommended approach for obtaining better resolved trees by increasing the number of geographical synapomorphies (Morrone & Escalante, 2002). We used TNT, version 1.5-beta, with Sectorial Searches, Ratchet, Drift, and Tree fusing algorithms set as default (Goloboff, Farris & Nixon, 2008), to search for most parsimonious trees. To reduce homoplasy during searches (Goloboff, 1993; Escalante *et al.*, 2007, 2013), implied weighting was used (with constant of concavity set to $k = 3$). Multiple search runs were performed by changing the seed from 1 to 100, which was shown to be a good approach for exploring supplementary sub-optimal islands, avoiding local optima, and retaining a high number of equally most parsimonious trees (Giribet, 2007).

BIOTIC ELEMENT ANALYSIS

Biotic element analysis provides two different predictions for testing: (1) taxa originating in distinct AoE by vicariance events belong to different biotic elements and (2) closely-related taxa that diverged through a vicariance event do not have overlapping ranges (Hausdorf, 2002; Hausdorf & Hennig, 2003; Hennig & Hausdorf, 2004, 2006). Because the second prediction implies the existence of phylogenetic information, we tested only the first prediction of vicariance.

We applied model-based Gaussian clustering implemented in package 'prabclus' (Hausdorf & Hennig, 2003; Hennig & Hausdorf, 2004) developed in the R environment (R Core Team, 2013–2016) to the endemics distribution data. We used the artificial grid-based dataset to correlate our results with those obtained from PAE. Default settings were used, with the 'kruskal' method for non-metric multidimensional scaling (four dimensions) and the maximum number of clusters set to 9. *Sensu* Hennig & Hausdorf (2006), we chose the distance-based '*geco*' coefficient, which is a generalized version of the Kulczynski index that also accounts for spatial autocorrelation. In addition, this coefficient is considered to be more independent against potential incomplete sampling.

To test the first prediction of vicariance, we employed a parametric bootstrap test and compared the clustering of distributions in our dataset against a null model by using 'distratio' statistics (i.e. the ratio between the 25% smallest and 25% largest distances between ranges of endemics) (Hennig & Hausdorf, 2004; Hausdorf & Hennig, 2006). We used a Monte Carlo simulation (1000 runs) to generate random distributions and to test whether the ratio in our dataset differs significantly from that expected under the null (homogeneity) hypothesis, thus indicating a significant spatial clustering. The resulting biotic elements were mapped in ARCGIS, version 9.3.1, as percentage of biotic element occurring in each quadrat.

IDENTIFYING BREAKS IN DISTRIBUTION

BARRIER, version 2.2 (Manni, Guérard & Heyer, 2004) was used to identify important breaks in the distribution of endemics, having previously been shown to be a suitable approach for species distribution pattern (Thiel-Egenter *et al.*, 2011). We first calculated the Kulczynski distance index between each pair of nOGUs, based on their endemic taxa composition. Considering the uneven distribution of SR, the Kulczynski index is recommended as a measure independent of differences in richness between spatial units (Hausdorf & Hennig, 2003). The resulting distance matrix was used to generate different sets of clusters ($k = 2–7$) by applying fuzzy clustering algorithm (Kaufman & Rousseeuw, 1990), implemented in the R package 'cluster', version 2.0.4 (Maechler *et al.*, 2016). This algorithm attempts to assign cluster membership probabilities to each analyzed unit,

by minimizing a dispersal function. We used three different membership exponent values ($r = 1.1–1.3$) in order to obtain increasingly crisper clusterings and to avoid arbitrary settings. Higher values ($r > 1.3$) were not used because they led to complete fuzziness and equivalence in membership probabilities. The maximum number of clusters was set to 7, corresponding to the total number of biotic elements previously identified. Finally, we used the cluster membership probabilities (for all $r \times k$ runs) to compute Euclidean distances between nOGUs, resulting in a total of 18 different distance matrices.

The centroid coordinates of all geographical units (see Supporting information, Appendix S3) were used to generate a Delaunay triangulation in BARRIER, version 2.2, which identifies the spatially neighbouring nOGUs. We searched for breaks in the distribution of endemics using Monmonier's maximum distance algorithm, applied to the Euclidean distance matrices obtained from clustering, which were associated with the centroids. We opted for $N = 6$ barriers, in accordance with the maximum number of clusters ($k_{max} - 1$) identified through fuzzy clustering. As an indicator of significance, the robustness value of each barrier was calculated by counting the number of times it develops between the same geographical units, when all distance matrices are used. The barriers were mapped in ARCGIS, version 9.3.1, and associated robustness values were used to calculate line densities using a moving window technique with a search radius of 0.1 decimal degrees. The lengths of the lines included in the search radius were multiplied by their robustness values, whereas the results are shown as a colour gradient.

RESULTS

Endemic plants diversity in the South-Eastern Carpathians and the main centres of endemism

The distribution of endemic plant diversity displayed a high level of spatial clustering across the South-Eastern Carpathians. Endemic species richness (SR) (Fig. 2A) and weighted endemism (WE) (Fig. 2B) mostly followed a similar pattern of insular distribution across the whole range, with highly correlated values (Spearman's correlation test, $\rho = 0.928$, $P < 0.001$) (Table 1). The highest 25% values for both indices (Fig. 2, white nOGU code labels; Table 1, values highlighted in grey) outlined five major centres of endemism (as spatially clustered hotspots of endemic plant diversity): (1) the Rodna, Maramureș, and Chyvchyny-Gryniava mountainous region; (2) the Moldavian calcareous belt (including the Ceahlău, Giurgeu-Hășmaș, and Rarău massifs); (3) the eastern part of the Southern Carpathians (including the Bucegi, Piatra Craiului, Bârsei, and Făgăraș Mountains); (4) the western part of the Southern Carpathians (including the Parâng, Retezat, and Piule-Piatra Iorgovalului massifs); and (5) the Mehedinți Mountains.

The highest values for endemic SR among the centres of endemism were recorded in the Rodna Mountains (50), the massifs of the Moldavian calcareous belt (Giurgeu-Hășmaș: 46 taxa; Ceahlău: 42 taxa), and the eastern part of the Southern Carpathians (Bucegi: 49 taxa; Făgăraș: 47 taxa; Piatra Craiului: 46 taxa). These nOGUs harboured over 72% of the total number of endemics (111), despite covering only 5.66% of the total investigated area (99 000 km^2) (see Supporting information, Appendix S3). Conversely, SR and WE differed in several important aspects. The first notable difference was reflected in the distribution of range-restricted taxa, where the Mehedinți Mountains possessed the highest weighted endemism (WE = 6.48), despite having much lower endemic species richness (SR = 31). Similarly, the Gilău-Muntele Mare and especially Cozia massifs had more range-restricted endemics (WE = 2.43 and 2.83, respectively), although harbouring a low number of endemics (SR = 24 and 17, respectively). In addition, the Gilău-Muntele Mare massif was the richest from the Apuseni Mountains, in terms of both SR and WE but, at the scale of the entire South-Eastern Carpathians, it did not emerge as a major centre of endemism. Second, the Călimani and especially Ciucaș Mountains had higher-ranked SR values (SR = 27 and 32, respectively) compared to their WE values (1.86 and 2.12, respectively), apparently not holding many range-restricted endemics.

