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Patterns of orchid species richness and composition in relation to geological substrates

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Summary: Terrestrial orchids in Europe are known for their great species richness that occurs on calcareous geological substrates. However, the knowledge of orchids that grow on other bedrock types is limited. In this paper, the richness and composition of orchid species in relation to geological substrates in the central Balkans (western Serbia) were investigated. For identification of floristic similarities among the geological substrates, the clustering method based on Jaccard distances and unweighted pair-group average hierarchical sorting strategy (UPGMA) was used. In addition, multi-response permutation procedure (MRPP) was applied to analyse the differences in orchid composition between bedrock types. Orchid species and subspecies were recorded on ten bedrock types. The greatest number of orchids was recorded on limestone-dolomite (52 species and subspecies), ophiolitic mélange (40) and schists-gneiss-phyllites (31). However, a significant number of orchid species and subspecies were recorded on carbonate clastites, ultramafics, andesite-dacite-porphyrite and Quaternary sediments. On the other hand, a smaller number of orchid species were recorded on acidic igneous rocks (quartz latite and granodiorite), indicating that they are less favourable for orchids. The cluster analysis of floristic similarities allowed the identification of seven sets of geological substrates. The greatest floristic similarity was identified between limestone-dolomite and ophiolitic mélange as well as schists-gneiss-phyllites and ultramafics. However, an analysis of the composition of orchid flora in terms of abundance and frequency of orchids indicated that carbonate substrates (limestone-dolomite and carbonate clastites) differ statistically from the highest number of bedrock types. *Cephalanthera damasonium*, *C. longifolia*, *Dactylorhiza sambucina*, *Epipactis helleborine*, *Gymnadenia conopsea*, *Neottia nidus-avis* and *Platanthera bifolia* were found to grow on nine bedrock types, suggesting their great ecological plasticity and adaptability. This study emphasizes the important role of geological substrates in the richness, composition and distribution of orchid species in the central Balkans, pointing out that many species, known to date to occur exclusively on calcareous substrates, also grow on different silicate substrates and ultramafics. The results indicate that differences in the species richness and composition of orchids which are related to the geological substrates of their habitats should be taken into account when designing orchid conservation and management strategies.

Keywords: Orchidaceae, bedrock types, diversity, flora, Serbia, Balkan Peninsula

Understanding how patterns of species richness and composition vary along geographical and ecological gradients is a central goal of many ecological and biogeographical studies (Zelnik & Čarni 2013; Zhang et al. 2015; Tsiftsis et al. 2019b). On a large geographic scale, diversity patterns of species are determined mainly by historical factors and macroclimate, whereas on a regional scale factors such as altitude, meso- and microclimate, light, vegetation type, disturbance and biotic factors influence the richness and abundance of species (Tsiftsis et al. 2008, 2019a, 2019b; Bystriakova et al. 2015; Djordjević et al. 2016a, b). Furthermore, geological substrates and consequently the soil properties represent important factors in determining diversity patterns of both plant communities and species (Barbaro et al. 2004; Holzinger et al. 2008; Kooijman 2010; Cachovanová et al. 2012; Zelnik & Čarni 2013; Fenu et al. 2014; Hahm et al. 2014). It is known that soil pH, the concentrations of basic cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and acidic cations (H^{+}, Al^{3+}) significantly affect the composition of plant communities (Ewald 2003). In general, numerous studies showed that the relationship between vascular plant species richness and base rich and calcareous soils in Europe is often positive, representing one of the most consistent gradients of species richness (Pärtel 2002; Ewald 2003). This pattern is explained by physical and chemical properties of the soil, the evolutionary history as well as the species pool size (Zobel et al. 1998; Pärtel 2002; Ewald 2003). Namely, it was hypothesized that high species richness on calcareous soils in Europe may be explained by the fact that carbonate substrates were widely distributed during the Quaternary period and that this influenced the evolution of many plant species on high pH soils (Grubb 1987). It should be noted that preferences of plant species for a certain bedrock type and soil are not independent of responses to other ecological factors. Thus, plant species that prefer calcareous substrates often grow on dry habitats and full-light conditions (Ewald 2003). Knowledge of species preferences in relation to geological substrates and soil properties may be useful for planning the conservation of species and their habitats, especially when it comes to sensitive and rare species in areas exposed to land-use changes and various aspects of anthropogenic factors.

The family Orchidaceae which includes 749 genera with approximately 28,000 species is considered to be one of the largest families in the plant kingdom (Christenhusz & Byng 2016; Chase et al. 2017). Although orchids occur throughout the world in different habitat types, most of the species are concentrated in tropical areas of the world. In Europe, all orchid species are terrestrial, inhabiting forests and herbaceous vegetation types (Delforge 2006). Since many orchid species are endangered primarily due to habitat loss and strong anthropogenic influences that lead to environmental changes, the knowledge of their habitat preferences is necessary in order to organize an adequate conservation. Numerous studies in Europe have highlighted that calcareous substrates are most suitable for the growth and survival of orchids, and that most orchids grow on these substrates (G.I.R.O.S. 2009; Landi et al. 2009; Pierce et al. 2014; Tsiftsis & Antonopoulos 2017; Tsiftsis et al. 2008, 2019b). However, the knowledge about the richness and composition of orchid species that grow on other bedrock types is limited. Moreover, some studies showed that some orchids inhabit environmentally polluted habitats, such as mining areas and fly ash deposits which represent extreme environments (Jurkiewicz et al. 2001; Esfeld et al. 2008), suggesting their ecological plasticity and tolerance. Although many studies provided an insight into the physical and chemical properties of the soil on which orchids grow (Vakhrameeva et al. 2008; Tsiftsis et al. 2008, 2012; Jacquemyn & Hutchings 2010; Jersáková et al. 2011, 2015; Meekers et al. 2012; Jacquemyn et al. 2014; Kotilínek et al. 2015, 2018), fewer papers have given information about preferences of orchids in relation to geological substrates (Tsiftsis et al. 2008, 2019a; van der Ent & Wood 2013; Filimonova et al. 2019). Furthermore, information is often given in the broad sense as 'carbonate substrates', 'silicate substrates', 'alkaline substrates' and 'acidic substrates' (Holzinger et al. 2008; Brković et al. 2015), without providing the specificities of these substrates. This is especially important due to the fact that there are several types of silicate and carbonate geological substrates that differ from each other in age as well as in physical and chemical properties. Studies in Greece and western Serbia have shown that geological substrates play an important role in determining the patterns of distribution and abundance of orchids (Tsiftsis et al. 2008; Djordjević et al. 2016a, b). However, these studies indicated the need for future research on the relationship between geological substrates and orchids, both in terms of diversity patterns, and in the issue of their ecophysiology.

