Butterfly Species Richness and Diversity on Tourism Trails of Northeast Portugal[1]

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Abstract Butterfly species can be sensitive to ecosystem disturbance and, therefore, suitable to be used as indicators of habitat quality. We determined species richness and diversity of butterfly species along five tourist trails in the northeast region of Portugal. These trails were in different landscape structures, varying from urban areas to areas extensively managed for agriculture (i.e., vineyards, meadows) to natural areas (i.e., grasslands, rivers, forests). A total of 522 butterflies representing 45 species belonging to 34 genera and 5 families of Lepidoptera were recorded. Of the taxonomic families represented in the survey, the Nymphalidae were most numerous (362 specimens, 22 species) followed by Pieridae (86 specimens, 11 species) and Lycaenidae (58 specimens, 8 species). Four species have a conservation status, an indicator of the risk of extinction they face at present or in the near future [*Euphydryas aurinia* (Rottemburg, 1775), *Phengaris alcon* (Denis & Schiffermüller, 1775), *Hipparchia semele* (L., 1758) and *Melanargia lachesis* (Hübner, 1790)], and these represent 6.9% of the total species identified. Among the five trails, diversity parameters varied with high values of species richness and diversity, low dominance of species, and moderate evenness of distribution. Additionally, butterfly species comparison among the trails revealed that Alvão and Vale do Corgo trails have most of the species in common, especially from Pieridae and Nymphalidae, while the Marão trail has more species associated exclusively to this trail. These results were also supported by hierarchical clustering performed with an average linkage aggregation method using Jaccard distance and by comparison between proportions of butterflies among trails within each family.

Key Words butterfly assessment, species diversity, species richness

Of the total number of butterfly species in Europe, almost one-third are in decline and 10% are threatened with extinction (van Swaay et al. 2012). Agricultural practices, pesticides, habitat fragmentation, and climate change are implicated as major causative factors (Koh 2007; Pang et al. 2016; Sánchez et al. 2010). This decline can have serious negative consequences, not just for biodiversity itself but also on the balance of ecosystem health.

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Butterfly species are used as indicators of environmental health, as they are sensitive to habitat and climate changes and, therefore, responses of species richness and abundance of species related to specific areas can have a direct consequence in habitat quality and loss of ecosystem services provision (Pang et al. 2016; Peters et al. 2016). These insects are considered a group of "model" organisms with application in several research areas such as pest control, embryology, mimicry, evolution, genetics, population dynamics, and biodiversity conservation (McGeoch 1998; Pang et al. 2016). In fact, butterflies are a group of organisms of great value (ecological, economic, educational, and scientific). For example, they are pollinators of several flower species that cannot be pollinated by bees, and the disappearance of butterflies can compromise the reproductive success of those flower species. Also, they are an important food resource for many predatory species and, therefore, their scarcity can impact birds and other groups that use them as food resources. Because of their attractive appearance and life cycle associations with other organisms, they are a popular group for ecotourism, adding value in this market (Gross 2014; Pang et al. 2016).

Portugal is home to 33% of all butterfly species in Europe, and the northern part of the country has a rich variety of butterfly species with a high number of endemic species (Sánchez et al. 2010). We conducted a detailed inventory to document the butterfly species richness and diversity at five tourism trails in the Northeast region of Portugal. This was the first step toward a more complete plan to identify butterfly species communities, ecology, and distribution in the region. At the time of this study, no information was known from these sites regarding butterfly abundance and distribution. Therefore, the present study aimed to: (a) provide detailed inventories of specific locations and habitats known to support butterfly species; (b) contribute to the knowledge of the butterfly species communities, ecology, and distribution in the region; (c) generate information to support future nature conservation actions; and (d) enhance public understanding of the need for conservation and appreciation of butterfly habitats.

