Article

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Spatial phylogenetic diversity of pteridophytes in Latium (Central Italy): a tool for conservation planning at regional scale

Abstract

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The spatial phylogenetic metrics of pteridophyte diversity and endemism may provide useful information to prioritize protected areas for conservation planning. We analysed spatial patterns of phylogenetic diversity (PD) and phylogenetic endemism (PE) of native pteridophytes across Latium, to recognise diversity and endemism centres. and to discuss implications for conservation in the region. We identified the main PD and PE centres that should be considered as valuable biodiversity hotspots, due to their high evolutionary and conservation value. Most, but not all of them correspond to protected areas Monitoring programmes of the rarest taxa are proposed.

Key words: phylogenetic endemism, ferns, protected areas, ecology optimization, biodiversity, hotspot priority.

Introduction

Presently, we are facing the sixth species mass extinction on Earth (Barnosky & al. 2011), biodiversity being currently threatened by human-induced climate change and other anthropogenic factors such as alien species invasions, habitat loss and fragmentation, pollution and over-consumption of natural resources (Myers & al. 2000; Sarkar & al. 2006; Devictor & al. 2012; Fenker & al. 2014; Jantz & al. 2015; Arnan & al. 2016; Pyšek & al. 2020). Therefore, measuring and quantifying the spatial patterns of biodiversity is a very important goal for conservation planning (Arnan & al. 2016), also useful in identifying areas of high conservation priority as well as in optimization of conservation strategies (Ceballos & Ehrlich 2006; Diniz-Filho & Telles, 2006; Lostia 2018). Biodiversity studies traditionally use species as a unit of analysis (i.e., species richness: SR). SR, the number of species in a given area (Faith 1992; Magurran & McGill 2011), was traditionally used to define areas of conservation importance (MacArthur & Wilson 1967; Connell 1978; Gotelli & Colwell 2001). However, SR alone is not sufficient to assess the biodiversity of an area, because it provides limited insight into the underlying characteristics of taxa and, fails to incorporate information about the relationships among species (Lee & Mishler 2014; Rosauer & Jetz 2015; Lean & Maclaurin 2016). Species endemism is another commonly used biodiversity metric (Mittermeier & al. 2011). Endemism refers to the geographic restriction of a species (Crisp & al. 2001) and is a key factor to map the distribution of biological diversity and to identify areas of priority for conservation, such as biodiversity hotspots (Myers & al. 2000; Crisp & al. 2001; Rosauer & Jetz 2015). Endemism is also a good estimator of extinction risks, since geographically restricted species are irreplaceable and cannot be found anywhere else (Gaston & Fuller 2009; Gudde & al. 2013). Unfortunately, also endemism-related metrics are often measured by looking at species alone, this approach giving an incomplete picture of endemism itself (Mishler & al. 2014). The traditional biodiversity metrics do not reveal areas where a few species represent a significant amount of evolutionary history (Mooers 2007; Yek & al. 2009), they only consider the terminal taxa on the phylogenetic tree, without taking into account the evolutionary relationships among taxa (Lee & Mishler 2014; Rosauer & Jetz 2015).

Knowledge of phylogenetic relationships may illuminate the origin of diversity patterns as well as the drivers that gave rise to them (Hawkins & al. 2014; Lagomarsino & al. 2016). Taking species phylogenetic relatedness into consideration in conservation assessment is also crucial because closely related species are often more similar functionally and ecologically than distantly related ones (i.e., phylogenetic autocorrelation; Gittleman & Kot 1990). Spatial phylogenetics combines species occurrence data with molecular phylogenetics to recover information about the spatial distribution of phylogenetic diversity (PD) and phylogenetic endemism (PE) across geographic regions. Faith (1992) described PD as the sum of the lengths of the phylogenetic branches separating all species occurring in a region. Accordingly, an area with low PD has less genetic diversity than an area with high PD (i.e., more distantly related species). PE is the sum of branch lengths weighted by the proportion of the range of each branch that is found in the area; in other words, high PE values are found where long branches on the phylogenetic tree are restricted to a small geographic range (Rosauer & Jetz 2015). Areas of high PE are very important to conserve, as they harbour evolutionary history that is at risk of extinction (Jetz & al. 2012; Rosauer & Jetz 2015; Laity & al. 2015). The evolutionary history of species is relevant for various measures of endemicity, such as neo-endemism (i.e., recently evolved) or paleo-endemism (i.e., anciently evolved), and is informative about the biogeographic history of an area (Casal-Lopez & al. 2018), aiding in identifying areas of potential biological interest (Nieto-Blázquez & al. 2017). Mishler & al. (2014) proposed a quantitative measure to clearly distinguish centres of neo- and paleo-endemism and to discover areas that are centres of both neo- and paleo-endemism (named centres of mixed-endemism). All these phylogenetic metrics offer an improvement over traditional biodiversity metrics, because they incorporate the evolutionary relationships of taxa, thus providing a more accurate assessment of biodiversity (Diniz-Filho & al. 2013; González-Orozco & al. 2015).

Several studies have been provided both at the national and regional level. A first main monograph was published by Fiori (1943), and later other authors, such as Arrigoni, Ciampolini, Chiarugi, Ferrarini, Marchetti, Nardi, Pichi-Sermolli, Pignatti, Ricceri, Tommei, Zangheri provided general studies on pteridophyte taxonomy, morphology and distribution.

At regional level, several studies exist for the following administrative regions of Italy: Molise (Lucchese 1995), Liguria (Bernardello & Martini 2004), Abruzzo (Conti & al. 2011), Friuli Venezia Giulia (Martini & al. 1977; Bona & al. 2005), Emilia-Romagna (Bonafede & al. 2016), Trentino-Alto Adige (Prosser & al. 2019), Veneto, Friuli Venezia Giulia e Trentino-Alto Adige (Bona & al. 2005), Sicily (Troìa & al. 2012), Tuscany (Arrigoni 2016; Carta & al. 2018) and Central Italy (Mayer 2017).