When compared with the total area of each nOGU (see Supporting information, Appendix S3), the highest densities of endemics were observed in the massifs predominantly or largely represented by limestone bedrocks and carbonate conglomerate bedrocks. These were located in both the Eastern and the Southern Carpathians (in descending order of values: the Buila-Vânturarița, Piule-Piatra Iorgovanului, Piatra Craiului, Bucegi, Ceahlău, and Bârsei Mountains), outranking the alpine massifs of Rodna and Făgăraș dominated by crystalline bedrock.

AREAS OF ENDEMISM

The searches performed through PAE resulted in 647 equally parsimonious trees [best score: 43, length (L) = 468, consistency index (CI) = 0.23, retention index (RI) = 0.62] that were used to obtain the strict consensus cladogram (Fig. 3A). A total of 32 geographical synapomorphies and autapomorphies were identified in the consensus cladogram, of which 17 outlined four major AoE (Fig. 3B, Table 2). Additionally, three nested areas were characterized by autendemic taxa (Fig. 3A,
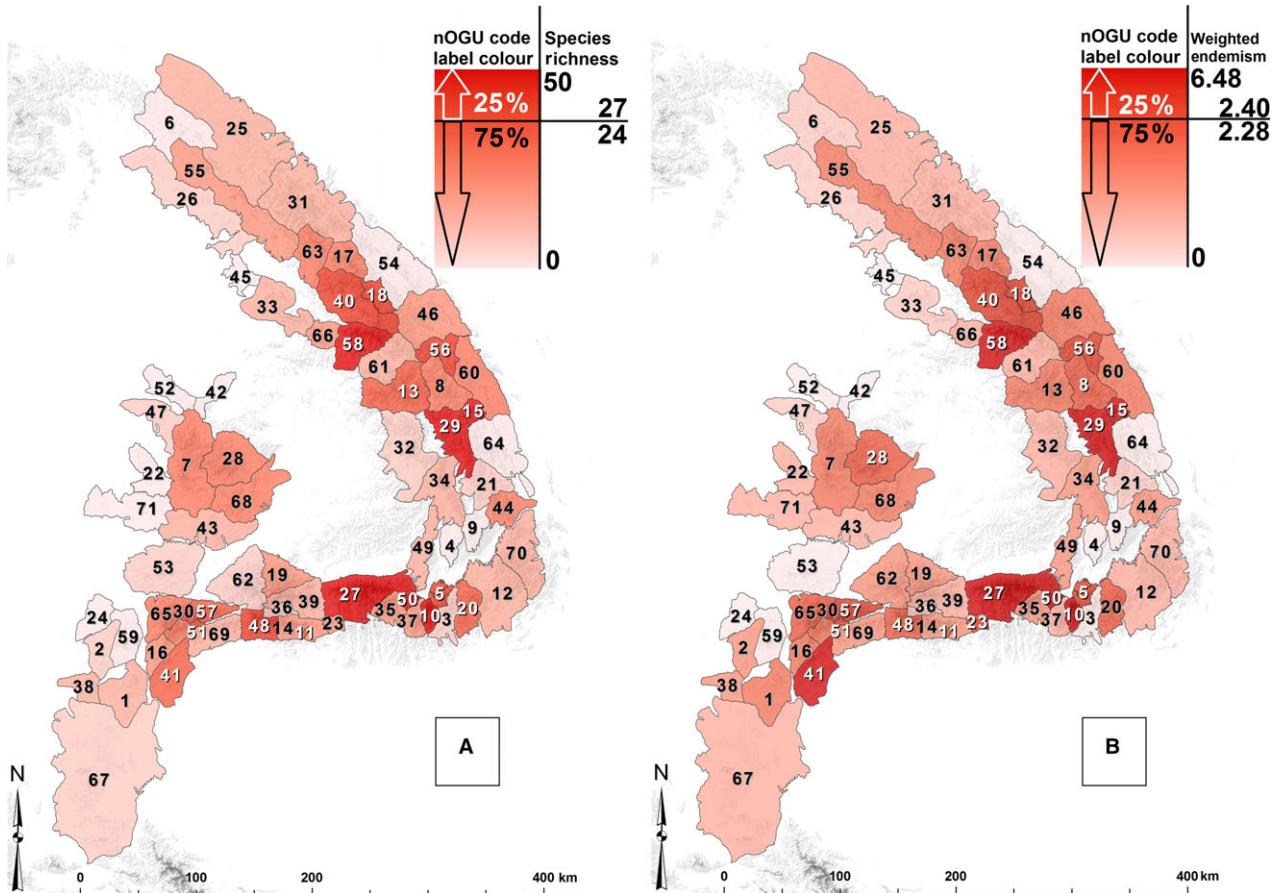
Figure 2. Patterns of endemic plant taxa richness (A) and weighted endemism (B) in the South-Eastern Carpathians. Values for species richness/operational geomorphological unit (nOGU) ranged from 50 endemic taxa in the Rodna Mountains (nOGU code 58) to 0 endemic taxa/nOGU in the Baraolt Mountains (nOGU code 4). Values for weighted endemism/nOGU ranged from 6.48 in the Mehedinți Mountains (nOGU code 48) to 0 in the Baraolt Mountains (nOGU code 4). Codes for each nOGU represented on the maps correspond to Table 1. nOGUs with the top 25% values for each index are labelled in white, whereas nOGUs with the bottom 75% values for each index are labelled in black.

star symbol; Table 2, A2.1, A3.1, and A4.1). Autapomorphies are not commonly used to identify AoE in PAE cladograms because they are not informative for the quadrats relationships. However, two or more geographical autapomorphies may appear synapomorphic when a different scale of analysis with smaller sized OGUs would be applied. Therefore, we also considered them as diagnostic geographical apomorphies. The four AoE identified in the present study corresponded to the following mountainous regions (Fig. 3A, B):

A1: The Danubian mountainous region (quadrat 135), including the Almăjului Mountains and the Iron Gates area, south of the Mehedinți Mountains. Four geographical autapomorphies characterized it: *Cephalaria uralensis* subsp. *multifida*, *Prangos carinata*, *Stipa danubialis*, and *Tulipa hungarica*.

A2: The western part of the Southern Carpathians (quadrats 207–208), corresponding to the Retezat–

Țarcu–Godeanu mountain group and the Piule–Piatra Iorgovanului calcareous massif, was supported by two synendemic taxa (*Centaurea phrygia* subsp. *ratezatensis* and *Carduus kerneri* subsp. *lobulatiformis*). One nested area of endemism (A2.1, quadrat 208) included here had three autendemic taxa (*Anthemis kitaibeli*, *Barbarea lepuznica*, and *Festuca pachyphylla*).

A3. The eastern part of the Southern Carpathians (quadrats 209–212 and 246–247), delimited the Southern Carpathians east of the Jiului valley, and had two synendemic taxa (*Aquilegia transsilvanica* and *Festuca bucegiensis*). Similar to A2, one nested area of endemism was included in A3, corresponding to the isolated massifs of Cozia and Buila-Vânturarița (A.3.1, quadrat 210), with two autendemic taxa (*Rosa villosa* subsp. *coziae* and *Stipa crassiculmis* subsp. *heterotricha*).