In this study, the preferences of orchid species and subspecies in relation to bedrock type in the central Balkans (western Serbia) were studied. We hypothesised that the greatest number of orchid species and subspecies grows on carbonate geological substrates, and that a smaller number of orchid taxa occur on acidic igneous rocks and ultramafics due to stressful growth conditions. The objectives were: (a) to determine orchid species and subspecies richness in relation to bedrock type, (b) to explore the similarities in the composition of orchid flora between certain bedrock types, (c) to analyse the differences in orchid composition between bedrock types in terms of abundance and frequency of orchids, and (d) to identify the orchid species that grow on the largest number of geological substrates.

Materials and methods

Study area. The territory of western Serbia lies in the north-central part of the Balkan Peninsula and covers an area of ca. 18,000 km^2. The altitude ranges from 65 m to 2,154 m. In the study area, different geological substrates are present, both by age and by chemical composition, i.e. various sedimentary, igneous and metamorphic rocks, whereas the specificity is high presence of carbonate substrates (limestone, dolomite and carbonate clastites), ultramafics and ophiolitic mélange (Chiari et al. 2011; Gawlick et al. 2017). According to Stevanović & Stevanović (1995), there are four bedrock types in western Serbia: (a) silicate bedrocks of acidic to neutral pH (igneous, metamorphic, sedimentary), (b) silicate bedrocks of basic to ultra-basic reaction (serpentinites and peridotites), (c) carbonate bedrocks of neutral to basic reactions (clastic, sedimentary), and (d) loess and Pleistocene sediments and deposits. The climate of the study area is humid temperate. The mean annual temperature varies from ca. 3.0°C in the regions over 1500 m of altitude to 11.6°C in the warmest parts, whereas annual precipitation varies between 726.4 mm in lower regions and ca. 1500 mm in mountain areas (Djordjević et al. 2016a).

Data collection. During chorological and ecological research of orchids of western Serbia, orchid abundance and geological substrate were determined at each locality. The identification of orchid taxa was performed according to Delforge (2006), whereas nomenclature followed the World Checklist of Kew Gardens (WCSP 2019). During field observations in the period between 1995 and 2019, data on 51 orchid species and subspecies from 2188 localities were collected. In addition to the field survey, the preferences of orchids in relation to geological substrates were supplemented by literature sources as well as by checking herbarium material deposited in the Herbarium of the Institute of Botany and Botanical Garden 'Jevremovac', University of Belgrade [BEOU] and the Herbarium of the Natural History Museum in Belgrade [BEO]. In total, literature data on 46 species and subspecies from 550 localities, and herbarium data on 43 species and subspecies from 195 localities were used. Consequently, the dataset is compiled of 55 orchid species and subspecies recorded at 2933 localities.

During field observations the abundance of each orchid species was determined by counting the total number of flowering individuals at the sample area (ca. 2500 m^2) of each site. Then, the abundance of each orchid species was expressed through a four-point scale, used by Tsiftsis et al. (2008): (1) 1–5 individuals, (2) 6–20 individuals, (3) 21–50 individuals and (4) > 50 individuals. In addition, literature data related to orchids with Braun-Blanquet (1964) values were converted to a four-point scale as follows: (1) r, (2) +, (3) 1, (4) 2.

Geological substrates were determined on the basis of field observations and the geological maps of the study area in a 1:100,000 scale (available on the Internet: http://geoliss.mre.gov.rs/

OGK/RasterSrbija/). The substrates were grouped into 10 classes based on the supplemented classification according to Djordjević et al. (2016a): (1) andesite-dacite-porphyrite; (2) carbonate clastites, which include carbonate sandstones, sands, shales, gravels and clays, conglomerates and marls; (3) flysch; (4) granodiorite; (5) limestone-dolomite, which includes different types of limestone and dolomite; (6) ophiolitic mélange and sandstones from the Carboniferous and Permian periods, which include diabase, gabbro, spilite, and cherts, sandstones, shales and marls of the Jurassic period, and sandstones from the Carboniferous and Permian periods; (7) quartz latite; (8) Quaternary sediments, which include proluvial and alluvial deposits, eluvial-deluvial sediments and river terraces; (9) schists-gneiss-phyllites; and (10) ultramafics (serpentinites and peridotites).

Data analysis. The degree of similarity in the composition of orchid flora in western Serbia was determined on the basis of the similarity index according to Jaccard (1928). The similarity (S) was calculated based on the formula: $S = Sij / St \times 100$, where Sij is the number of orchid species and subspecies which are shared between both sets of geological substrates, whereas St is the total number of orchid species and subspecies in both substrates (shared and un-shared). For identification of hierarchical floristic similarities among the 10 bedrock types, clustering based on Jaccard distances and unweighted pair-group average hierarchical sorting strategy (unweighted pair group method with arithmetic mean) was used.

In addition, we used multi response permutation procedure (MRPP; Quinn & Keough 2002), a nonparametric method analogous to the analysis of variance, to analyse the differences in orchid composition between bedrock types. This procedure was used to test the null hypothesis of no differences in orchid composition between each pair of the bedrock types of the study area. In this analysis, we used data not only on the presence / absence of species, but also concerning orchid abundance through a four-point scale (see data collection). All analyses were performed in R version 3.5.1 (R Core Team 2013).

Results

Orchid species and subspecies richness in relation to geological substrates. The orchid species and subspecies richness in relation to geological substrates in western Serbia (the Central Balkans) is presented in Fig. 1, whereas the detailed preferences of orchids in relation to geological substrates are shown in Table 1. The highest number of orchid species and subspecies in western Serbia was recorded on limestone and dolomite (52). The orchids found on ophiolitic mélange and sandstones from the Carboniferous and Permian periods are in the second place with 40 species and subspecies, whereas the orchids recorded on schists-gneiss-phyllites are in the third place with 31 species and subspecies. A significant number of orchid species and subspecies were recorded on carbonate clastites, ultramafics, andesite-dacite-porphyrite as well as Quaternary sediments, whereas the smallest number of orchid taxa was recorded on quartz latite, granodiorite and flysch (Fig. 1, Table 1).