For the Latium region, a single paper about the phytogeography of ferns is that by Lusina (1951). Thus, the present paper is the first study about the diversity and distribution of pteridophytes for this region.

Latium is one of the 20 administrative regions of Italy; it is located at the centre of the Italian peninsula facing to the Tyrrhenian sea. Central Italy is considered one of the most important hotspots within the Mediterranean region both for endemic vascular plants (van Gils & al. 2012) and for invertebrates (Balletto & al. 2010). During the late Pleistocene and the early Holocene, the vegetation history of Latium has faced a very complex scenario with several arboreal phases due to volcanic activities and climatic glacial oscillations (Giardini 2007). During the Late Glacial and the early Holocene, the Central Apennines have acted as a crucial refugium for several tree genera (Brown & al. 2013; Krebs & al. 2019). A highly inclusive biogeographical study of the distribution of the present vascular flora of Latium was recently provided by Lucchese (2017, 2018). The plant diversity of Latium is very high (almost 3,000 native taxa) mainly due to the highly heterogeneous landscape with a high variation in topography and climate (Lucchese 2017). Pteridophytes are a suitable group for biogeographic studies because of their long evolutionary history, wide distribution, high variation of ecological niches and independence from biotic pollination and distribution vectors (Kessler 2010). With the term "pteridophytes" we intend here all the spore-producing vascular plants, which belong to two different clades commonly called lycopods and ferns. Besides producing spores, both clades share a similar life cycle, and were usually joined within a singular monophyletic group named "pteridophytes". However, it has recently been found that these clades do not share a common evolutionary ancestor or ancestral group, since ferns are more closely related to seed plants than to lycopods (Christenhusz & Chase 2014). In this study, however, we have decided to join lycopods and ferns in a single group. The same approach and similar methodology have been previously adopted by Carta & al. (2018) for the neighbouring Tuscany Region, hosting 94 taxa and largely richer than Latium. Within the pteridophyte pool of taxa which occurs in the Latium region, both ancient taxa, that show long terminal branches of the phylogenetic tree (e.g., *Selaginella*, *Isoetes*, *Huperzia*, *Botrychium* and *Ophioglossum*), and more recent taxa with short terminal branches (e.g., *Polystichum*, *Equisetum*, *Polypodium* and *Cystopteris*) coexist. In both cases there are taxa that occur widely within the region and others that are present in more restricted areas. Thus, phylogenetic metrics of pteridophyte diversity and endemism of Latium may provide very useful information to prioritize protected areas for conservation planning.

Accordingly, the aims of this study were: (1) to estimate, analyse and map the PD and PE spatial patterns of native pteridophytes across Latium; (2) to identify and distinguish centres of diversity and endemism that should be prioritised in conservation planning; (3) to compare the centres of endemism and to discuss the implications for conservation.

Material and methods

Geographic data

Latium (in italian “Lazio”) extends for 17.227 km^2 within the latitudinal band of 42°53’-40°42’ N-S and longitudinal one of 11°20’-14°10’ W-E. This region is a part of the Central Apennines (0-2.458 m) with carbonate, flysch and volcanic deposits, a part of the peri-Mediterranean orogenic belt built up mainly in Neogene times as a consequence of the collision and convergence between the European and African macroplates (Cosentino & al. 2010).

5,148 geo-referenced unique occurrence data of 61 native pteridophyte were extracted from the geodatabase of the vascular flora of Latium, that includes field (5,538 occurrences), literature (4,867 occurrences) and herbarium (713 occurrences) data (Lucchese 2017). Only recently confirmed and accepted occurrence data were included, excluding doubtful ones. Field surveys were carried out by Lucchese from 1980 until today, recording species on cards within “quadrant” units of CCEF system (Cartography of Central European Flora; Ehrendorfer & Hamann 1965; Niklfeld 1971; Pignatti 1978) that consist of equal-area cells of 5’ x 3’ E longitude and N latitude (approx. 7 km x 5,5 km). In these occasions, we have to pay tribute to Pignatti for starting the flora survey in Lazio (Pignatti 1978). The completeness of the source geodatabase is very high (Lucchese 2017), thus the selected pool of pteridophyte records may be considered as a presence-absence geodata and not only as a presence data. Native pteridophytes occur in 533 of the total 544-cells grid that covers the Latium region. Distribution data of subspecies of *Asplenium obovatum*, *A. ceterach* and *A. trichomanes* were merged at species level because of their restricted taxonomic distinctiveness. In contrast, we decided to maintain separate distribution data of three hybrids (*Polystichum* x *bicknellii*, *Equisetum* x *moorei* and *Asplenium* x *tyrrhenicum*), that are well differentiated from their parental taxa.

Phylogenetic structure

All the occurrence data were geo-referenced and projected within the CCEF grid in equal-area cells of 0,08333 x 0,05 decimal degrees of E longitude and N latitude (7 km x 5,5 km). A phylogenetic tree of the native pteridophytes of Latium (Fig. 1) was assembled using Phylomatic (Webb & Donoghue 2005). As a source mega-tree we used the dated phylogeny for the Central European Flora (Durka & Michalski 2012). In order to define the phylogenetic relationship within the *Lycophytes*, we also considered additional phylogenetic information on *Isoetes* and *Selaginella* (Smith & al. 2006; Christenhusz & al. 2011).

Phylogenetic biodiversity analyses

The Biodiverse software (version 3.1; Laffan & al. 2010) was used to calculate species richness (SR), weighted endemism (WE), phylogenetic diversity (PD), phylogenetic endemism (PE), relative phylogenetic diversity (RPD) and relative phylogenetic endemism (RPE) for each grid cell.