Table 1. Species richness (SR) and weighted endemism (WE) values across the 71 operational geomorphological units (nOGUs)

nOGU code	nOGU name	SR	WE	nOGU code	nOGU name	SR	WE
58	Rodna	50	6.19	46	Obcinele Bucovinene	16	1.46
10	Bucegi	49	4.88	70	Vrancei	14	0.64
27	Făgăraș	47	4.62	12	Buzăului	14	0.49
50	Piatra Craiului	46	5.28	69	Vâlcan-Oslea	12	0.90
29	Giurgeu-Hășmaș	46	5.04	43	Metaliferi	12	0.70
15	Ceahlău	42	4.41	1	Almăjului	11	1.79
5	Bârsei	39	3.35	31	Gorgany	11	0.55
56	Rarău-Giumalau	37	2.94	34	Harghita	10	0.82
40	Maramureș	35	3.54	49	Perșani	10	0.81
48	Parâng	34	2.67	61	Suhard-Bârgău	10	0.60
18	Chyvchyny-Gryniava	33	3.04	25	Eastern Beskyds	10	0.60
20	Ciucaș-Grohotiș	32	2.12	39	Lotrului	10	0.50
41	Mehedinți	31	6.48	33	Gutâi	10	0.43
57	Retezat	30	3.63	38	Locvei	9	0.94
11	Buila-Vânturarița	30	2.28	2	Aninei	8	0.91
51	Piule-Piatra Iorgovanului	28	2.40	32	Gurghiu	8	0.51
13	Călimani	27	1.86	47	Pădurea Craiului	8	0.38
8	Bistriței	24	2.52	36	Latorița	8	0.32
28	Gilău-Muntele Mare	24	2.43	26	Eastern Volcanic Ridge	7	0.43
17	Chornohora	24	1.81	3	Baiului	7	0.21
65	Țarcu	23	2.09	62	Șureanu	6	1.13
7	Bihor-Vlădeasa	23	1.46	21	Ciucului	6	0.26
63	Svydovets	22	1.76	67	Transdanubian	5	0.66
30	Godeanu	22	1.41	53	Poiana Ruscă	5	0.15
60	Stânișoarei	22	1.35	71	Zarand	4	0.63
68	Trascău	21	1.81	6	Bieszczady	4	0.25
44	Nemira	21	0.85	59	Semenic	4	0.20
35	Iezer-Păpușa	20	1.17	9	Bodoc	4	0.12
55	Polonyna Krasna-Boržava-Rivna	19	1.66	54	Pokutsko-Bukovynski Karpaty	3	0.19
16	Cernei	19	1.54	45	Oaș	3	0.16
19	Cibin	19	1.13	64	Tarcău	3	0.09
66	Țibles	18	0.74	42	Meseș	2	0.12
37	Leaota	18	0.69	52	Plopiș	2	0.07
23	Cozia	17	2.83	22	Codru-Moma	1	0.50
14	Căpățânii	17	0.97	4	Baraolt	0	0.00
				24	Dognecea	0	0.00

The nOGUs were arranged in descending order of SR, with the top 25% values for each index framed and highlighted in grey; their corresponding codes are shown as white nOGU labels in Fig. 2A for SR and Fig. 2B for WE.

A4. The Pocutico-Marmarossian mountainous region (quadrats 390–391 and 426–427), corresponding to the Rodna, Maramureș, and Chyvchyny-Gryniava Mountains, had two perfectly sympatric endemics (*Festuca versicolor* subsp. *dominii* and *Minuartia verna* subsp. *oxypetala*). A nested area of endemism, overlapping the eastern part of the Maramureș Mountains and the Chyvchyny-Gryniava Mountains, had two autendemic taxa (*Cochlearia borzaeana* and *Nigritella carpatica*).

Notably, A3 also had a branch (quadrats 247, 211, and 212) with partially sympatric taxa (*Dianthus callizonus*, *Primula wulfeniana* subsp. *baumgarteniana*, and *Saxifraga mutata* subsp. *demissa*). Another two quadrat groups in the PAE cladogram had a similar structure. The first one corresponded to the calcareous mountains of the Eastern Carpathians (quadrats 320 and 356; represented by the Hășmaș-Cheile Bicazului, and Ceahlău massifs) and was characterized by three partially sympatric taxa (*Cyanus pinnatifidus* subsp. *sooanus*, *Astragalus pseudopurpureus*, and *Festuca*
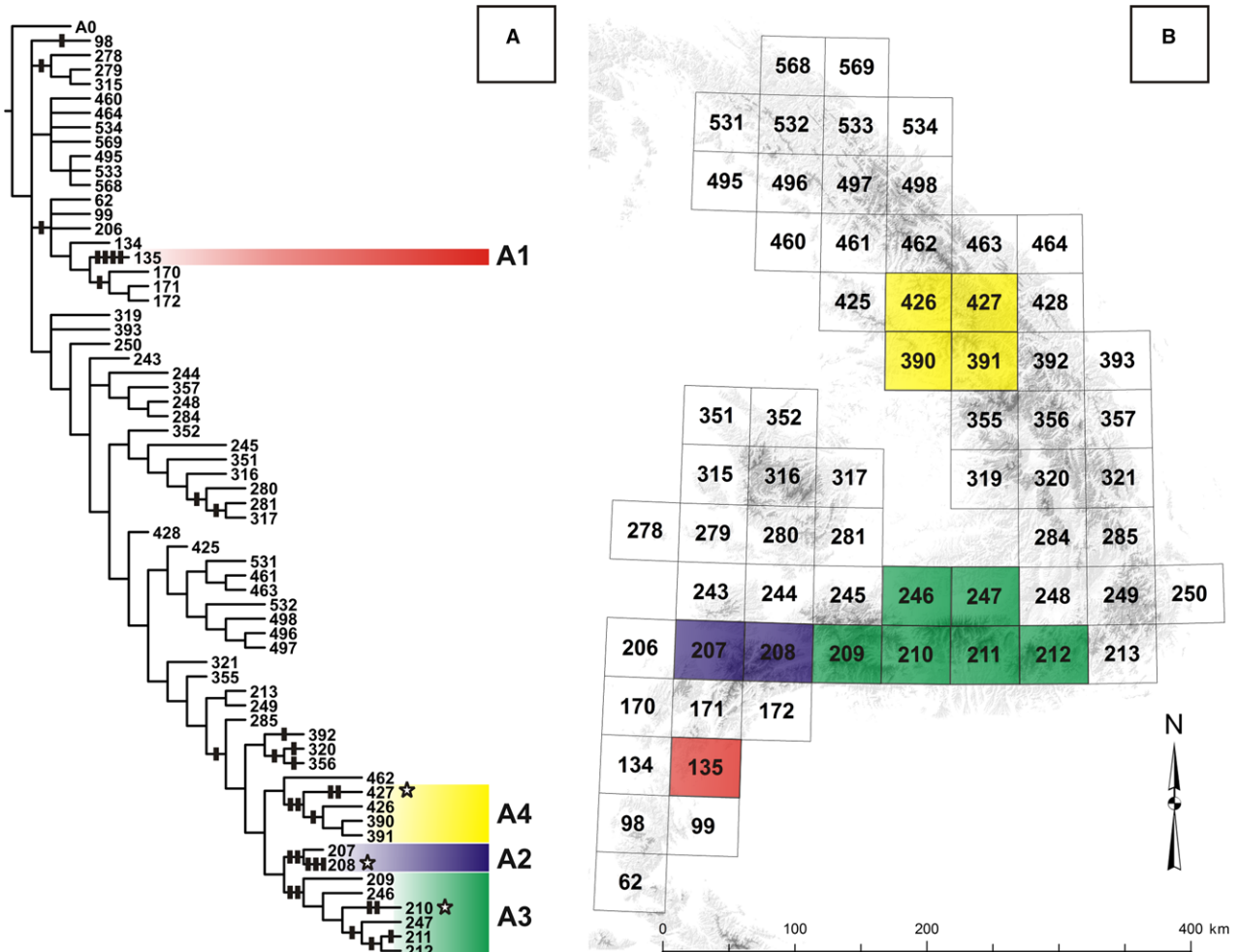
Figure 3. Areas of endemism (A1–A4) in the South-Eastern Carpathians resulted from parsimony analysis of endemicity. The distribution of endemics was recorded in a grid system with quadrats of 24′ latitude × 40′ longitude (aproximately 45 × 50 km). A, consensus cladogram obtained from parsimony analysis of endemicity. Area codes correspond to Table 2 (a star symbol represents nested areas of endemism). Geographical synapomorphies and autapomorphies are represented with black marks on the cladogram's branches (for taxa names, see Table 2). B, spatial representation of areas of endemism, symbolized in colours corresponding to cladogram 3A.