Materials and Methods

Study areas. The surveys were conducted in the municipality of Vila Real, North of Portugal. Data were collected along the trails of Alvão, Douro Vinhateiro, Marão, Mineiro, and Vale do Corgo (Fig. 1). The Alvão trail includes areas of the site Alvão-Marão (belonging to the Natura 2000, a network of nature protection areas in the territory of the European Union), the Alvão Dam, and two rural villages (Barreiro and Lamas d'Olo). The landscape is largely scrub, cultivated fields, and marshes as well as oak and pine forests. The Douro Vinhateiro trail is located in the populated Douro region where the landscape has vineyards, shrubs, cork trees, and other orchards. The Marão trail vegetation is characterized mainly by shrubs (i.e., heather, gorse) and forested areas of willows, ash, and alder but is dominated by oak and chestnut trees. Some hazel, pear angry, hawthorn, and holly species also occur there. The Mineiro trail is mainly a matrix of intense agricultural patches mixed with grassland and livestock and some forested portions alternating between the areas of pine and oak. Evidence of historic mining activities may be seen. The Vale do Corgo Norte trail starts in an urban environment that gradually changes to forest zones with

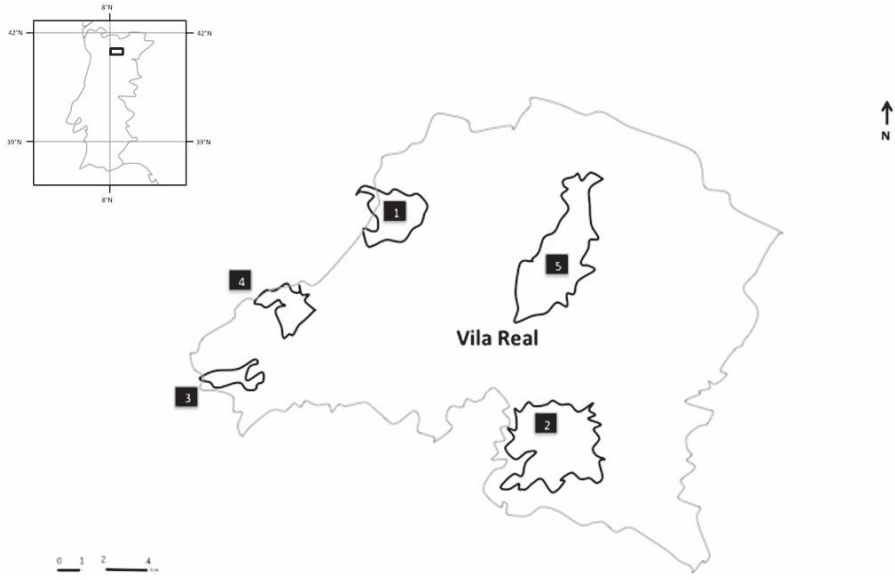

Fig. 1. Location map of the study area with the five trails in the municipality of Vila Real, Northeast region of Portugal. Legend: 1 = Alvão trail, 2 = Douro Vinhateiro trail, 3 = Marão trail, 4 = Mineiro trail, 5 = Vale do Corgo trail.

patches of oak dagger and cattle grazing. Part of this trail is adjacent to a river with riparian flora. Other trail characteristics such as trail extension, altitude, and geographic coordinates are presented in Table 1.

Butterfly census. Butterfly field surveys were conducted during July 2013. Counts on each trail were made between 9:30 a.m. and 12:30 p.m. by using transects of 500 m representing a gradient of vegetation. Specimens were captured using butterfly nets, and individual specimens were identified on site using Haahtela et al. (2011),

Table 1. Trail information (name, point counts, geographical coordinates, and altitude).

Trail name	Point counts	Geographical coordinates		Altitude (m)
		Latitude	Longitude	
Alvão	17	41.3573 N	7.7954 W	1,068
Douro Vinhateiro	17	41.2944 N	7.7388 W	385
Marão	19	41.2794 N	7.9145 W	956
Mineiro	17	41.2886 N	7.8531 W	883
Vale do Corgo	18	41.3063 N	7.7440 W	544

Maravalhas (2003), and Tshikolovets (2011). For each sampling site, data collected were date, location, time of collection, and number of each species collected.

Data analysis. Species richness (Menhinik index), species diversity (Shannon index), component of dominance (Simpson dominance index), and relative abundance of different species at a sampling site (Pielou's evenness index) (Magurran 1988) were determined for each trail using the PAST software version 3 (Hammer et al. 2001). Comparisons in species composition between different sites were estimated through Nonmetric Multidimensional Scaling (NMDS) ordination. NMDS results were then used to plot samples in "ecological space" using a dissimilarity matrix based on species composition. Function meta MDS from Vegan package in R (Oksanen et al. 2015) was used with presence–absence of data by Jaccard Distance. Ggplot2 and grepel packages were used for plot edition to avoid overlapping of labels. Additionally, several hierarchical clusterings were performed to identify clusters of sites and verify the consensus clustering solution. Numerical results show the solution for average linkage aggregation method using Jaccard distance.