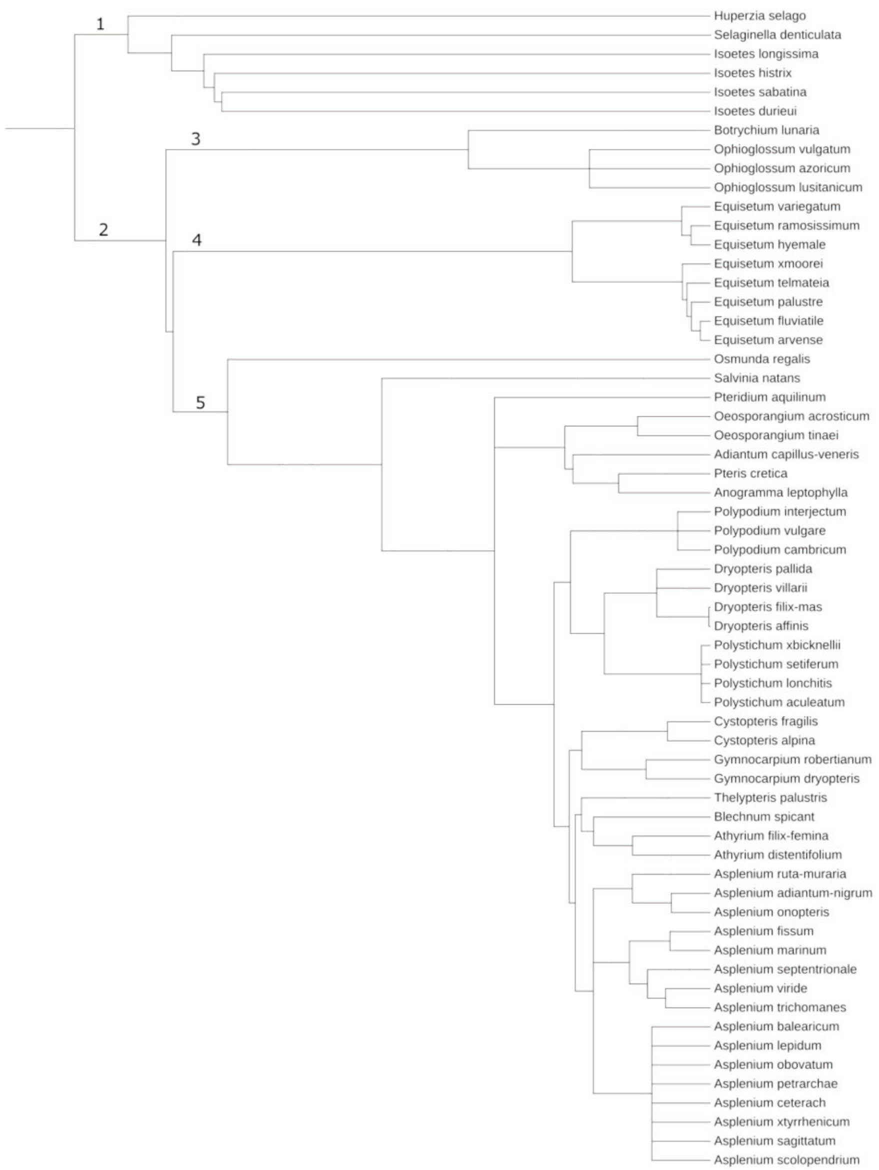

Fig. 1. Phylogenetic tree of native Pteridopyhtes of Latium. Notes: lycopods and ferns clades do not share a common evolutionary ancestor or ancestral group, since ferns are more closely related to seed plants than to lycopods (Christenhusz & Chase 2014). However, within this study we have decided to follow the old phylogenetic approach joining lycopods and ferns in a unique monophyletic clade. Branches of clades marked with numbers are: 1) *Lycophytes*; 2) *Pteridophytes* 3) *Psilotopsida*; 4) *Equisetopsida*; 5) *Polypodiopsida*.

The last two metrics compare respectively PD and PE observed values, calculated on the actual phylogenetic tree, with estimated values calculated on a comparison tree that retain the actual topology but has all branches of equal length (Mishler & al. 2014; Carta & al. 2018). The statistical significance of PD, PE, RPD and RPE were assessed using a randomization test (999 trials) with a null model that randomly reassign species occurrence to grid cells without replacement and that keeps constant both the total number of grid cells for each species and the SR of each grid cell (Laffan & Crisp 2003). The estimated values of PD, PE, RPD and RPE obtained from the randomisation test formed a null distribution for each cell that was used in two-tailed non-parametric tests to obtain the significance of the observed values. Thus, we identified, for each index, which cells have significantly higher and lower values than the null model.

Assessment of pteridophyte centres of diversity and endemism

To identify and distinguish different centres of endemism we followed the CANAPE (Categorial Analysis of "Neo-" And "Paleo-" Endemism) two-step procedure (Mishler & al. 2014). In the first step, to establish which grid cells are centres of endemism, all cells that show significantly high values (one tailed, $\alpha = 0.05$) for either the numerator or denominator of RPE were selected. Grid cells that passed the first step but that have $SR < 4$ were removed from the selection. In the second step, the RPE significance (two tailed, $\alpha = 0.05$) of the selected areas was evaluated in order to classify grid cells: (i) as centres of neo-endemism if they show significant low values of RPE, indicating the major presence of range-restricted taxa having short terminal branches; (ii) as centres of paleo-endemism if they show significant high values of RPE, indicating the major presence of range-restricted taxa having long terminal branches; (iii) as centres of mixed endemism if they do not show significance for RPE values, indicating the co-occurrence of range-restricted taxa with both short- and long- terminal branches. These latter centres of Mixed Endemism were further subdivided and named as centres of Super Endemism if both the numerator and the denominator of the RPE ratio are highly significant.