gautieri subsp. *lutea*). The second one was located in the east of the Apuseni Mountains (quadrats 280, 281 and 317) and was characterized by two partially sympatric taxa (*Centaurea reichenbachii* and *Sorbus dacica*), with one reversal for *C. reichenbachii*.

BIOTIC ELEMENTS

The result of the 'distratio' statistical test was significantly smaller ($t = 0.313$) than expected by chance (Monte Carlo simulation, $t = 0.373$, ranging from 0.339 to 0.410, $P < 0.001$). This indicates an important spatial clustering of endemic species ranges. The biotic element analysis resulted in assigning 45 (40% of

total) significantly co-occurring endemic taxa to seven biotic elements. The remaining 66 taxa (60% of total) were included in the noise component (Fig. 4). Two of the biotic elements (BE1 and BE3) showed comparable distribution patterns, being spread over most of the quadrats in our dataset, without any clear geographical indication. However, BE1 was mostly linked with the sub-alpine and alpine vegetation belts from the Eastern and Southern Carpathians.

The remaining five biotic elements were spatially clustered. These were characterized by clear core areas of distribution, with the majority of included species occupying one to few quadrats, and only a few species being over-dispersed (see Supporting information, Appendix S4):

Table 2. Comparison between areas of endemism (A1–A4; Fig. 3) and biotic elements (BE1–BE7; Fig. 4)

Biotic elements	AoE	nested AoE	Synendemic & autendemic taxa from the areas of endemism	Partially sympatric taxa additionally included in the biotic elements
BE 1				Achillea oxyloba subsp. schurii, Cerastium arvense subsp. lerchenfeldianum, Chrysosplenium alpinum, Carduus kerneri subsp. kerneri, Dianthus glacialis subsp. geldius, Doronicum carpaticum, Erysimum witmanni subsp. transsilvanicum, Festuca porcii, Melampyrum saxosum, Papaver alpinum subsp. corona-sancti-stephani, Phyteuma vagneri
BE 2	A4		Festuca versicolor subsp. dominii, Minuartia verna subsp. oxypetala	Armeria pocutica, Euphorbia carpatica, Lychnis nivalis, Saussurea porcii, Soldanella rugosa
		A4.1 ☆	Cochlearia borzaeana, Nigritella carpatica	
BE 3				Cerastium transsilvanicum, Campanula rotundifolia subsp. kladniana, Dianthus carthusianorum subsp. tenuifolius, Genista tinctoria subsp. oligosperma, Noccaea dacica subsp. dacica, Scabiosa lucida subsp. barbata, Viola declinata
BE 4	A1		Cephalaria uralensis subsp. multifida, Prangos carinata, Stipa danubialis, Tulipa hungarica	Campanula crassipes, Minuartia hirsuta subsp. cataractarum
BE 5				Dianthus callizonus, Ornithogalum orthophyllum subsp. acuminatum, Primula wulfeniana subsp. baumgarteniana, Saxifraga mutata subsp. demissa, Silene dinarica
BE 6	A2		Centaurea phrygia subsp. ratezatensis, Carduus kerneri subsp. lobulatiformis	
		A2.1 ☆	Anthemis kitaibeli, Barbarea lepuznica, Festuca pachyphylla	
BE 7				Astragalus pseudopurpureus, Festuca gautieri subsp. lutea
	A3		Aquilegia transsilvanica, Festuca bucegiensis	
		A3.1 ☆	Rosa villosa subsp. coziae, Stipa crassiculmis subsp. heterotricha	

The diagnostic taxa (synendemic and autendemic for areas of endemism) are shown based on their inclusion in the biotic elements. A star symbol (★) corresponds to Fig. 3A and represents nested areas of endemism.

BE2 delimited the northern part of the Eastern Carpathians. It included nine endemic taxa, the sympatric taxa of A4, together with *Armeria pocutica*, *Euphorbia carpatica*, *Saussurea porcii*, *Soldanella rugosa*, and *Lychnis nivalis*. Although characterized by a large area of distribution, many component species (5) were confined to the Rodna, Maramureș, and Chyvchyny-Gryniava Mountains.

BE4 included all synendemic taxa from the Danubian area of endemism (A1), together with *Minuartia hirsuta* subsp. *cataractarum* and *Campanula crassipes*, with the latter being more widespread.

BE5, with a core area occupying two quadrats (211 and 212), included five partly sympatric endemic taxa distributed in the eastern part of the Southern Carpathians (the Bucegi, Piatra Craiului, Bârsei, and Făgăraș Mountains). Three of the species characterizing it (*D. callizonus*, *P. wulfeniana* subsp. *baumgarteniana*, and *S. mutata* subsp. *demissa*) appeared as isolated apomorphies in the PAE cladogram (quadrats 211, 212, and 247 from A3 area of endemism), whereas *Ornithogalum orthophyllum* subsp. *acuminatum* and *Silene dinarica* were not discriminant for any quadrat or group of quadrats in the cladogram.

BE6 was distributed over the Retezat, Țarcu, Godeanu, and Piule-Piatra Iorgovanului massifs. With five endemics, it was completely species coincident with the A2 area of endemism.

BE7, occurring in the Giurgeu-Hășmaș and Ceahlău massifs, was defined by two taxa (*A. pseudopurpureus* and *F. gautieri* subsp. *lutea*). These were not diagnostic in the PAE cladogram as a result of partial sympatry.

BARRIER ANALYSIS

Barrier analysis reflected the spatial segregation of massifs from the South-Eastern Carpathians. The robustness value obtained for each barrier segment was proportional to the number of times the barriers developed over the same path when all distance matrices were used in the calculations. The absolute values ranged from 1 to 17 (with line densities varying from 0.6 to 175.88) and outlined three major breaks in the distribution of endemics (Fig. 5).