Preference of orchids of western Serbia in relation to geological substrates. *Cephalanthera damasonium*, *C. longifolia*, *Dactylorhiza sambucina*, *Epipactis helleborine*, *Gymnadenia conopsea*, *Neottia nidus-avis* and *Platanthera bifolia* were recorded on nine geological substrates, whereas *Anacamptis coriophora*, *A. morio*, *Dactylorhiza incarnata*, *D. saccifera*, *Neotinea ustulata*, *Neottia ovata* and *Traunsteinera globosa* were found growing on eight bedrock types (Table 1). Five orchid

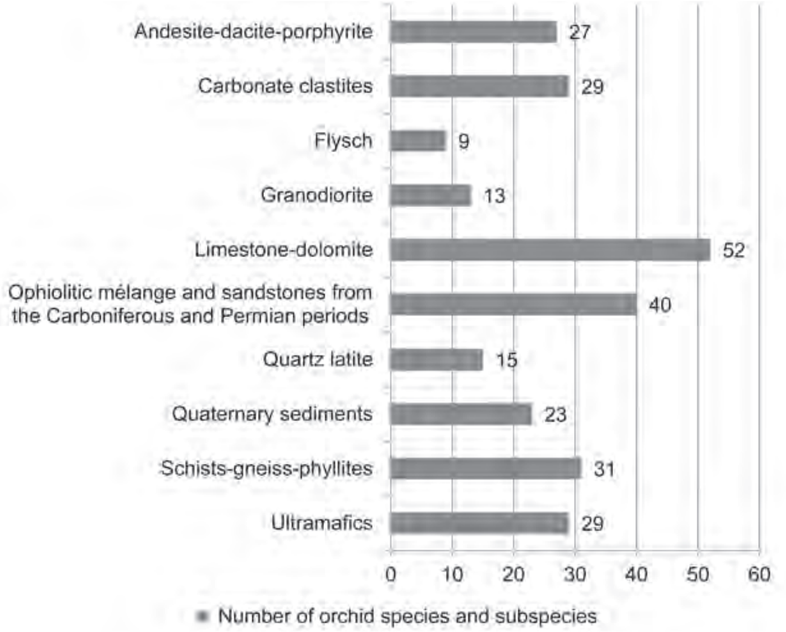

Figure 1. The orchid species and subspecies richness in relation to geological substrates in the Central Balkans (western Serbia).

species and subspecies were recorded on seven geological substrates, six orchid species were found on six geological substrates, whereas other orchid species were recorded on a smaller number of geological substrates (Table 1). Among the orchid species that grow on a small number of geological substrates, nine species were recorded on one geological substrate, eight species were found on two geological substrates, whereas three species and subspecies were found on three geological substrates (Table 1).

Analysis of the similarity of orchid flora among geological substrates. The results of the analysis of floristic similarities among geological substrates are presented in Fig. 2 and Table 2. The results of the cluster analysis of floristic similarities among certain bedrock types allowed the identification of seven sets of geological substrates: (1) flysch, (2) granodiorite, (3) quartz latite, (4) Quaternary sediments, (5) andesite-dacite-porphyrite and carbonate clastites, (6) limestone-dolomite and ophiolitic mélange and sandstones from the Carboniferous and Permian periods, and (7) schists-gneiss-phyllites and ultramafics (Fig. 2). It is noticeable that flysch is separate, which means that this geological substrate is the most different compared to other geological substrates when looking at the floristic composition of orchids. The greatest similarity of the orchid flora was determined between the following subsets: a) limestone-dolomite and ophiolitic mélange and sandstones from the Carboniferous and Permian periods (76.92%), b) andesite-dacite-porphyrite and carbonate clastites (75.00%), and c) schists-gneiss-phyllites and ultramafics (66.67%). Moreover, the greatest floristic similarities were found between the following pairs of subsets: a) limestone-dolomite and ophiolitic mélange and sandstones from the Carboniferous and Permian periods, and c) schists-gneiss-phyllites and ultramafics (Fig. 2).

Differences in orchid composition between bedrock types. Multi-response permutation procedure (MRPP) yielded variable results about the relationships between different bedrock types regarding their orchid flora (Table 3). Carbonate substrates (limestone-dolomite and

Table 1. Representation of orchids on the geological substrates in the Central Balkans (western Serbia): 1 – taxon is recorded on the specific geological substrate; 0 – taxon is not recorded on the specific geological substrate.

Orchid species and subspecies	Geological substrates										
	Andesite-dacite-porphyrite	Carbonate clastites	Flysch	Granodiorite	Limestone-dolomite	Ophiolitic mélange and sandstones from the Carboniferous and Permian periods	Quartz latite	Quaternary sediments	Schists-gneiss-phyllites	Ultramafics	Total
Anacamptis coriophora (L.) R.M. Bateman, Pridgeon & M.W. Chase	1	1	0	0	1	1	1	1	1	1	8
Anacamptis laxiflora (Lam.) R.M. Bateman, Pridgeon & M.W. Chase	0	0	0	0	0	0	0	1	0	0	1
Anacamptis morio (L.) R.M. Bateman, Pridgeon & M.W. Chase	1	1	1	0	1	1	0	1	1	1	8
Anacamptis palustris (Jacq.) R.M. Bateman, Pridgeon & M.W. Chase	0	0	0	0	0	0	0	1	0	0	1
Anacamptis papilionacea (L.) R.M. Bateman, Pridgeon & M.W. Chase	0	0	0	0	1	0	0	0	0	1	2
Anacamptis pyramidalis (L.) Rich.	1	1	0	0	1	1	0	1	1	1	7
Cephalanthera damasonium (Mill.) Druce	1	1	1	1	1	1	0	1	1	1	9
Cephalanthera longifolia (L.) Fritsch	0	1	1	1	1	1	1	1	1	1	9
Cephalanthera rubra (L.) Rich.	1	1	0	1	1	1	0	0	1	1	7
Corallorhiza trifida Châtel.	0	0	0	0	1	1	1	0	1	1	5
Dactylorhiza cordigera (Fr.) Soó	0	0	0	0	1	1	0	0	0	0	2
Dactylorhiza fuchsii (Druce) Soó subsp. *fuchsii*	1	0	0	0	1	1	1	0	0	0	4
Dactylorhiza incarnata (L.) Soó subsp. *incarnata*	1	1	0	0	1	1	1	1	1	1	8
Dactylorhiza maculata (L.) Soó subsp. *maculata*	0	0	1	1	1	1	1	0	1	1	7
Dactylorhiza maculata subsp. *transsilvanica* (Schur) Soó	0	0	0	0	1	1	0	0	1	1	4
Dactylorhiza saccifera (Brongn.) Soó subsp. *saccifera*	1	1	0	0	1	1	1	1	1	1	8
Dactylorhiza sambucina (L.) Soó	1	1	0	1	1	1	1	1	1	1	9
Dactylorhiza viridis (L.) R.M. Bateman, Pridgeon & M.W. Chase	0	1	0	0	1	1	1	0	1	1	6
Epipactis atrorubens (Hoffm.) Besser	0	1	0	0	1	1	0	0	0	1	4
Epipactis distans Arv.-Touv.	0	0	0	0	1	0	0	0	0	0	1
Epipactis helleborine (L.) Crantz subsp. *helleborine*	1	1	0	1	1	1	1	1	1	1	9
Epipactis leptochila (Godfery) Godfery subsp. *neglecta* Kümpel	0	0	0	0	1	1	0	1	1	0	4
Epipactis microphylla (Ehrh.) Sw.	1	1	0	1	1	1	0	0	1	0	6
Epipactis muelleri Godfery subsp. *muelleri*	0	0	0	0	1	0	0	0	0	0	1
Epipactis palustris (L.) Crantz	1	1	0	0	1	1	0	1	1	0	6
Epipactis pontica Taubenheim	0	0	0	0	1	0	0	0	1	0	2
Epipactis purpurata Sm.	0	0	0	0	1	1	0	0	0	0	2