Finally, biodiversity hotspots for conservation planning (*sensu* Myers & al. 2000) were identified by comparing and selecting those grid cells which are centres of diversity and/or endemism. Preliminary insights on the conservation status of the most rare pteridophyte populations were also provided.

Results

Phylogenetic biodiversity analyses

Maps of SR, WE, PD and PE are shown in Fig. 2. PD and SR values are significatively positively correlated ($R^2 = 0.615$), but the two spatial patterns show different trends.

Higher SR values are mainly located in Temperate mountain areas, with some isolated exceptions in volcanic sub-coastal areas and in the Mediterranean zone. Higher PD values are mainly located in Mediterranean areas and in some internal areas which include water bodies, with some isolated exceptions in Temperate mountain areas and in volcanic sub-coastal areas.

Spatial differences between these two patterns may be explained by two factors: (i) ancient taxa (e.g., *Lycophytes*) show relatively more restricted distributions that are mainly placed in the Mediterranean zone, as for *Selaginella* or *Isoetes*; *Huperzia selago* is restricted (singleton) only in high mountain dwarf (*Vaccinium*) heaths in the Laga district; (ii) recent taxa (e.g., *Eupolypods* which include *Polypodiineae* and *Aspleniineae*) have relatively wider distributions, mainly in Temperate internal areas.

PE and WE are significatively positively correlated ($R^2 = 0.568$), with very similar spatial patterns. Higher WE and PE values do not show a typical trend but are sparsely located in several isolated areas with very different lithology, elevation and climatic zones (Table 1). A first area with very high values of WE and PE is placed on the Western and South-Western slopes of Pizzitello, Pizzo di Sevo and Cima Lepri Mounts (Laga Mountains) on siliceous and marly-arenaceous soils from Flysch. Within this area, three singletons occur (*Equisetum variegatum*, *Huperzia selago* and *Athyrium distentifolium*), as well as other very restricted taxa (e.g., *Gymnocarpium dryopteris, Polystichum* x *bicknellii* and *Cystopteris alpina*). Another centre of endemism is the area near the eastern shore of the volcanic Bracciano Lake where a very important singleton occurs (*Isoetes sabatina*), which is not just a "relative endemism", but rather a very restricted endemic taxon that actually occurs just in this site and not elsewhere in the world (i.e., "absolute endemism").

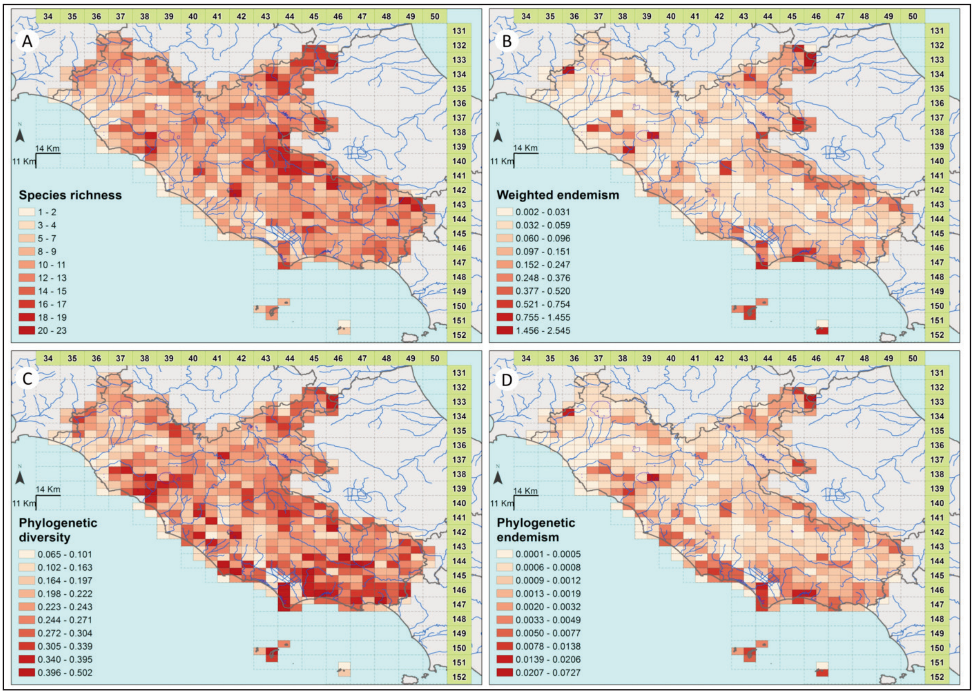

Fig. 2. Maps of basic and phylogenetic biodiversity patterns of native pteridophytes in Latium. Notes: A: SR (species richness) values; B: WE (weighted endemism); C: PD (phylogenetic diversity); D: PE (phylogenetic endemism).

Table 1. Hotspot locations, very rare taxa list, PD, RPD, PE, RPE index values and results of the CANAPE classification for the selected grid cells showing higher PE values. Notes: grid cells code format is provided as in Lucchese (2017); bold taxa indicate singletons; significance of index values is marked with ** and *** for p values > 0,95 and > 0,975 respectively.