The highest distance values between neighbouring nOGUs, based on floristic differences, were recorded in the eastern and western parts of the Southern
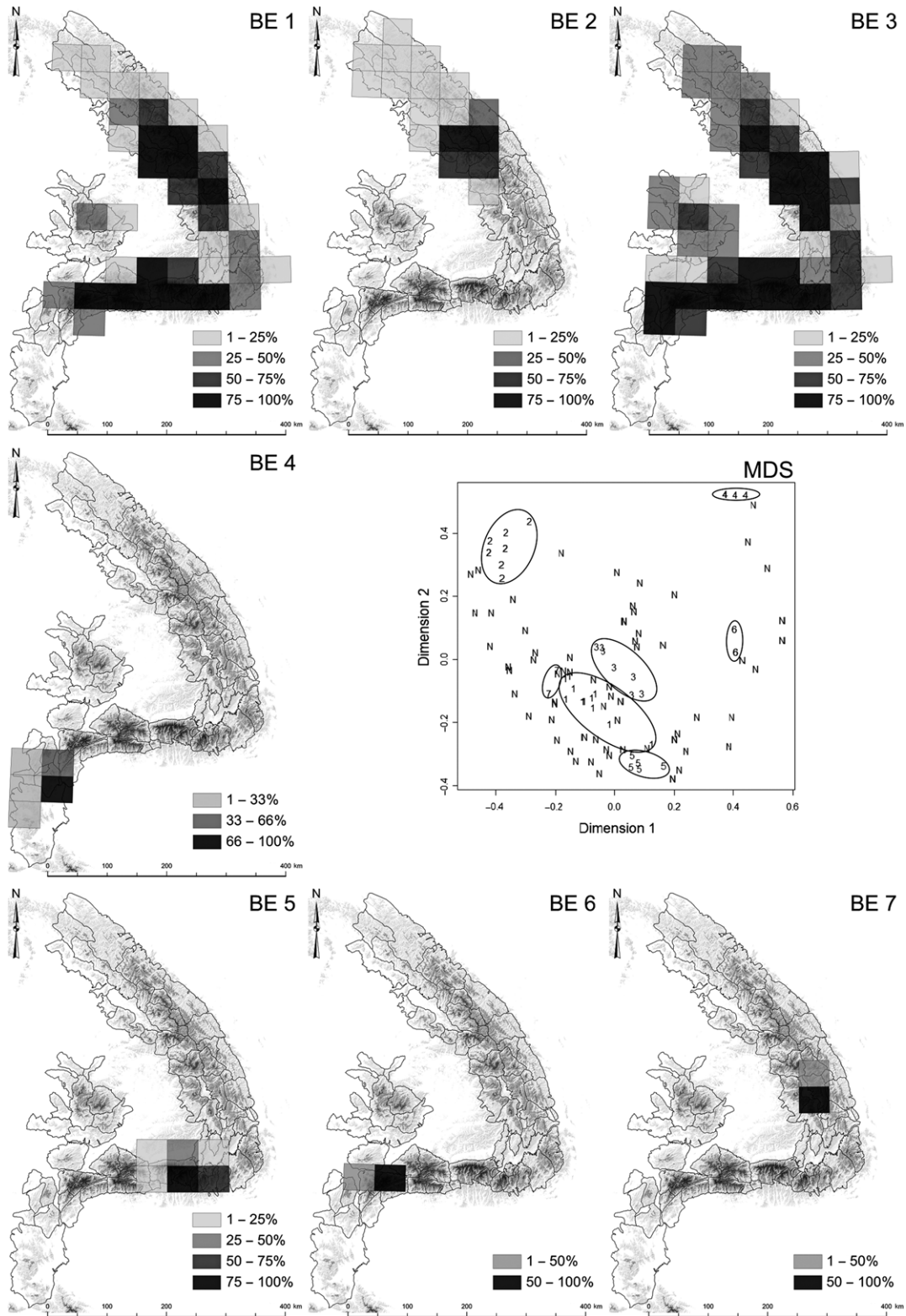
Figure 4. Biotic elements based on endemic taxa in the South-Eastern Carpathians. The biotic elements (BE1–BE7) are numbered according to Table 2 and labels from the multidimensional scaling analysis (MDS), where numbers 1–7 indicate biotic element, whereas *N* is the noise component. Grey shades on the maps correspond proportionally to the percentage of each biotic element occurring in the quadrat.
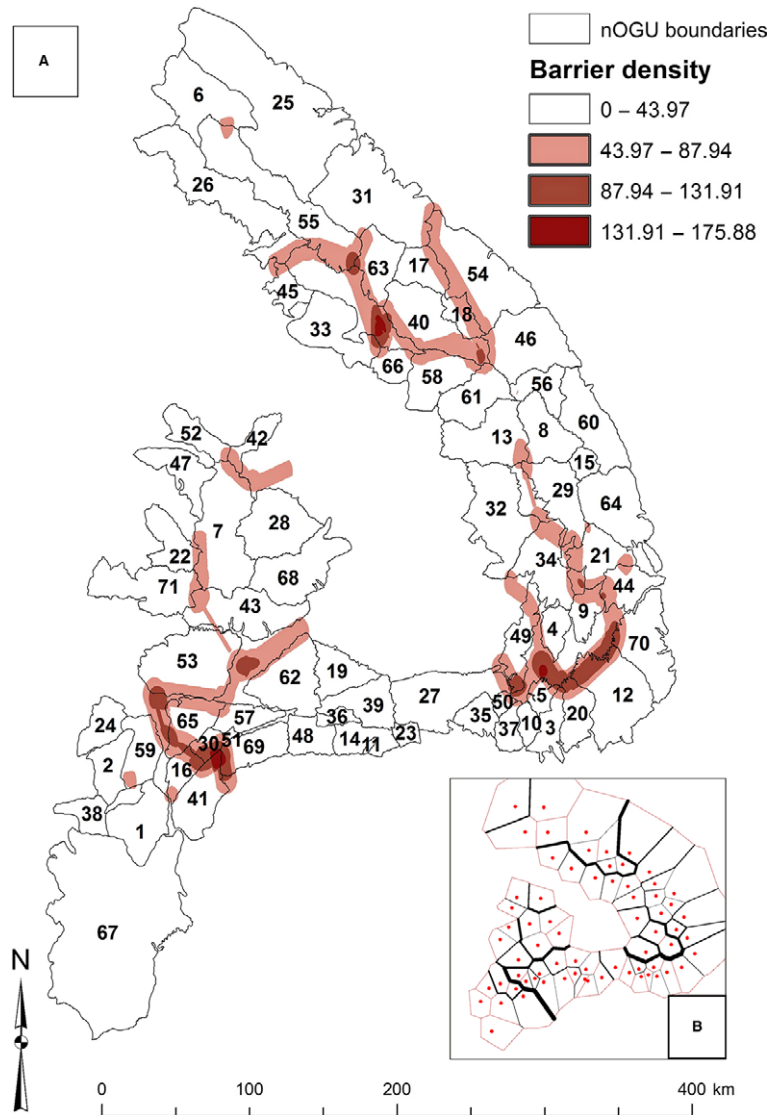
Figure 5. Major breaks in the distribution of South-Eastern Carpathian endemic plant taxa. A, barrier density mapped according to the robustness of breaks are shown with gradual colours. Labels of operational geomorphological units (nOGUs) correspond to Table 1. B, robustness of breaks as output of BARRIER, version 2.2. Thicker lines indicate a higher robustness value (for details on calculations, see Material and Methods); red dots mark the centroid location from which the Delaunay triangulation was constructed.