Orchid species and subspecies	Geological substrates										Total
	Andesite-dacite-porphyrite	Carbonate clastites	Flysch	Granodiorite	Limestone-dolomite	Ophiolitic mélange and sandstones from the Carboniferous and Permian periods	Quartz latite	Quaternary sediments	Schists-gneiss-phyllites	Ultramafics	
Epipogium aphyllum Sw.	0	0	0	0	1	0	0	0	0	0	1
Goodyera repens (L.) R. Br.	0	0	0	0	1	0	0	1	0	1	3
Gymnadenia conopsea (L.) R. Br.	1	1	1	0	1	1	1	1	1	1	9
Gymnadenia frivaldii Hampe ex Griseb.	0	0	0	0	0	0	0	0	1	0	1
Gymnadenia odoratissima (L.) Rich. subsp. *odoratissima*	0	1	0	0	1	0	0	0	0	0	2
Gymnadenia rhellicani (Teppner & E. Klein) Teppner & E. Klein	0	0	0	0	1	1	1	0	1	1	5
Himantoglossum calcaratum (Beck) Schltr. subsp. *calcaratum*	1	1	0	0	1	1	0	1	1	0	6
Limodorum abortivum (L.) Sw.	1	1	0	1	1	1	0	0	1	1	7
Neotinea tridentata (Scop.) R.M. Bateman, Pridgeon & M.W. Chase subsp. *tridentata*	1	1	0	0	1	1	0	0	1	1	6
Neotinea ustulata (L.) R.M. Bateman, Pridgeon & M.W. Chase	1	1	1	0	1	1	0	1	1	1	8
Neottia cordata (L.) Rich.	0	0	0	1	1	1	1	1	0	1	6
Neottia nidus-avis (L.) Rich.	1	1	1	1	1	1	0	1	1	1	9
Neottia ovata (L.) Bluff & Fingerh.	1	1	0	0	1	1	1	1	1	1	8
Ophrys apifera Huds.	1	0	0	0	1	1	0	0	0	0	3
Ophrys insectifera L.	0	0	0	0	1	1	0	0	0	0	2
Ophrys scolopax subsp. *cornuta* (Steven) E.G. Camus	1	1	0	0	1	1	0	1	0	0	5
Ophrys sphegodes Mill. subsp. *sphegodes*	0	0	0	0	1	0	0	0	0	0	1
Orchis mascula (L.) L.	0	0	0	0	1	1	0	0	0	1	3
Orchis militaris L. subsp. *militaris*	0	0	0	0	1	1	0	0	0	0	2
Orchis pallens L.	1	1	0	1	1	1	0	0	0	0	5
Orchis purpurea Huds. subsp. *purpurea*	1	1	0	0	1	1	0	0	0	0	4
Orchis simia Lam. subsp. *simia*	1	1	0	0	1	0	0	0	1	0	4
Orchis spitzelii Saut. ex W.D.J.Koch subsp. *spitzelii*	0	0	0	0	1	0	0	0	0	0	1
Platanthera bifolia (L.) Rich.	1	1	1	1	1	1	0	1	1	1	9
Platanthera chlorantha (Custer) Rchb.	1	1	0	1	1	1	0	0	1	1	7
Pseudorchis albida (L.) Á. Löve & D. Löve subsp. *albida*	0	0	0	0	1	0	0	0	0	0	1
Spiranthes spiralis (L.) Chevall.	0	1	0	0	1	0	0	0	0	0	2
Traunsteinera globosa (L.) Rchb.	1	0	1	0	1	1	1	1	1	1	8
Total	27	29	9	13	52	40	15	23	31	29	

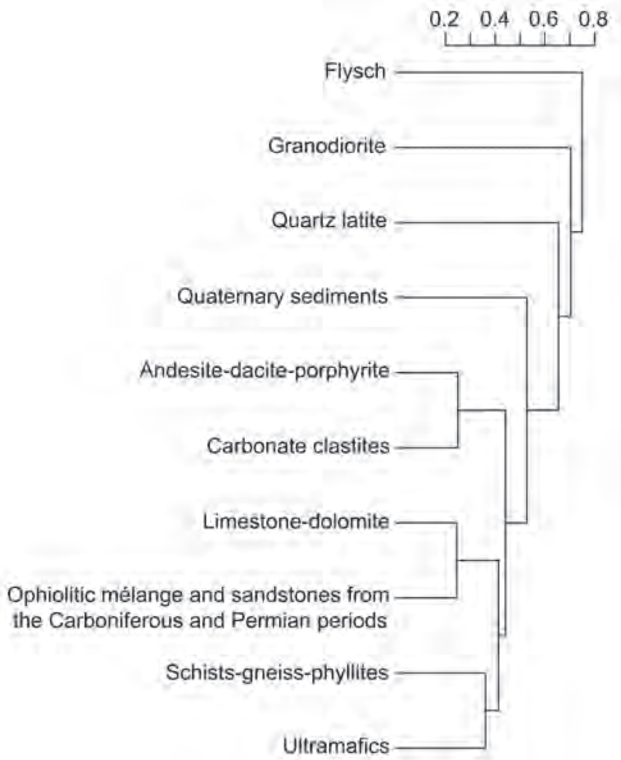

Figure 2. Cluster analysis of the floristic similarities among the ten geological substrates in the Central Balkans (western Serbia) based on the orchid species and subspecies occurrence. Similarity coefficient used: Jaccard.