Mark	Hotspot location	Rare taxa	Grid cell	PD	RPD	PE	RPE	CANAPE
A	Laga Mountains	***Equisetum variegatum*** ***Huperzia selago*** ***Athyrium distentifolium*** *Gymnocarpium dryopteris* *Polystichum x bicknellii* *Cystopteris alpina*	13346-1 13245-4 13346-3	0,371 0,312 0,347	0,8 0,657 0,627	0,072 *** 0,011** 0,01	2,043** 1,132 0,523	SUPER MIX
B	Bracciano Lake (eastern shore)	***Isoetes sabatina*** *Isoetes durieui* *Asplenium adianthum-nigrum*	13839-4	0,321**	0,97***	0,051***	5,88***	PALEO
C	Lamone Reserve	***Asplenium septentrionale*** ***Ophioglossum azoricum***	13436-1	0,285	0,695	0,02***	0,674	SUPER
D	Fondi Lake	*Salvinia natans* *Thelypteris palustris* *Osmunda regalis* *Asplenium adianthum-nigrum* *Ophioglossum lusitanicum* *Equisetum x moorei* *Isoetes histrix* *Isoetes durieui*	14646-3 14645-4	0,355*** 0,429***	1,267*** 0,995***	0,018*** 0,018***	1,32 1,266	SUPER MIX
E	Sabaudia surroundings	*Thelypteris palustris* *Osmunda regalis* *Ophioglossum lusitanicum* *Ophioglossum vulgatum* *Isoetes histrix* *Isoetes durieui* *Isoetes longissima*	14644-1 14744-1 14644-3	0,502*** 0,415*** 0,459***	1,225*** 1,255*** 1,081***	0,017*** 0,011*** 0,01**	2,385*** 2,211** 2,82***	PALEO MIX PALEO
F	Near Cerveteri	***Oeosporangium tinaei*** *Osmunda regalis*	13938-4	0,447**	0,874	0,014**	1,193	MIX
G	Terracina	***Asplenium sagittatum*** *Asplenium petrarchae* *Oeosporangium acrosticum*	14745-2	0,293	0,85	0,011***	0,902	SUPER
H	Ponza Island	*Asplenium balearicum* *Asplenium obovatum* *Osmunda regalis*	15043_4	0,42***	1,167***	0,01***	1,536	MIX
I	Circeo Promontory	***Asplenium marinum*** *Osmunda regalis*	14744-3	0,425***	0,953***	0,01	0,991	
L	Near Nettuno	*Osmunda regalis* *Isoetes histrix* *Isoetes durieui* *Isoetes longissima*	14542-1	0,439***	1,11***	0,009**	3,395***	PALEO
M	Duchessa Mountains	***Asplenium lepidum*** *Asplenium fissum* *Cystopteris alpina* *Dryopteris villarii*	13745-4	0,276	0,581	0,008	0,68	
N	Zannone Island	***Asplenium x tyrrhenicum*** *Asplenium obovatum*	15246-2	0,169	0,871	0,008***	0,867	SUPER
O	Near S. Vittorino and Gallicano	*Pteris cretica*	14042-4 14142-2	0,285 0,274	0,66 0,694	0,005 0,005	0,632 0,656	

Other very important areas with high values of WE and PE are located in the volcanic district of the Lamone Reserve (two singletons: *Asplenium septentrionale* and *Ophioglossum azoricum*), on the Fondi coastal Lake (one doubleton: *Salvinia natans*, and other very rare taxa e.g., *Thelypteris palustris*, *Osmunda regalis* and *Isoetes* spp. near Sabaudia in a lowland forest (very rare taxa e.g., *Thelypteris palustris*, *Osmunda regalis*, *Ophioglossum lusitanicum*, *O. vulgatum* and *Isoetes* spp.), near Cerveteri on coastal trachytic outcrops (one singleton: *Oeosporangium tinaei*, and other very rare taxa e.g., *Osmunda regalis*), near Terracina on limestone rocks (one singleton: *Asplenium sagittatum*, and other very rare taxa e.g., *Asplenium petrarchae* and

Oeosporangium acrosticum), on pumice and tuff deposits in the Ponza island (where very rare taxa occur e.g., *Asplenium balearicum*, *A. obovatum* and *Osmunda regalis*), in the Circeo Promontory on sea limestone cliffs (one singleton: *Asplenium marinum*, and other very rare taxa e.g., *Osmunda regalis* in bodies of water), near Nettuno (very rare taxa e.g., *Osmunda regalis* and *Isoetes* spp.), in the Duchessa Mountains on limestone rocks (one singleton: *Asplenium lepidum*, and other very rare taxa e.g., *Asplenium fissum, Cystopteris alpina* and *Dryopteris villarii*), in the Zannone island on pumice and tuff deposits (one singleton: *Asplenium* x *tyrrhenicum*, and other very rare taxa e.g., *Asplenium obovatum*) and near San Vittorino and Gallicano on volcanic tuffs (one doubleton: *Pteris cretica*).

Spatial patterns of significance values of PD, PE, RPD and RPE based on randomization analyses are shown in Fig. 3.

Areas of significantly high PD and PE values include mainly Mediterranean coastal and insular sectors of Latium and some isolated internal areas located near some river valleys; areas of significantly low PD and PE values include mainly Temperate mountain sectors and volcanic areas located between Temperate and Mediterranean zones.