Carpathians. The first one delimited, along the Olt river valley in Brașov Depression, the Făgăraș, Piatra Craiului, Bârsei, Bucegi, and Ciucaș Mountains from the northern ranges. This break in the distribution of endemics described a path that continued north between the volcanic and calcareous regions of the Eastern Carpathians. The second one developed south of the Țarcu and Godeanu Mountains, separating them from the Cerna and Mehedinți Mountains, and continued north between the Retezat and Poiana Ruscă Mountains, finally delimiting the southern Apuseni Mountains from the Șureanu Mountains. A third notable break in distribution appeared in the

northern part of the Eastern Carpathians and delimited the Rodna Mountains from the Maramureș Mountains. It continued east of the volcanic ridge and separated the Maramureș, Chyvchyny-Gryniava, Chornohora, and Svydovets mountain group from the surrounding ranges.

DISCUSSION

Several key factors are presumed to have generated endemics and led to their uneven distribution in space (Jetz *et al.*, 2004; López-Pujol *et al.*, 2011a).

Among them, some factors promoted relictualism (allowing the survival of species in certain areas). Climatic gradients and environmental heterogeneity increased habitat diversity and led to a higher occupancy of the ecological space through niche fragmentation, consequently decreasing extinction rates (Hobohm, 2014). Additionally, climatic stability over time allowed species with weak dispersal ability to survive (Jansson, 2003; Sandel *et al.*, 2011), a phenomenon observable especially in the glacial refugia areas (Schönswetter *et al.*, 2005; Meédail & Diadema, 2009). Conversely, other factors promoted the divergence of lineages. Dispersal barriers and habitat insularity led to spatial isolation by stopping genetic interchange among disjunct populations, which in turn promoted speciation (Kruckeberg & Rabinowitz, 1985; Young *et al.*, 2002; Hobohm, 2014). Finally, endemics with disjunct populations may be the result of long distance dispersal events or the absence of divergence among segregated populations (Schönswetter *et al.*, 2003; Tribsch & Schönswetter, 2003). All of these factors might have played a role in shaping the currently observed spatial structure of endemics in the South-Eastern Carpathians.

Diversity patterns

Evaluation of endemic diversity in the South-Eastern Carpathians depends on three equally important factors: (1) the choice of taxa; (2) the available knowledge on their chorology at the time of analysis; and (3) the limits, type, and size of spatial units employed in the statistics. Variable results have been obtained in previous studies with regard to the absolute numbers of endemics for each unit (Pawłowski, 1970; Hendrych, 1982; Heltmann, 1985; Negrean & Oltean, 1989; Hurdu *et al.*, 2012a; Tasenkevich, 2014; Kliment *et al.*, 2016; Mráz *et al.*, 2016). The different results recorded in the present study compared to previous studies mostly ensued from the choice of taxa employed. However, the relative distribution of endemics diversity obtained among different studies (Pawłowski, 1970; Tasenkevich, 2014; Kliment *et al.*, 2016; Mráz *et al.*, 2016) is comparable to the present study, outlining arguably similar patterns of endemism. Our results indicate some noteworthy aspects with regard to endemic plant taxa richness and rarity:

1. The highest 25% values/massif for both SR and WE are spatially clustered, outlining five major centres of endemism, distributed in the Eastern and Southern Carpathians (Fig. 2). These are characterized mainly by three different types of physiographical and climatic features. First, the presence of extensive alpine areas in three of the centres (including Retezat, Parâng, Făgăraș, and Bucegi massifs in the Southern Carpathians or Rodna massif in the Eastern Carpathians) is reflected also in the high number of endemic orophytes occurring there. The existence of glacial refugia in the South-Eastern Carpathians has been previously suggested for the survival of alpine plants, and for explaining the distribution of disjunct populations (Mráz *et al.*, 2007; Ronikier, 2011). Although long-distance dispersal can also be a cause of disjunctions, glacial refugia have played a major role in shaping the current distribution of alpine endemics, usually range-restricted specialists that are weak competitors (Schönswetter *et al.*, 2003), and which survived in distinct refugia congruent to the centres of endemism in the Alps (Tribsch & Schönswetter, 2003). Second, it is known that the distribution of endemics is tightly linked with the existence of calcareous bedrock (Casazza *et al.*, 2008; Essl *et al.*, 2009). Being unevenly distributed throughout the South-Eastern Carpathians, carbonate conglomerates bedrocks or limestone bedrocks appear either as the dominant type (e.g. in the Piule-Piatra Iorgovanului, Buila-Vânturarița, Piatra Craiului, Bucegi, Ciucaș, Ceahlău or Giurgeu-Hășmaș massifs) or scarcely in the geological structure of other alpine, mostly siliceous mountains (e.g. the Rodna, Făgăraș or Parâng Mountains) (Badea *et al.*, 1983). With the highest density of endemics being found in these calcareous areas, bedrock type appears to be the main factor driving the diversity of endemics in the South-Eastern Carpathians. Similarly driven patterns were found not only in the South-Eastern Carpathians (Coldea *et al.*, 2009), but also in the Western Carpathians (Mráz *et al.*, 2016). Third, there appears to be a strong influence of the thermophilous elements of Balkan and Mediterranean origin in the flora of the South-Eastern Carpathians (Coldea, 2003). This is especially notable in the south-western part of the Southern Carpathians, where the highest value for weighted endemism from all the analyzed nOGUs was recorded in the Mehedinți Mountains. Moreover, this region's florogenetic transient nature and phytogeographical affinities with the Carpathian and Moesian floristic provinces is supported by the marginal distribution of both Balkan and Carpathian elements (Hurdu *et al.*, 2012a).

2. By contrast, regions with low endemics diversity in the South-Eastern Carpathians are usually associated with a poorer manifestation of all these drivers of diversity: lower altitudes with a high coverage of forest habitats and a reduced proportion of calcareous bedrock. These are

characteristic for the transitional zones edging each subunit, either within the South-Eastern Range (the Plopiș and Meseș Mountains in the north of the Apuseni Mountains; the Perșani, Baraolt, and Bodoc Mountains, separating the Eastern and Southern Carpathians) or at the border with neighbouring regions (the Transdanubian Carpathians in Serbia, between the Carpathians and the Stara Planina Mountains or the Eastern Beskids in the northern part of the Eastern Carpathians, at the border with the Western Carpathians). Similar low values have been recorded in the Apuseni Mountains, where the highest diversity of endemics was linked to the presence of calcareous habitats and rock screes in the Gilău-Muntele Mare massif (especially in Scărița-Belioara cliffs) or the Trascău Mountains with similar geological structure.

3. Previous studies compared the subunits of the CMR in terms of endemic SR and discussed their main distribution patterns. Tasenkevich (2014) indicated a total of 468 endemic and 36 subenedemic taxa for the entire Carpathians, with the highest diversity being registered in the Eastern Carpathians (142 endemic and subendemic taxa), followed by the Southern Carpathians (121). By contrast, our results indicate higher richness values in the Southern Carpathians (87 endemic taxa) compared to the Eastern Carpathians (78 taxa). These differences may arise for several reasons. First, there are differences in both the selection of taxa and included areal types (we accounted neither for sub-endemics, nor for pan-Carpathian endemics). Second, based on the distribution of the fifth biotic element, and an equally important barrier identified north of Ciucaș, we included this massif in the Southern Carpathians. Similar to our results, Heltmann (1985) observed higher values for endemic SR in the Southern Carpathians compared to the Eastern Carpathians.