carbonate clastites) were found to differ statistically from the highest number of bedrock types. Both geological substrates differ statistically from schists-gneiss-phyllites, ophiolitic mélange and sandstones from the Carboniferous and Permian periods as well as Quaternary sediments. In addition, limestone-dolomite differs statistically significantly from ultramafics. Moreover, limestone-dolomite and carbonate clastites are also statistically significantly different from each other. Orchid flora recorded on Quaternary sediments and ophiolitic mélange differs statistically from three bedrock types, whereas orchids found on flysch, granodiorite, quartz latite, schists-gneiss-phyllites and ultramafics differ statistically from two bedrock types (Table 3). Orchid composition on andesite-dacite-porphyrite differs the least from orchid composition recorded on other bedrock types, i.e. only from Quaternary sediments (Table 3).

Discussion

The richness and composition of orchid species and subspecies in relation to geological substrates. This study shows that most orchid species and subspecies of western Serbia grow on limestone and dolomite, thereby confirming our hypothesis. Our results are in agreement with previous studies that highlighted the importance of carbonate substrates for the development and survival of orchids (Delforge 2006; Tsiftsis et al. 2008; Landi et al. 2009; Pierce et al. 2014; Leuschner & Ellenberg 2017; Procházka et al. 2017; Tsiftsis & Antonopoulos 2017). According to Vakhrameeva et al. (1991) about two thirds of terrestrial orchids in Russia and other countries of the former Soviet Union occur on limestone. In addition, Tsiftsis et al. (2019b) found that richness of tuberous orchid species increases with increasing percentage of

Table 2. Similarity matrix of orchid flora among geological substrates in the Central Balkans (western Serbia) (in percentages).

Geological substrate	Andesite-dacite-porphyrite	Carbonate clastites	Flysch	Granodiorite	Limestone-dolomite	Ophiolitic mélange and sandstones from the Carboniferous and Permian periods	Quartz latite	Quaternary sediments	Schists-gneiss-phyllites	Ultramafics
Andesite-dacite-porphyrite										
Carbonate clastites	75.00									
Flysch	24.14	22.58								
Granodiorite	33.33	35.48	29.41							
Limestone-dolomite	51.92	55.77	17.31	25.00						
Ophiolitic mélange and sandstones from the Carboniferous and Permian periods	63.41	60.47	22.50	32.50	76.92					
Quartz latite	27.27	25.71	20.00	21.74	28.85	37.50				
Quaternary sediments	51.52	48.57	33.33	24.14	38.89	46.51	35.71			
Schists-gneiss-phyllites	61.11	62.16	29.03	33.33	56.60	65.12	39.39	50.00		
Ultramafics	47.37	52.63	31.03	35.48	55.77	64.29	46.67	48.57	66.67	

calcareous substrates in grid cells in Greece, and especially in southern parts of this country. Moreover, the studies of the total flora of certain areas (Ewald 2003; Tyler 2003; Brković et al. 2015), the flora of endemic plants (Essl et al. 2009; Tomović et al. 2014) and grassland communities (WallisDeVries et al. 2002; Krauss et al. 2004) also point out that most vascular plant species in Europe occur on carbonate substrates. A large number of orchid species on limestone and dolomite in western Serbia may be explained primarily by the physical and chemical characteristics of carbonate soils as well as the significant area size of this substrate. Limestone and dolomite are represented from the lowland to high-altitude areas in western Serbia, which additionally explains a large number of orchid species. However, a smaller number of orchid species that occur on carbonate clastites (Fig. 1, Table 1) and the fact that this bedrock type differs statistically significantly from limestone-dolomite (Table 3) may be explained by the fact that this bedrock type occupies smaller areas of western Serbia, mainly at lower altitudes. Many orchids known as characteristic species of calcareous habitats were also recorded on non-carbonate geological substrates in the study area. For example, *Epipactis palustris*, *E. pontica*, *E. purpurata*, *Gymnadenia conopsea*, *G. rhellicani*, *Himantoglossum calcaratum* subsp. *calcaratum* and *Neotinea ustulata* in western Serbia were found to grow on limestone-dolomite and different types of silicate substrates, whereas previous studies indicated that these species occur mainly or

Table 3. Results of the multi-response permutation procedure among orchid flora of the different bedrock types.

Geological substrate	Andesite-dacite-porphyrite	Carbonate clastites	Flysch	Granodiorite	Limestone-dolomite	Ophiolitic mélange and sandstones from the Carboniferous and Permian periods	Quartz latite	Quaternary sediments	Schists-gneiss-phyllites	Ultramafics
Andesite-dacite-porphyrite										
Carbonate clastites	-0.0135									
Flysch	0.0246	-0.0048								
Granodiorite	0.0116	-0.0217	0.0436**							
Limestone-dolomite	-0.0006	0.0184***	0.0021	0.0003						
Ophiolitic mélange and sandstones from the Carboniferous and Permian periods	-0.0099	0.0229**	0.0017	-0.0014	0.014**					
Quartz latite	0.0058	-0.0168	0.0404**	0.041**	0.0003	-0.0018				
Quaternary sediments	0.0094**	0.0091*	0.0179	0.0065	0.008*	0.0044	-0.0063			
Schists-gneiss-phyllites	-0.0162	0.0182**	-0.0005	-0.0078	0.0184***	0.0119	-0.011	-0.0007		
Ultramafics	-0.0262	0.0013	-0.0051	-0.0064	0.0563***	0.021***	-0.0071	-0.013	-0.007	

Significance levels: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

exclusively on calcareous substrates (Tali et al. 2004; Delforge 2006; Meekers et al. 2012; Jacquemyn et al. 2014).