Associations between taxa and trails were determined using chi-square tests, with Monte Carlo correction due to the presence of small expected frequencies. Z-tests were used to compare column proportions considering adjusted *P*-values (Bonferroni method) to determine which taxa had significant proportions between sites. Species richness counts from each trail were pooled to obtain rarefaction curves for comparison of estimated species richness between the habitats. Sampling completeness was calculated as a ratio of the observed species richness to the richness estimate (Sorensen et al. 2002).

Results

Butterfly fauna. A total of 522 butterflies representing 45 species belonging to 34 genera and 5 families were recorded in the study (Table 2). A maximum of 26 species and 168 individuals of butterflies were recorded in Mineiro in contrast with a minimum of 15 species and 52 individuals recorded in Marão trail. No significant differences were detected (KW-H $= 4.355$, df $= 4$, $P = 0.360$) in comparing the median number of total individuals observed per species among the sites surveyed.

Of the 45 species observed, 16 species (35.56%) were recorded only at one of the five trails and were considered 'unique' species. Eight of those were labelled as 'singleton' species, that is, only one individual per species was observed (Table 2). Another 6 species (13.33%) commonly occurred at all five trails. Furthermore, of the total number of species recorded, four (8.88%) had a conservation status. Those were: *Euphydryas aurinia* (Rottemburg, 1775), a species listed in the Habitats Directive Annex 2 (Council of European Union 1992) and Bern Convention Annex 2 (Council of European Union 1982); *Phengaris alcon* (Denis & Schiffermüller, 1775), a species listed by the IUCN (IUCN 2014) (International Union for Conservation of Nature) Red List of Threatened Species (tm) and considered as "Vulnerable"; and *Hipparchia semele* (Linnaeus, 1758) and *Melanargia lachesis* (Hübner, 1790), which are species listed at the SPEC (van Swaay et al. 1999) (Species of European Concern) as 4a – Global distribution restricted to Europe, but not threatened. Species in this category are endemic to Europe and, therefore, are of conservation concern because their distribution is restricted to Europe.

Table 2. The butterfly species found on the five touristic trails in Northeastern Portugal. Abbreviations: M = Marão trail, DV = Douro Vinhateiro trail, Mi = Mineiro trail, A = Alvão trail, VC = Vale do Corgo trail.

Family	Genera	Species	Sites					Total (no. individuals)
Hesperiidae	*Hesperia*	*H. comma* (L.)	A	M	Mi			8
	Thymelicus	*T. sylvestris* (Poda)	A					5
Lycaenidae	*Aricia*	*A. cramera* (Eschscholtz)			Mi	DV	VC	6
	Lampides	*L. boeticus* (L.)	A		Mi		VC	5
	Leptotes	*L. pirithous* (L.)	A	M	Mi	DV	VC	30
	Lycaena	*L. phlaeas* (L.)					VC	1
	Phengaris	*P. alcon* (Dennis & Schiffermüller)		M				1
	Plebejus	*P. argus* (L.)	A		Mi			12
	Satyrium	*S. esculi* (Hübner)	A			DV		2
		S. spini (Dennis & Schiffermüller)					VC	1
Nymphalidae	*Arethusana*	*A. arethusa* (Schiffermüller)	A		Mi			4
	Argynnis	*A. adippe* (Dennis & Schiffermüller)	A		Mi			2
		A. pandora (Dennis & Schiffermüller)	A	M			VC	9
	Brintesia	*B. circe* (F.)	A			DV	VC	5
	Coenonympha	*C. arcania* (L.)	A	M	Mi	DV	VC	20
		C. dorus (Esper)					VC	4
		C. glycerion (Borkhausen)	A					4
		C. pamphilus (L.)			Mi	DV		21
	Euphydryas	*E. aurinia* (Rottemburg)	A	M	Mi		VC	15

Table 2. Continued.