4. Our results mostly agree with the previous study of Pawłowski (1970), with a few noteworthy differences. In his comprehensive study of plant endemism in the Alps and the Carpathians, Pawłowski (1970) delimited one minor and three major centres of endemism in the South-Eastern Carpathians, including in the Central-European Floristic Province: the Apuseni Mountains, the Pocutico-Marmarossian floristic district, the Bistrito-Moldavian floristic district, and the entire Southern Carpathian range. However, based on our analyses, there appears to be a clear differentiation between the two edges of the southern range, which does not support their inclusion in the same centre of endemism. Furthermore, we did not find any arguments to support enlisting the Apuseni Mountains as a centre of endemism, as a result of their low values in measured diversity indices. Also, our results suggest that the Danubian mountainous region (including the Mehedinți Mountains) emerged as a second distinct centre of endemism in the south-western part of the Southern Carpathians, which might have played a major role in the preservation of thermophilous flora.

AREAS OF ENDEMISM AND BIOTIC ELEMENTS OF THE SOUTH-EASTERN CARPATHIANS

PAE has been criticized as a method for identifying AoE as a result of its implied assumptions. One major critique relates to its inability to detect AoE in cases where perfect sympatry is absent (Brooks & van Veller, 2003). Furthermore, a drawback of PAE resides in the lack of phylogenetic information included in the analyses (Brooks & van Veller, 2003). To overcome the homopatry constraint, BEA has been proposed as an alternative for analyzing the clustering of endemics distribution areas (Hausdorf, 2002; Hausdorf & Hennig, 2003; Hennig & Hausdorf, 2004, 2006). However, BEA has been shown to perform in a counter-intuitive manner, by omitting exactly the cases of complete overlap of distributions (Casagranda *et al.*, 2012). Such a situation was revealed in our analyses with respect to the nested area of endemism delimiting the Cozia and Buila-Vânturarița massifs (A.3.1). Conversely, PAE was unable to identify an OGU group characterized by one synapomorphy, with two disjunct autapomorphies in terminal quadrats (quadrats 320 and 356), which was recovered by BEA. There are two main implications resulting from these differences. First, the partial sympatry might be a result of the size of the OGUs because choosing a different size would translate into subtle changes in the range of species and the degree of sympatry. Previous studies have outlined the scale-dependency of biogeographical analyses (Morrone & Escalante, 2002). Second, the partial sympatry can also be a consequence of evolutionary processes, such as post-speciation dispersal or extinction events, a partial response to vicariance (Brooks & van Veller, 2003) or even environmental filtering, although the latter may play a lesser role than geographical isolation of endemics in mountain systems (Gehrke & Linder, 2014). Furthermore, the partial sympatry observed in the biotic elements (BE2, BE4, BE5, BE6), with core distribution areas overlapping the AoE (A1–A4), could be the result of several interplaying factors. The distributional cores might have acted as

'cradles of evolution' and 'museums' for both Pleistocene alpine endemics and older thermophilous relict endemics (especially in the calcareous massifs), from which different dispersal events might have occurred, as observable in the densities of biotic elements distribution.

The South-Eastern Carpathian plant endemism is characterized by two distinct evolutionary phenomena: recent speciation and relictualism. First, recent florogenetic processes are reflected in the emergence of new taxa. The Pleistocene climatic fluctuations might have led to an increased change in favourable habitat availability, which in turn acted differentially on species ranges because of their unequal dispersal capacity and adaptive potential. Concretely, these differences could have promoted range expansion in some endemics, at the same time as increasing isolation through range-contraction for others. Consequently, such range dynamics could have led to a disjunction of populations with or without posterior speciation. This is notable in the distribution of alpine endemics, either restricted to one mountain or with disjunct populations in distant massifs of the South-Eastern Carpathians.

Second, the distribution of endemics in the South-Eastern Carpathians, in addition to current ecological conditions and past major climatic events, could also have been influenced by older geological events. The palaeo-islands existing during the mid-Miocene (13–14 Mya), which gradually converged throughout the Pliocene (1.8–5.3 Mya) with the regression of the Paratethys Sea (Harzhauser & Piller, 2007), were indicated as temporally differentiated uplifts of landmasses in the northern part of the Eastern Carpathians, and south-eastern and south-western parts of the Southern Carpathians (Popov *et al.*, 2004). This complex geological history of the Carpathians in the mid to late-Miocene period could have led to the isolation of lineages that might be linked to the distribution of extant endemics. The archipelago configuration of the Carpathian palaeo-islands during the mid-Miocene corresponds geographically to the A2, A3 and, A4 AoE, and the BE6, BE5, BE2, and BE7 biotic elements, respectively.

Disjunction of A2 and A3 AoE could be supported by the hypothesis of a differential uplift of landmasses during the Miocene, which in turn led to isolation processes in the western and eastern parts of the Southern Carpathians. A comparable west–east meridional disjunction was observed in the biogeography of the *Gammarus balcanicus* amphipod, where two different lineages evolved separately in the eastern and western ends of the Southern Carpathians during the Miocene (Copilaş-Ciocianu & Petrusek, 2016). Additionally, four taxa characterizing BE5 in the eastern part of the Southern

Carpathians (*D. callizonus*, *P. wulfeniana* subsp. *baumgarteniana*, *S. mutata* subsp. *demissa* and *S. dinarica*) are partly sympatric, characterized by reversals in the PAE cladogram (A3 area of endemism). This case could indicate smaller scale isolation processes or dispersal/extinction events in the eastern Southern Carpathians. Similarly, an older uplift in the Eastern Carpathians (Popov *et al.*, 2004) overlaps the A1 area of endemism (which includes the Rodna, Maramureş, and Chyvchyny-Gryniava Mountains).

Finally, migration processes caused by past climatic or geological events coupled with the existence of a dispersal barrier might be the cause that led to the high number of narrow endemics (4) found in the Danubian (A1) area of endemism, where all taxa but *T. hungarica* are restricted to the Iron Gates region.

MAJOR BREAKS IN THE DISTRIBUTION OF ENDEMICS

Three important breaks emerged in the distribution of endemic plants from the South-Eastern Carpathians. Two of them are in agreement with the spatial structure uncovered by both PAE and BEA in the Southern Carpathians. These are situated in the eastern and western parts of the Southern Carpathians and appear to follow abrupt changes in the distribution of alpine environments.

First, the westernmost break zone delimits the A2 area of endemism (and subsequently biotic element 6) from the neighbouring northern Poiana Ruscă Mountains and, more interestingly, separates A2 from a different area of endemism in the south (A1–Danubian area of endemism). When regarded in terms of floristic distinctiveness, this major break is defined by several characteristics. On the one hand, the Danubian mountainous region is an important contact zone with the adjacent mountains from the northern Balkan Peninsula, and is considered as a migration corridor through which floristic elements repeatedly dispersed in and from the Carpathians (Stevanović, 1996; Peev & Delcheva, 2007; Ronikier, 2011; Šingliarová, Hodálová & Mráz, 2011; Puşcaş & Choler, 2012; Kuzmanović *et al.*, 2013; Surina *et al.*, 2014). However, the lower altitudes of this region generate an abrupt transition towards the alpine environment from the Retezat, Ţarcu, and Godeanu Mountains, which might have acted as an important barrier for some alpine plants (Ronikier & Zalewska-Gałosz, 2014; but see Puşcaş & Choler, 2012; Šingliarová *et al.*, 2011). On the other hand, recent molecular studies reflected an important differentiation along the Danube, between the northern Balkan Peninsula Mountains (Stara Planina) and the Carpathians (Kuzmanović *et al.*, 2013). Alternative phases of range expansion and contraction in

termophilous plants might have acted differentially on their dispersal capacity, leading to the emergence of stenochoric endemics. The range limits of narrow endemics such as *C. uralensis* subsp. *multifida*, *M. hirsuta* subsp. *cataractarum*, *P. carinata*, *S. danubialis*, and *T. hungarica*, confined to the Danubian area of endemism (A1), suggest such dispersal limitations are caused by a putative barrier and long-term isolation from the surrounding regions that could have promoted their divergence.