This study shows that a significant number of orchids occur on ophiolitic mélange and sandstones from the Carboniferous and Permian periods (Fig. 1, Table 1), which may be explained primarily by the fact that this bedrock type includes a heterogeneous complex of rocks (Festa et al. 2012) and that it is widespread, from lowland to highland areas of western Serbia. Namely, the ophiolitic mélange is a volcanogenic-sedimentary formation (the old name is 'diabase-chert formation') (Đorđević et al. 1991; Chiari et al. 2011; Gawlick et al. 2017) that in most cases contains diabase, basic igneous rock which is composed of calcium-rich plagioclase feldspat (mostly labradorite) and pyroxene (augite), as well as chert, a chemically precipitated sedimentary rock mainly composed of quartz, the mineral form of silicon dioxide SiO_2 (Perry & Lefticariu 2003). Although there are differences in the adoption and accumulation of Si in different plant species, its role is known in alleviating abiotic and biotic stress (Epstein 2009), which may additionally explain the great species richness of orchids on these substrates. Previous studies showed that Si has an important role in alleviation of abiotic stresses: UV radiation and temperature extremes (Liang et al. 2007), drought (Gong et al. 2005) as well as the toxicity of metals such as cadmium, manganese, zinc, copper and aluminium, but also the lack of essential elements (nitrogen, phosphorus, potassium and iron) in higher plants (Liang et al. 2007; Mali & Aery 2008; Song et al. 2011). The results indicate that ophiolitic mélange is suitable for the Balkan endemic *Himantoglossum calcaratum* subsp. *calcaratum*, the two subendemics of the Carpathians and the Balkans (*Dactylorhiza cordigera* and *D. maculata* subsp. *transsilvanica*) as well as for some Central European and boreal orchids (*Dactylorhiza viridis*, *Epipactis leptochila* subsp. *neglecta*, *Epipactis purpurata*, *Neottia cordata* and *Ophrys insectifera*). A great similarity in the composition of orchid species recorded on ophiolitic mélange and limestone-dolomite in terms of presence / absence of species (Fig. 2, Table 2) may be explained by the heterogeneous composition of ophiolitic mélange, the large altitude range at which these substrates occur, by the presence of calcium in diabase, and the fact that in the study area the ophiolitic mélange is often widespread in the immediate vicinity of limestone. However, an analysis of the composition of orchid flora in terms of abundance and frequency of orchids between ophiolitic mélange and carbonate substrates (limestone-dolomite and carbonate clastites) indicated significant differences between them (Table 3), suggesting that the population sizes of orchids differ significantly among these substrates.

Many orchids in western Serbia were recorded on substrates that include metamorphic rocks (schists, gneisses and phyllites; Fig. 1, Table 1). On these geological substrates, mainly in high-altitude areas, some of the rarest orchids of western Serbia were found. It is especially important to point out that *Gymnadenia frivaldii* and *G. rhellicani* were recorded on Mt Golija (western Serbia) on phyllites, substrates primarily composed of mica, quartz, chlorite, sericite, biotite and albite (Mottana et al. 1978). The previous study showed that these two species are indicator species of schists, gneisses and phyllites (Djordjević et al. 2016a). In addition, many boreal and Central European orchids in western Serbia were recorded on this bedrock type (*Corallorhiza trifida*, *Dactylorhiza maculata* subsp. *maculata*, *D. maculata* subsp. *transsilvanica*, *D. viridis*, *Goodyera repens*, *Neottia cordata*, *Epipactis leptochila* subsp. *neglecta* and *E. pontica*). Previous floristical studies in Serbia, on Mt Kopaonik (Lakušić 1993), on the Vlasina plateau (Ranđelović & Zlatković 2010) and on Mt Šarplanina (Duraki 2008), as well as in northeastern Greece (Tsiftsis et al. 2008) also showed that certain orchid species grow on this bedrock type. Statistically significant differences in the composition of the orchid flora, regarding abundance and frequency of orchids, between schists-gneisses-phyllites and carbonate substrates (Table 3) may be explained by differences in the soil physical and chemical properties, primarily by larger water-holding capacity of schists-gneisses-phyllites, and the fact that this bedrock type has less presence, mainly at higher altitudes of the investigated area.

A significant number of orchids in western Serbia were found on ultramafics (Fig. 1, Table 1), which is not fully in line with our hypothesis. Previous studies regarding orchids that occur on ultramafics are rare: van der Ent & Wood (2013) found a significant number of orchids on serpentine in Malaysia (Kinabalu Park), whereas Filimonova et al. (2019) noted *Epipactis atrorubens* on this bedrock type in the Middle Urals, Russia. Ultramafic (serpentine) soils have a low content of macronutrients (N, P and K), high concentrations of Ni, Cr and Co and an unfavourable ratio of Ca to Mg, (Stevanović et al. 2003; Brady et al. 2005; Harrison et al. 2006). In general, the reduced specificity of the extreme ultramafic soils may explain why certain orchid species, which are characteristic primarily for carbonate substrates, have significant populations on ultramafics (Djordjević et al. 2016a, 2016b). Namely, the extreme effects of ultramafic soils are manifested in the case of skeletal soils. However, when the soils are moist or well-developed, their specificity is significantly reduced primarily due to migration and removal of ions as well as to the production of humus (Proctor & Woodell 1971; D'amico & Previtali 2012). Thus, the study on ultramafics of the western Italian Alps showed that higher forest productivity occurs on well-developed soils due to the migration and removal of Mg and Ni, since the removal of Mg and Ni leads to a higher concentration of Ca compared to Mg, as well as lower Ni concentrations in comparison to the typical soils developed on ultramafics (D'amico & Previtali 2012). In the central Balkans (western Serbia) ultramafics are often situated on the plateaus and slightly inclined terrains, which enables better soil development and longer water retention.