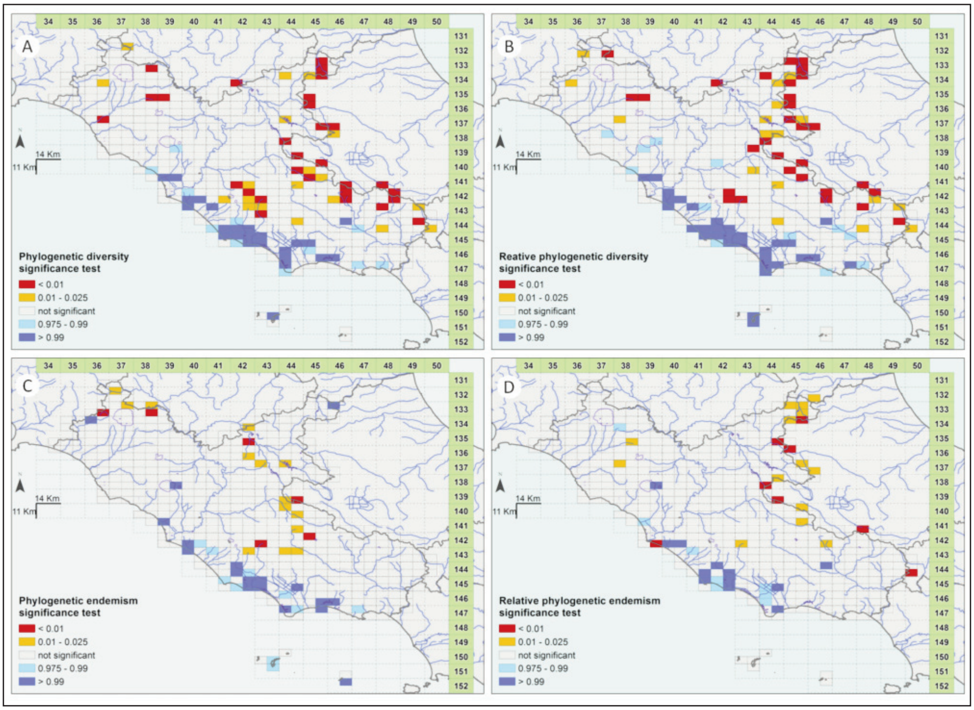

Fig. 3. Maps of the significance values resulting from a randomization test of native pteridophytes in Latium. Notes: transparent cells contain no data; grey cells indicate not significant values or areas with less than 4 taxa; red and orange grid cells contain significantly lower PD, RPD, PE and RPE values than expected; light blue and dark blue grid cells contain significantly higher PD, RPD, PE and RPE values than expected (as indicated).

Three important exceptions that do not follow the above-mentioned trends are represented by Laga Mountains, Lamone and Bracciano Lake. The significantly high PE values reached by these non-Mediterranean areas may probably be explained by the relative high presence of singletons and other range-restricted taxa in the observed distribution which are not likely estimated within the null distribution. Surprisingly, the Duchessa Mountains, the areas near San Vittorino and Gallicano and the Circeo promontory show non-significant PE values. In these cases, despite the presence of singleton, doubleton and other very rare taxa, the observed values fall between the 2-tails of the null distribution.

The spatial pattern of the RPD significance values shows the same general trend observed for PD. Also, the spatial pattern of the RPE significance values shows the same general trend as PE, albeit with some exceptions: (i) Lamone, Ponza and Ventotene islands show significantly high values only in PE; (ii) the surroundings of Ostia and Castelfusano show significantly low values only in RPE; (iii) the Laga Mountains show significantly high PE values but significantly low RPE values.

Assessment of pteridophyte centres of diversity and endemism

A total of 47 grid cells have been identified as centres of phylogenetic endemism. Applying the CANAPE procedure, we identified 1 centre of neo-endemism, 18 centres of paleo-endemism, 23 centres of mixed-endemism and 5 centres of super-endemism (Fig. 4). The grid cell identified as being dominated by neo-endemism is located along the coastal surroundings of Ostia, in the lowland Castel Fusano forest.

This area only includes 4 taxa, *Adiantum capillus-veneris*, *Equisetum telmateia*, *E. ramosissimum*, that are very common, and *E. fluviatile* that only occurs in 5 grid cells. Despite these taxa have relatively short terminal branches within the phylogenetic tree, we believe that this area cannot be a valuable centre of endemism (see Figs. 2b, 2d and 3c). Centres of paleo-endemism are mainly located along the coastal Mediterranean areas, except for the Eastern shore of Bracciano Lake which are placed on a volcanic area between the Mediterranean and the Temperate climatic zones. Several rare taxa with relatively long terminal branches, *e.g.*, *Isoetes* spp. and *Osmunda regalis*, occur in these areas. Severe human-made land use changes (*e.g.*, drainage and reclamation of coastal swamps and marshes, and deforestation) probably have reduced the distribution of these taxa during the last Century. Thus, we believe that these areas may represent proper centres of paleo-endemism.

Centres of mixed endemism are also mainly located in Mediterranean areas, but with several isolated exceptions in internal and mountainous areas within the Temperate climatic zone. A large number of rare pteridophytes with both short (e.g., *Thelypteris palustris*, *Oeosporangium acrosticum*, *Asplenium petrarchae* and *A. obovatum*) and long terminal branches (e.g., *Isoetes* spp. and *Osmunda regalis*) co-exist in most of these areas.

Centres of super-endemism are located in areas that are extremely different in both climates, lithologies and elevation belts. A first centre of super-endemism is the area of the Laga Mountains, having three singletons and other very rare taxa.

Changes in the occupancy area of rare species are usually due to artificial (*e.g.*, human-made land use changes) and natural (e.g., competition or isolation) processes, but they may also be due to the restrictedness of suitable niches. Unlike the other mountainous massifs of Latium, which are mainly formed by carbonate lithology, the Laga Mountains are composed by arenaceous, pelithic-arenaceous and marly litofacies. Thus, the singular occurrence of acidophilous taxa e.g., *Huperzia selago*, *Gymnocarpium dryopteris* and *Athyrium distentifolium* in this site is mainly a consequence of its peculiar lithology, probably as glacial relicts. Another important centre of super-endemism is the volcanic area of Lamone, where two very valuable singletons co-exist: *Asplenium septentrionale*, with a relatively short terminal branch, and *Ophioglossum azoricum*, with a long branch. *Asplenium septentrionale* is a typical circumboreal acidophilous and orophilous species which is very rare in the Italian peninsula and usually occurs at higher elevations (more than 1,200 m). The population of the Lamone Reserve is placed at 300 m, thus this taxon may be considered as an acidophilous glacial relict and the Lamone site may be interpreted as a suitable climatic refuge. *Ophioglossum azoricum* is a typical W-European Atlantic acidophilous species.