Family	Genera	Species	Sites					Total (no. individuals)
	Hipparchia	*H. semele* (L.)			Mi			5
	Hyponephele	*H. lycaon* (Rottemburg)			Mi	DV		16
	Inachis	*I. io* (L.)			Mi			1
	Lasiommata	*L. megera* (L.)					VC	3
	Maniola	*M. jurtina* (L.)	A	M	Mi	DV	VC	38
	Melanargia	*M. lachesis* (Hübner)	A	M	Mi	DV	VC	118
		M. russiae (Esper)			Mi	DV	VC	3
	Melitaea	*M. cinxia* (L.)		M				1
		M. deione (Geyer)			Mi			1
	Pararge	*P. aegeria* (L.)		M	Mi			11
	Pyronia	*P. cecilia* (Vallantin)			Mi		VC	19
		P. tithonus (L.)			Mi	DV	VC	61
	Vanessa	*V. cardui* (L.)	A					1
Papilionidae	*Iphiclides*	*I. feisthamelii* (Duponchel)				DV	VC	2
	Papilio	*P. machaon* (L.)				DV		1
Pieridae	*Anthocharis*	*A. cardamines* (L.)					VC	3
	Aporia	*A. crataegi* (L.)		M				2
	Colias	*C. croceus* (Geoffroy)	A		Mi		VC	29
	Euchloe	*E. crameri* (Butler)	A	M			VC	3
	Gonepteryx	*G. rhamni* (L.)	A				VC	2
	Leptidea	*L. sinapis* (L.)		M	Mi		VC	3
	Pieris	*P. brassicae* (L.)	A	M	Mi	DV	VC	14
		P. napi (L.)	A	M	Mi	DV	VC	16
		P. rapae (L.)	A		Mi	DV		5
		P. mannii (Mayer)			Mi			3
	Pontia	*P. daplidice* (L.)	A			DV	VC	6

Table 3. Diversity parameters and species richness estimates of butterfly communities in the five trails in Northeastern Portugal.

Trails	Individuals (*n*)	Species (*n*)	Menhinik index	Shannon (H)	Dominance (D)	Evenness ($e^{H/S}$)	Chao 1	Sampling completeness (%)
Alvão	122	23	2.09	2.52	0.13	0.54	30.00	76.67
Douro Vinhateiro	59	17	2.21	2.19	0.17	0.52	72.00	23.61
Marão	52	15	2.08	2.37	0.12	0.71	18.75	80.00
Mineiro	168	26	1.94	2.55	0.13	0.51	34.33	72.82
Vale do Corgo	121	25	2.27	2.25	0.21	0.38	32.5	76.92
Overall	104	21	2.16	2.40	0.15	0.53	38.26	61.78

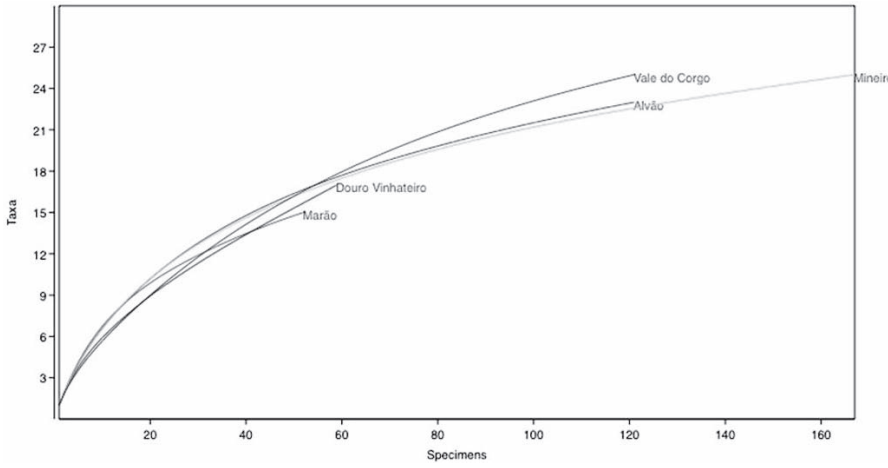

Fig. 2. Sample-based rarefaction curves of estimated species richness at five trails of Northeast Portugal.

Butterfly diversity. Family-wise distribution of butterflies showed that members of Nymphalidae dominated the sampling (22 species, 362 individuals) followed by Pieridae (11 species, 86 individuals), Lycaenidae (8 species, 58 individuals), Hesperiidae (2 species, 13 individuals), and Papilionidae (2 species, 3 individuals) (Table 2).