The presence of orchid species and subspecies on ultramafics in western Serbia may be explained by the physical and chemical characteristics of the soil, especially the low content of nutrients, bearing in mind that most orchids are sensitive to increased nitrogen, phosphorus and potassium content in the soil (Beyrle et al. 1991; Dijk & Olff 1994; Dijk et al. 1997; Silvertown et al. 1994; Hornemann et al. 2012). Furthermore, ultramafics favour the development of open habitats with a generally lower level of competition between plants (Kazakou et al. 2008), which corresponds to competitively weak orchids (Stuckey 1967). Among the orchids that have large populations on ultramafics, it is especially important to highlight the following: *Anacamptis morio*, *Dactylorhiza maculata* subsp. *transsilvanica*, *D. sambucina*, *Gymnadenia conopsea* and *Platanthera bifolia*. Moreover, a previous study showed that *Dactylorhiza maculata* subsp. *transsilvanica* and *Platanthera bifolia* represent the indicators of the ultramafic (serpentine) geological substrate (Djordjević et al. 2016a). The presence of large populations of these species may be explained by the temporal and spatial continuity of ultramafic open habitats, primarily due to the lack of strong agricultural influence and less pronounced competitive relations among plants (Djordjević et al. 2016b). Furthermore, it is assumed that mycorrhiza plays an important role in increasing orchid tolerance to high concentrations of heavy metals that are present in ultramafic soils. In general, all orchids of western Serbia, which are recorded on ultramafics, are considered as serpentine-facultative plants, bearing in mind that their populations are also recorded on other geological substrates. The floristic composition of orchids that were recorded on ultramafics is the most similar to the composition of orchids found on schists, gneisses and phyllites (Fig. 2, Table 2). Among the species that are common to these two groups of geological substrates, it is particularly important to highlight orchids that inhabit wet and moderately wet habitats (*Dactylorhiza incarnata*, *D. maculata* subsp. *maculata*, *D. maculata* subsp. *transsilvanica*, *D. saccifera, D. sambucina* and *D. viridis*). However, orchid flora of ultramafics, in terms of orchid abundance and frequency, significantly differs from limestone-dolomite and ophiolitic mélange (Table 3), which may be explained by differences in the soil physical and chemical properties of these substrates.

Many orchid species in western Serbia were found growing on intermediate igneous rocks (andesite, dacite, and porphyrite) (Fig. 1, Table 1). The composition of orchids recorded on andesite, dacite, and porphyrite is the most similar to the orchid composition found on carbonate clastites (Fig. 2, Table 2), which is related to the fact that these two bedrock types are usually in direct contact, and that both substrates are suitable for the existence of species that prefer xero-mesophilous habitats. A previous floristic study conducted on Mt Sokolovica (central Serbia) has also shown that significant number of orchid species occur on andesite (Tomović et al. 2005).

This study emphasizes the importance of Quaternary sediments, bearing in mind that this geological substrate is suitable for the existence of many orchids that prefer wet habitats (*Anacamptis palustris*, *Dactylorhiza incarnata* and *Epipactis palustris*) as well as for some rare boreal and Central European orchid taxa (*Goodyera repens*, *Neottia cordata* and *Epipactis leptochila* subsp. *neglecta*). The composition of orchid flora found on this bedrock type differs significantly from carbonate clastites, limestone-dolomite and andesite-dacite-porphyrite (Table 3), which may be explained by high soil moisture and high water-holding capacity of Quaternary sediments.

The results of this study show that a smaller number of orchid species in western Serbia were recorded on acidic igneous rocks (granodiorite and quartz latite), which confirm our hypothesis. Furthermore, these two geological substrates, according to the composition of orchid flora, were distinguished as separate groups (Fig. 2). This may be explained by the fact that acidic soils of pH < 4.5 contain free (dissolved) ions of heavy metals, especially iron and manganese as well as high concentrations of harmful Al^{3+} ions (Brunet et al. 1996; Tyler 2003). Consequently, habitats developed on acidic soils are inhabited by a smaller number of taxa, primarily because most species are not able to tolerate high concentrations of Al^{3+}, which break down cell divisions and prevent further growth of plants, but not because of the small amount of calcium carbonate in these soils (Tyler 2003). Statistically significant differences in the composition of the orchid flora, regarding abundance and frequency of orchids, between granodiorite and quartz latite (Table 3) may be explained by the fact that mainly forest orchids were recorded on granodiorite, whereas orchids of herbaceous vegetation types were found mostly on quartz latite (Table 1). Previous studies showed that in Greece some orchid species also occur on acidic igneous rocks – granite, granodiorite, rhyolite and quartz-monzonite (Tsiftsis et al. 2008, 2019a), whereas many orchids on Mt Kopaonik (central Serbia) were recorded on granite (Lakušić 1993), pointing out the significance of these geological substrates for some orchids on the Balkan Peninsula. The smallest number of orchid species in western Serbia was recorded on flysch (Fig. 1, Table 1), which may be explained by the small size of the areas that this geological substrate occupies in western Serbia. Otherwise, flysch represents a series of sedimentary rocks in which the most common are marls, clay shales, sandstones, conglomerates and limestone (Dzulynski & Walton 1965). Furthermore, due to the smallest number of recorded orchid species, the floristic composition of orchids recorded on flysch differed most from the floristic composition of orchids of other geological substrates (Fig. 2, Table 2).

In general, many orchids of central or northern European origin were found on non-carbonate substrates in western Serbia, whereas orchids from different chorological groups, including a significant number of Mediterranean and sub-Mediterranean orchids were recorded on carbonate substrates. Similarly, previous floristic studies in the Balkans showed that silicate substrates are suitable for many boreal species (Vukojičić et al. 2014), and Arctic-Alpine species (Stevanović et al. 2009). Additionally, a significant number of orchid species and subspecies that prefer moderately wet and wet habitats were found on non-calcareous substrates, which may be explained by the fact that most non-calcareous substrates, i.e. silicate substrates, have a high water retention capacity. Therefore, our results suggest that non-carbonate geological substrates may play an important role in the conservation of orchids due to climate change and global warming. Overall, our results indicate that different soil resource (water and nutrient) availability across geological substrates is significant in association between richness and composition of orchid species and geological substrates. Similarly, Fayolle et al. (2012) noted that the strong relationship between tree species distribution and bedrock types suggests that different soil resource availability is important for these patterns. Furthermore, due to less agricultural practices on non-carbonate substrates, especially on ultramafics, these substrates and their habitats could be regarded as potential orchid reserves.

Which orchids grow on a large number of geological substrates? Among the orchids that were recorded on a large number of geological substrates in western Serbia, it is important to emphasize the species that were found on nine and eight geological substrates (Table 1), suggesting that these orchid species have the greatest ecological plasticity and adaptability as well as the smallest specialization levels in relation to geological substrate in the study area. The results of this study are in accordance with previous studies that showed that the following orchid species are not specialized in relation to geological substrates and soil properties: *Anacamptis morio*, *Cephalanthera damasonium*, *C. longifolia*, *Dactylorhiza sambucina*, *Epipactis helleborine*, *Neottia nidus-avis*, *Neottia ovata* and *Platanthera bifolia* (Buttler 1991; Delforge 2006; Jersáková et al. 2015; Kotilínek et al. 2015).