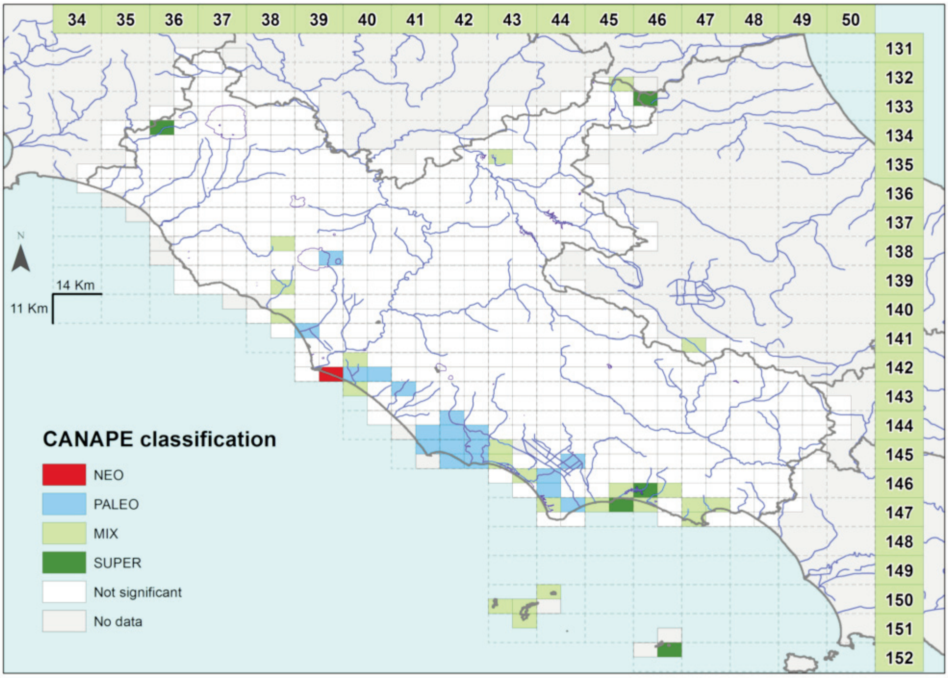

Fig. 4. Map of centres of endemism that also denote potential biodiversity hotspots for conservation planning (*sensu* Myers & al. 2000). Notes: grey cells contain no data; white cells are not significant or show less than 4 taxa; red cells indicate centres of neo-endemism that contain significantly lower RPE values than expected; blue cells indicate centres of paleo-endemism that contain significantly higher RPE values than expected; light green cells indicate centres of mixed-endemism that show not significantly lower or higher RPE values than expected; dark green cells indicate centres of super-endemism that are special cases of “mixed” showing the most highly significantly values of the numerator and denominator of the RPE ratio.

The few individuals found at the Lamone Reserve are highly isolated from the main distributional range, thus this population should be considered as the main target for regional and local conservation planning. Recently, Peruzzi & al. (2015) proposed a new taxonomic attribution, referring this population to a new pentaploid hybrid named *Ophioglossum* x *pseudoazoricum* (a reverse hybrid of the *O. azoricum* x *O. vulgatum* combination). Accordingly, this hybrid should be considered as extremely rare (occurring only in few localities of southern France, Tuscany and Latium) and a biogeographically valuable taxon of the regional flora. A third centre of super-endemism includes Terracina and its surroundings, with the occurrence of *Asplenium sagittatum*, a Mediterranean taxon which has gone extinct in several localities of Latium and Italy during the last two Centuries. Recently, the IUCN risk category for this taxon in Latium has been assessed as Critically Endangered (Iocchi & Lucchese 2018). Thus, also this taxon should be considered as a topic target for regional and local conservation planning.

Another centre of super-endemism is the eastern sector of the Fondi Lake.

Several very rare aquatic and semi-aquatic taxa occur within this area. Among these, the most endangered is probably *Salvinia natans*, an Eurasiatic taxon with a very long terminal branch in the phylogenetic tree. The populations near the Fondi Lake are the southernmost of the natural range in the Italian peninsula and are also very isolated (the nearest population is in Tuscany, 200 km away). Several human-induced threats (e.g., alien species invasions, water pollution and water capture for agricultural irrigation) might cause the local extinction of this taxon in the next decades. Thus, we believe that this area should be considered as a high-priority in conservation planning.

The last centre of super-endemism is the Zannone island, with only 5 taxa, including *Asplenium × tyrrhenicum* (the natural hybrid of *A. balearicum × A. onopteris*) and *Asplenium obovatum*. Both are endemisms, mainly occurring on Mediterranean islands. Accordingly, their distribution within Latium is restricted to the Pontine Archipelago. They show relatively short terminal branches within the phylogenetic tree, indicating a relatively recent origin, that in both cases took place by polyploidy and hybridization (Sessa & al. 2018). Thus, we believe that this area should be considered as a very important centre of endemism.

Discussion

The use of phylogenetic metrics, that take into consideration the evolutionary relationships between taxa, offers an improvement over traditional biodiversity pattern assessments that consider species diversity alone. *Lycophytes* include several ancient lineages formed by rare taxa with relatively longer terminal branches, which occur mainly in the Mediterranean zone of Latium (*Selaginella* and *Isoetes*). *Pteridophytes* may be further subdivided in three subclades: *Psilotopsida*, *Equisetopsida* and *Polypodiopsida*. The first clade includes rare, ancient taxa with relatively longer terminal branches (i.e., *Botrychium* and *Ophioglossum*), whereas within the *Equisetopsida,* all taxa have relatively shorter terminal branches (i.e., *Equisetum*). Most pteridophytes (43 of the 61 selected taxa) are part of the *Polypodiopsida* clade. Also, within this clade, ancient taxa with relatively longer terminal branches (e.g., *Osmunda*, *Salvinia*, *Oeosporangium*, *Pteridium* and *Adianthum*) mainly occur in the Mediterranean zone of Latium, whereas several recent taxa with relatively shorter terminal branches (e.g., *Polystichum*, *Dryopteris*, *Cystopteris* and some orophilous *Asplenium*) mainly occur in the Temperate zone of Latium. These different spatial trends explain the observed dissimilarities between the spatial patterns of SR and PD, highlighting the relatively higher phylogenetic value of several Mediterranean areas, as well as of some internal grid cells which host water bodies (*i.e.*, rivers and lakes). We may define as centre of phylogenetic diversity of native pteridophytes the following Mediterranean areas: (i) Sabaudia (lake and forest), Fondi Lake surroundings, Circeo Promontory and Ponza island; (ii) Nettuno (Foglino and Padiglione forests); (iii) Cerveteri, surroundings; (iv) Scauri-Minturno, surroundings. Higher PD values are also observed in the following internal areas which include water bodies: Liri valley near Ceprano, Amaseno valley near Priverno and Albano and Nemi lakes.