Overall, the five trails showed a high species richness and diversity as well as a moderate evenness distribution of butterflies and very low dominance of species (Table 3). Nevertheless, when comparing the diversity parameters of butterflies among trails, there were some variations observed (Table 3). More specifically, Mineiro showed maximum diversity ($H = 2.55$) and, together with Marão and Alvão, presented the least amount of dominance of butterflies ($D = 0.12$, $D = 0.13$, and $D = 0.13$, respectively), whereas the habitat with the populated landscape (Douro Vinhateiro) showed the minimum diversity ($H = 2.19$) and the second highest value of dominance of butterflies ($D = 0.17$) (Table 3). Evenness of distribution in all the study sites was found to be from relatively low to moderate ($e = 0.38$–0.71). Species diversity was lower in trails within more-populated areas and with higher levels of land-use matrix (e.g., Douro Vinhateiro and Vale do Corgo) than in more-natural landscapes (e.g., Marão and Alvão) (Table 3).

Richness estimates. Estimation of species richness using Chao 1 in the five trails showed expected richness values that were very close to the observed values, with exception to Douro Vinhateiro (Chao $1 = 72.0$, $S = 17.0$) (Table 3).

The overall estimate of species richness was higher by 16.26 species than the observed value. This difference was also evident on the overall sampling completeness, which ranged between 23 and 80% among the five trails and with an overall value of 62%. Rarefaction curves from the five trails showed quick rises for the Douro Vinhateiro and Marão trails and the rarefaction curves of the other three trails approached gently asymptote (Alvão, Mineiro, and Vale do Corgo) (Fig. 2).

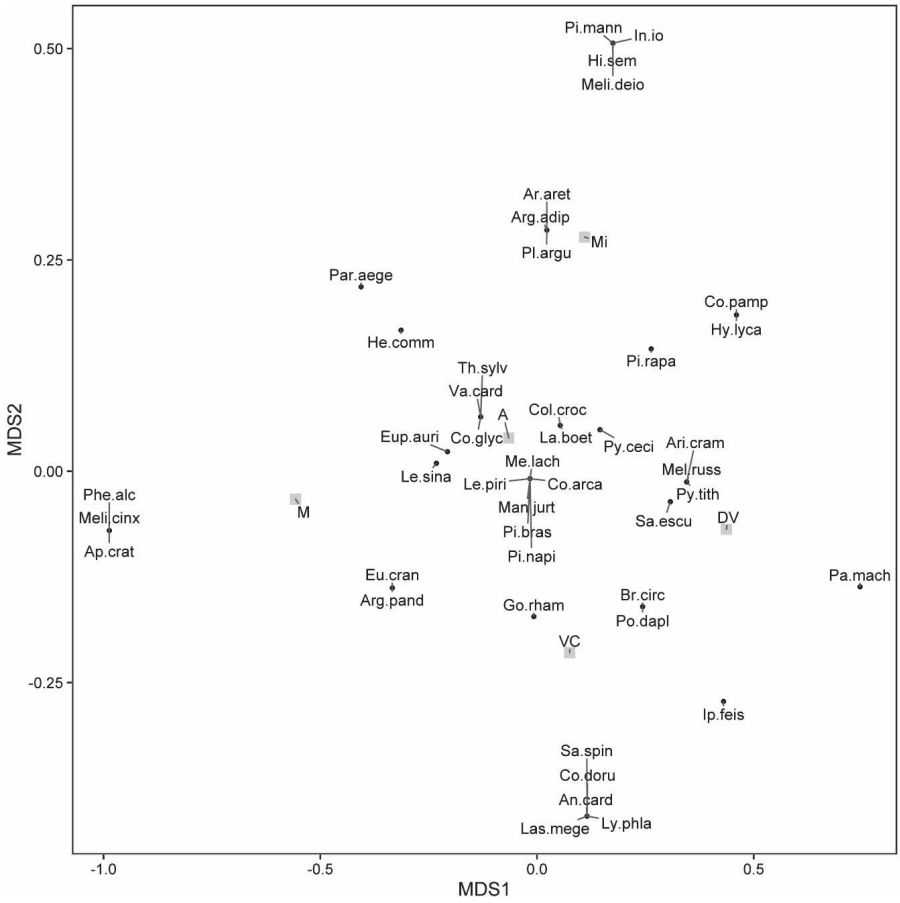

Fig. 3. Species composition similarity between different five trails of Northeast Portugal.

Butterfly species composition. Comparisons in species composition similarity among different trails estimated through NMDS ordination revealed that Alvão, Vale do Corgo, Mineiro, and Douro Vinhateiro trails had more species similarity while the Marão trail had more species associated exclusively to it than was shared with other trails (Fig. 3).