A second barrier delimiting the eastern part of the Southern Carpathians (corresponding to the A3 area of endemism) from the Eastern Carpathians, followed a similar pattern of abrupt break in the alpine zone. It appears that our findings are not in agreement with previous results obtained by Negrean & Oltean (1989), which indicated, based on floristic similarity, a more probable link between the Făgăraș, Piatra Craiului, Bucegi, and Ciucaș Mountains and the calcareous massifs from the Eastern Carpathians. These differences may emerge from a different choice of endemic taxa employed in the analysis because we did not include the pan-Carpathian endemics. Narrow endemics probably differentiate the Southern Carpathians from the Eastern Carpathians along this border, whereas the pan-Carpathian endemics may outline stronger affinities of the massifs from the eastern part of the Southern Carpathians with the Eastern Carpathians, suggesting a general pattern of distribution that extends to the west of this break in distribution. However, not only based on the emergence of the floristic break zone between the Eastern and Southern Carpathian subunits, but also considering the complete allopatric distribution of BE5 and BE7, we consider both the Ciucaș and Baiului Mountains as being part of the Southern subunit of the Carpathians.

Finally, a third major break in the distribution of endemics delimited the Rodna Mountains from the northern ranges. Its placement does not correlate with previously described phylogeographical patterns (Mráz *et al.*, 2007; Pușcaș *et al.*, 2008; Ronikier, Cieślak & Korbecka, 2008), which uncovered a different, more northern break in the genetic structure of alpine plants; but see also Ronikier, Schneeweiss & Schönswetter (2012) who reported a very divergent and rare lineage for the arctic-alpine plant *Ranunculus glacialis* in Rodna Mountains. This emerged as even more surprising because it develops inside the A4 area of endemism, which apparently includes highly sympatric taxa at the level of the quadrat. However, two key factors are the probable cause of the positioning of this barrier between the Rodna and Maramureș Mountains. First, the natural distribution of many South-Eastern Carpathian endemics

does not extend north of the Rodna Mountains (including *F. versicolor* subsp. *dominii* and *L. nivalis*, which are taxa confined to the Rodna Mountains). Additionally, the nested area A4.1 is differentiated based on endemic taxa occurring in the eastern Maramureș and Chyvchyny-Gryniava Mountains (*C. borzaeana* and *N. carpatica*). Second, the Rodna Mountains detain the most ample development of alpine zone in the entire Eastern Carpathians, where many alpine taxa with disjunct areal (East–South) occur, thus implying the existence of a possible major glacial refugium in this massif (Ronikier *et al.*, 2012). All of these findings suggest that the Rodna Mountains may represent a contact zone between northern and southern ranges of taxa, acting both as a dispersal barrier and a refugium during the Pleistocene glaciations. This hypothesis also is supported by the phylogeographical patterns found in two boreal tree species, which indicate a contact zone between the northern and southern migration routes: *Abies alba* (Gömöry *et al.*, 2012) and *Picea abies* (Tollefsrud *et al.*, 2008).

CONCLUDING REMARKS

The present study has addressed the problem of spatiality in the structure of plant endemism from the South-Eastern Carpathians through the complementary use of several widely applied methods in quantitative biogeography: diversity patterns analysis, PAE, BEA, and barrier analysis. The results obtained from all of the methods used were mostly spatially convergent, with a few discrepancies that might be explained by the biogeographical particularities retained by each method. Both endemic SR and rarity hotspots mostly coincided with the major AoE identified through PAE and with the distribution cores of biotic elements. This suggests a highly structured distribution pattern of South-Eastern Carpathian endemic plants. We argue that the geographical structure of endemism in the South-Eastern Carpathians holds an important historical component, with a potentially strong influence of past geological and climatic events that acted on their evolution during the Neogene Period. This influence, along with the current geomorphological and climatic features of the South-Eastern Carpathians, their alpine and geological insularity (especially for carbonate conglomerate and limestone bedrocks), climatic gradients, and a relatively low influence of Pleistocene glaciations, may represent important drivers that led to the current distribution of plant endemics. This specific combination of factors probably allowed the long-term survival and promoted diversification of endemic plants in the AoE.

Spatial analysis of endemism has major implications in biogeography. First, AoE are the basic units in cladistic biogeography (Morrone, 2009, 2014a). Although it is a 'pattern-orientated' approach, their delimitation through PAE is a first step in biogeographical analysis (Morrone, 2014b). Further inferences can be made about the causes of evolutionary processes that led to spatial convergence of similar ranges in the AoE (Morrone & Escalante, 2002; Riddle & Hafner, 2006). Such inferences are possible by including phylogenetic (e.g. relatedness of taxa, times of divergence) and ecological information (e.g. climatic niche, dispersal ability) (Webb *et al.*, 2002; Wiens & Donoghue, 2004; Crisp, Trewick & Cook, 2011; Morlon, 2014; Noguera-Urbano, 2016). Furthermore, hypotheses on the origin and range dynamics of endemic flora from the South-Eastern Carpathians can be tested by also including palaeoenvironmental data (Escalante *et al.*, 2007) in a defined evolutionary model. Second, endemics distribution is one of the major criteria applied in biogeographical regionalisation, their usage at different taxonomical levels serving for a hierarchical delimitation of floristic regions, provinces and districts (Kreft & Jetz, 2010). Third, similar analyses conducted at the scale of the entire CMR, and by including also non-endemic taxa, might better outline the causes of plant endemism and its potential influences with respect to neighbouring floristic provinces. Finally, areas with high endemism values are of major importance in designating priority areas for conservation (Whittaker *et al.*, 2005). Moreover, recent studies have demonstrated the relative incongruence of AoE with areas of high phylogenetic diversity, which are important for conserving evolutionary histories of floras (Forest *et al.*, 2007; Gonzaléz-Orozco *et al.*, 2015). Consequently, a combined use of quantitative biogeographical and phylogenetic approaches would be recommended for establishing appropriate strategies on biodiversity conservation in the context of both climate change and human influence on natural mountain habitats, and further would serve to optimize the network of protected areas in the region. In all of the aspects noted above, the results of the present study will open several new avenues in the research of South-Eastern Carpathian endemics.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Presence (1)/absence (.) of endemic plant taxa in the South-Eastern Carpathians, recorded in the 24′ latitude × 40′ longitude quadrats.

Appendix S2. Presence (1)/absence (.) of endemic plant taxa in the South-Eastern Carpathians, recorded in the operational geomorphological units (nOGU).

Appendix S3. Species richness (SR) and area of operational geomorphological units (nOGUs).

Appendix S4. Biotic element analysis results.