Which orchids grow on a small number of geological substrates? Among the orchids that were found on few geological substrates in western Serbia, *Epipactis distans*, *E. muelleri*, *Epipogium aphyllum*, *Ophrys sphegodes*, *Orchis spitzelii* and *Pseudorchis albida* were recorded exclusively on limestone-dolomite. Previous studies have also shown that *Epipactis distans*, *E. muelleri*, *Ophrys sphegodes* and *Orchis spitzelii* mainly grow on carbonate substrates, i.e. on alkaline soils (Buttler 1991; Delforge 2006; G.I.R.O.S. 2009). *Epipogium aphyllum* grows mainly on carbonate substrates in Greece (Tsiftsis & Antonopoulos 2017), whereas in Italy it occurs on carbonate and silicate substrates (G.I.R.O.S. 2009). According to Jersáková et al. (2011), *Pseudorchis albida* mainly grows on limestone, but it was also recorded on ultramafics and different acidic substrates in Europe. This orchid was recorded in Greece exclusively on limestone (Tsiftsis & Antonopoulos 2017), whereas in Serbia it grows also on silicate substrates (Lakušić 1993; Ranđelović & Zlatković 2010).

Gymnadenia frivaldii was found exclusively on schists-gneiss-phyllites in western Serbia, which is in accordance with previous studies that showed that this species mainly grows on silicate substrates (Buttler 1991; Delforge 2006; Djordjević et al. 2017; Tsiftsis & Antonopoulos 2017). Similarly, this orchid was also recorded on metamorphic rocks in other areas of Serbia (Mt Šarplanina and the Vlasina Plateau; Duraki 2008; Ranđelović & Zlatković 2010), whereas on Mt Kopaonik (central Serbia) was found on acidic igneous rock-granite (Lakušić 1993). The results indicate that *Anacamptis laxiflora* and *A. palustris* in western Serbia prefer habitats developed on Quaternary sediments. According to Delforge (2006), these two species grow in moist soils with an alkaline or slightly acidic reaction.

Gymnadenia odoratissima and *Spiranthes spiralis* were recorded in western Serbia exclusively on carbonate substrates (limestone-dolomite and carbonate clastites). Many authors also found that *Gymnadenia odoratissima* mainly grows on limestone (Diklić 1976; Buttler 1991; Delforge 2006; Tsiftsis & Antonopoulos 2017). According to Jacquemyn & Hutchings (2010), Leuschner & Ellenberg (2017) and Tsiftsis & Antonopoulos (2017), *Spiranthes spiralis* grows mainly on limestone, but it was also recorded on gravel and sandy substrates as well as on acidic substrates. In western Serbia, *Dactylorhiza cordigera*, *Epipactis purpurata*, *Ophrys insectifera* and *Orchis militaris* were found growing on limestone-dolomite and ophiolitic mélange. Previous studies showed that *Dactylorhiza cordigera* grows on both alkaline and acidic substrates (Delforge 2006), on schists (Duraki 2008; Ranđelović & Zlatković 2010) as well as on granite (Lakušić 1993). According to Lang (2004), Delforge (2006), Rankou (2011) and Antonopoulos & Tsiftsis (2017), *Epipactis purpurata* grows on carbonate substrates, on neutral and acidic soils, as well as on soils with high clay content, whereas *Ophrys insectifera* grows on alkaline and neutral substrates, most commonly on limestone. Many authors noted that *Orchis militaris* grows primarily on limestone and the alkaline soils (Farrell 1985; Buttler 1991; Baumann et al. 2006; Delforge 2006; Leuschner & Ellenberg 2017; Tsiftsis & Antonopoulos 2017).

In western Serbia, *Anacamptis papilionacea* was found on limestone and ultramafics, which is in line with previous studies that showed that this species grows on limestone and alkaline substrates (Buttler 1991; Delforge 2006). *Epipactis pontica* was found in the study area on limestone and schists-gneiss-phyllites. In Europe, this species was primarily found on limestone (Davies et al. 1984), but recent studies indicate that it also inhabits habitats on silicate substrates. Namely, in Bulgaria the populations of this species were recorded on gneiss and limestone (Petrova & Venkova 2006), whereas in Greece it was recorded on rhyolites, gneisses and granites (Tsiftsis et al. 2008).

Among orchids that were found growing on three bedrock types in western Serbia (Table 1), *Goodyera repens* was recorded on limestone, Quaternary sediments and ultramafics. In Italy, this species grows mainly on limestone (G.I.R.O.S. 2009), and in Greece it was recorded on limestone and acidic igneous rocks-granodiorites (Tsiftsis & Antonopoulos 2017). *Ophrys apifera* was found in western Serbia on limestone-dolomite, andesite-dacite-porphyrite and ophiolitic mélange, whereas Buttler (1991), Lang (2004), Delforge (2006), Antonopoulos & Tsiftsis (2017) and Leuschner & Ellenberg (2017) found that this species mainly grows on alkaline soils, primarily on limestone. Populations of *Orchis mascula* in western Serbia were recorded on limestone-dolomite, ophiolitic mélange and ultramafics, whereas previous studies point out that this species also grows on limestone as well as on acidic, neutral and alkaline soils (Lang 2004; Delforge 2006).

Conclusion

The results of this study show that most orchid species and subspecies in the central Balkans grow on limestone-dolomite, confirming the general statement that calcareous substrates and habitats are most important for orchid survival and existence. Our results particularly indicate the importance of ophiolitic mélange and schists-gneiss-phyllites, bearing in mind that these bedrock types are suitable for many orchids, including some rare and endemic taxa. In addition, a surprisingly significant number of orchid species were recorded on ultramafics in western Serbia. Our results show that non-calcareous bedrock types (igneous and metamorphic rocks) and Quaternary sediments are significant for many orchids of central or northern European origin and for orchids that prefer wet and moderately wet habitats. Moreover, non-carbonate substrates and their habitats could be regarded as potential orchid reserves, due to less agricultural practices compared to carbonate areas. Overall, the differences in species richness and composition of orchids suggest environmental filtering due to different soil resource availability across geological substrates. It can be concluded that the heterogeneity of geological substrates significantly affects the diversity patterns of orchids. Furthermore, preferences of orchids in relation to geological substrates can be used as a background for conservation planning and the management strategies of orchids. Future research should examine the impact of different geological substrates on the orchid performances, such as ecophysiological characteristics, potential for trace element accumulation, reproductive success, as well as their role in determining effective orchid conservation strategies.

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