Additionally, the cluster analysis using Jaccard distance indicated that Alvão and Vale do Corgo trails presented the lowest average linkage similarity distance of 0.588, which represented the highest degree of similarity between trails studied. These two trails are also related to Mineiro trail, followed by Douro Vinhateiro with average linkage similarity distances of 0.611 and 0.627, respectively. Marão was separated from the other trails by an average linkage of similarity distance of 0.689, representing the lowest similarity among the five trails surveyed (Fig.4).

From the total number of families present in more than one trail, Pieridae and Nymphalidae expressed significantly different proportions among the trails (Table

Table 4. Families proportion in percentage per each trail and in the pooled data.*

	Site					
Family	Alvão	Marão	Mineiro	Douro Vinhateiro	Vale do Corgo	Total (%)
Pieridae	15.6_{a}	38.5_{b}	14.9_{a}	10.2_{a}	13.2_{a}	16.5
Nymphalidae	$68.9_{a,b}$	51.9_{b}	$68.5_{a,b}$	$74.6_{a,b}$	76.0_{a}	69.3
Lycaenidae	9.8_{a}	5.8_{a}	14.3_{a}	11.9_{a}	9.9_{a}	13.1
Hesperiidae	5.7_{a}	5.8_{a}	2.4_{a}	—**	—	2.5
Papilionidae	—	—	—	3.4_{a}	0.8_{a}	0.6

* Each subscript letter denotes a subset of site categories whose column proportions do not differ from each other at a $P = 0.05$.
** (—) denotes not applicable.

4). The proportion of Pieridae species was significantly higher in Marão when compared with all other sites. The proportion of Nymphalidae species was significantly higher in Vale do Corgo than in Marão (Table 4).

Discussion

The 45 species of butterflies recorded in this study represent a major proportion of the species that can be found in Portugal (33.83%), indicating the high potential of this region in terms of butterfly biodiversity and, therefore, with potential to indicate conservation issues in order to preserve species, communities, and habitats (Maravalhas 2003). Overall, species abundance and richness reveal that Nymphalidae iss the most-frequently encountered family followed by Pieridae and Lycaenidae. Nymphalids are the dominant group, probably due to their generalist polyphagous larvae which can survive in a large variety of habitats and also because the individuals of this family are known to be active fliers with a good dispersal ability and, therefore, able to exploit larger areas than individuals from other families. In fact, there are several butterfly species inventories that report this family as the dominant family (Majumder et al. 2013; Pang et al. 2016). Lower numbers of individuals belonging to species with typically small-body size in our survey might be attributed to these being more difficult to observe in the field (e.g., *Aricia cramera* (Eschscholtz), *Lampides boeticus* (L.), *Plebejus argus* (L.)). And similarly, species that are rapid fliers and at higher altitudes might be difficult to observe and collect (e.g., *Brintesia circe* (F.), *Arethusana arethusa* (Schiffermüller), *Hipparchia semele* (L.)) given our survey techniques and, therefore, be underestimated as per Pang et al. (2016).

The difference in butterfly species composition among sites found in these surveys suggests a response to comparative habitat (Hill et al. 1995; Majumder et al. 2013). From the total recorded species, 33.33% were observed only at one of the