Concerning the range restrictedness of taxa (or "relative endemism"), we focused on four overlapping main causes: (i) artificial impacts (e.g., human-made land use changes and water pollution); (ii) natural processes (e.g., competition, hybridization, and polyploidy); (iii) historical processes (e.g., relict isolation); (iv) rarity of suitable niches. We believe that the first two causes are mainly responsible for the restricted occurrence of several Mediterranean, very rare taxa (i.e., most aquatic or semi-aquatic pteridophytes such as *Isoetes* spp., *Salvinia natans*, *Ophioglossum* spp. and *Osmunda regalis*). In contrast, the latter two causes are mainly responsible for the restricted occurrence of some very rare taxa in Temperate areas (e.g., *Huperzia selago*, *Athyrium distentifolium* and *Equisetum variegatum* on the Laga Mountains and *Asplenium septentrionale* on the Lamone Reserve). Thus, the spatial patterns of WE and PE may mainly be due to the different effects (in space and time) of these overlapping causes. We identified a total of 47 centres of endemism, which should be considered as valuable biodiversity hotspots (sensu Myers & al. 2000) because of their high evolutionary and conservation value. Most of them match with protected areas (e.g., Gran Sasso and Laga Mountains National Park, Circeo National Park, "Selva del Lamone" Regional Natural Reserve; Bracciano and Martignano Regional Park), thus both habitat and species should benefit from adequate conservation actions (e.g., monitoring, threats and extinction risk assessment). Two other selected hotspots (marked with D and L in Table 1) encompass two "Natura 2000" sites ("Bosco di Foglino" IT6030047 and "Lago di Fondi" IT6040010).

Unfortunately, the spatial localization of the rarest populations does not always fall within the boundaries of protected areas. Moreover, several very rare taxa are not listed as "important species" in the standard data forms of the two sites, and therefore do not receive any specific attention. Even if suitable habitats of these very rare taxa formally should benefit from conservation actions, we believe that in these cases monitoring and extinction risk assessment should be performed at species level. We suggest providing monitoring programmes in order to understand population sizes and trends of hygrophilous pteridophytes which occur in aquatic habitats such as lake shores, swamps, marshes, and hygrophilous woods. These species may also be used as bioindicators, because they are very sensitive to specific abiotic factors such as water drainage and salinity changes, eutrophication and pollution. This is the case of *Salvinia natans,* which occurs at the Fondi Lake (outside the protected area boundaries). At the European level, *S. natans* is considered as NT ("Near threatened"; García Criado & al. 2017), whereas at the Italian national level its extinction risk is reported as VU ("Vulnerable"; Rossi & al. 2013). As suggested before, the Fondi Lake site is the southernmost in the Italian peninsula and it is also very isolated from the natural range of the species. Over the last Century, *S. natans* has disappeared from several sites of Latium and other Italian regions (Lucchese 2018). Scientific studies on the biology of this threatened species are needed, in order to better define the most important biotic and abiotic factors that may impact its occurrence, and to perform adequate conservation actions.

Another important hotspot is placed near Cerveteri (marked with F in Table 1). Unfortunately, the very rare taxa which occur within this area do not receive any specific protection or conservation actions. In this case, establishing a new protected reserve is needed, in order to provide an adequate management plan for conservation of the species. The occurrence within Latium of *Oeosporangium tinaei* (singleton) has been recently detected (Rosati & al. 2020). We have performed a preliminary field census in order to assess the population size and we have found just a single individual, thus the extinction risk at regional scale is extremely high. A specific study of the major threats which affect this taxon in the site is needed. The *O. tinaei* individual has been found inside a rocky fissure on a south-facing steep slope dominated by sparse Mediterranean dwarf shrubs and bushes. Goats and sheep grazing and the increase in fire frequency are the two reasonable major threats to this taxon. In this case, an urgent action of *ex-situ* conservation is needed, such as collecting and conserving spores in a seed bank.

During the last two Centuries, a high number of taxa became extinct in Latium, mostly in the Mediterranean zone (Lucchese 2018). Among these, 3 aquatic and semi-aquatic pteridophytes have been identified: *Pilularia minuta*, *Marsilea quadrifolia* and *Thelypteris limbosperma*. Following the precept "learn from the past to plan a better future", we suggest focusing the attention of researchers on the conservation of both hygrophilous species and water habitats occurring within the observed centres of endemism. A more detailed monitoring of population sizes and trends, as well as fully comprehensive overviews of the potential threats are needed. Moreover, habitat protection and restoration are also required, especially in aquatic habitats and wetlands that are highly affected by pollution, canalisation, eutrophication, drainage and changes in hydrological regime.

Conclusion

Very rare pteridophytes do not receive any specific protection or conservation actions. Spatial phylogenetic metrics of pteridophyte diversity and endemism allowed us to identify the main native pteridophytes PD and PE centres, valuable as biodiversity hotspots due to their high evolutionary and conservation value.

We suggest performing monitoring programmes to understand population sizes and trends and focusing research plan on the conservation of both hygrophilous species and water habitats occurring within the observed centres. Among the several species, we highlight that *Salvinia natans* and *Oeosporangium tinaei* should be priority species for Latium.

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