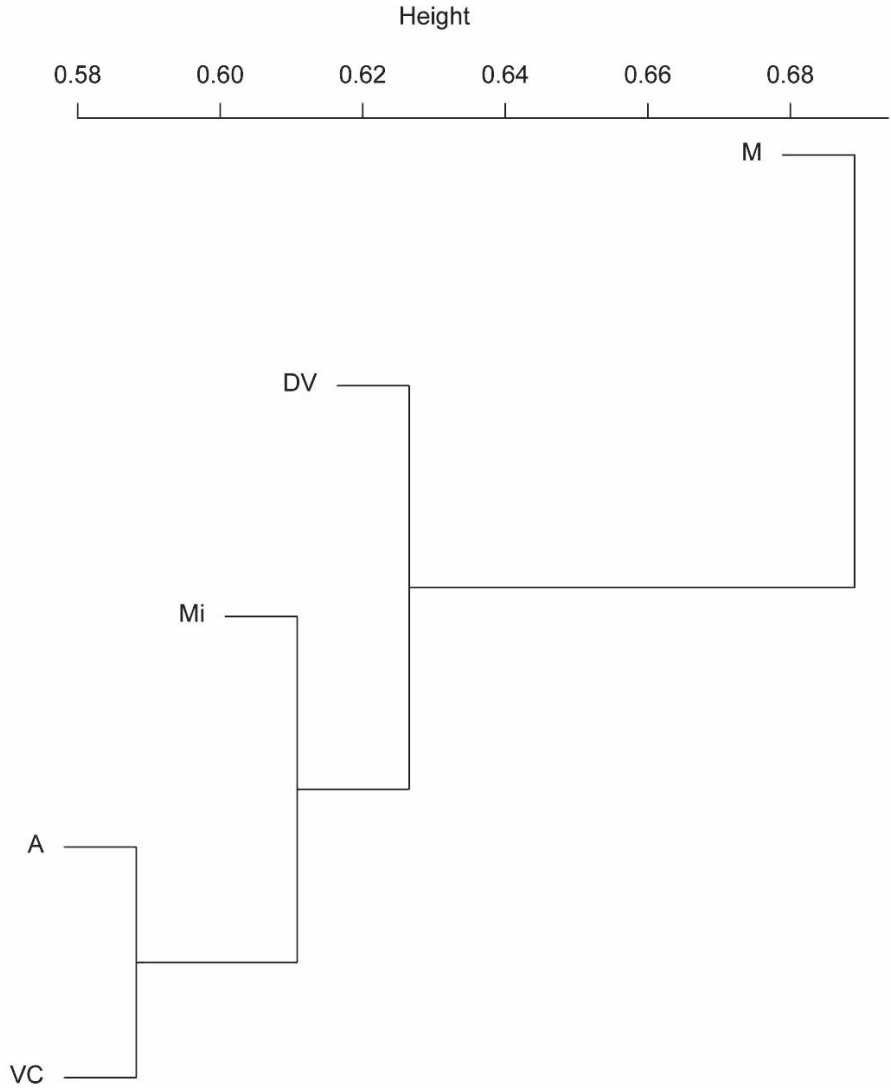

Fig. 4. Hierarchical cluster analysis between sites using Jaccard distance similarity as aggregation method. Abbreviations: M = Marão trail, DV = Douro Vinhateiro trail, Mi = Mineiro trail, A = Alvão trail, VC = Vale do Corgo trail.

five trails; that is, unique species indicating a restricted geographical distribution which can be related to low abundance and high habitat specificity. Species that are geographically restricted are considered as habitat-specific species with low ecological tolerance. These species usually occur only in undisturbed habitats and, therefore, have high conservation value in contrast with species that have high geographical distribution ability and, consequently, the high ecological tolerance

that can be found in a variety of vegetative complexes (Majumder et al. 2013; Spitzer et al. 1993). The presence or absence of butterfly species in an area can be directly attributed to the availability of food and shelter for larvae and also adults (Grossmueller and Lederhouse 1987; Majumder et al. 2013; Thomas 1995).

In our study, the maximum number of species and individuals were observed in areas where the landscapes were dominated by mixed landscapes of rural villages, with agriculture and pasture areas and also natural forests as well as marshes and grasslands (e.g., Alvão, Mineiro, and Vale do Corgo), in contrast to areas dominated by human populations and activities (i.e., vineyards, shrubs, cork trees, orchards) such as with the Douro Vinhateiro trail. These differences observed in butterfly species composition among the trails surveyed can be an effect of habitat heterogeneity and species diversity, which are determined largely by plant communities that occupy specific habitats and, consequently, are factors that influence the ecological distributions and interactions of the organisms that inhabit those areas (Atauri and Lucio 2001; Tews et al. 2004). Moreover, from the total number of species observed, four (6.90%) (i.e., *Euphydryas aurinia*, *Phengaris alcon*, *Hipparchia semele*, and *Melanargia lachesis*) were listed in the Habitats Directive Annex 2 (Council of European Union 1992) and Bern Convention Annex 2 (Council of European Union 1982), IUCN Red List of Threatened Species (IUCN 2014) and SPEC 4a – Global distribution restricted to Europe (van Swaay et al. 1999), but not Threatened. Another 8 species (17.78%) (i.e., *Arethusana arethusa*, *Argynnis adippe* (Denis & Schiffermüller 1775), *Coenonympha arcania* (L.), *Hyponephele lycaon* (Rottemburg), *Inachis io* (L.), *Melanargia russiae* (Esper), *Melitaea cinxia* (L.), and *Aporia crataegi* (L.)) are considered as moderately threatened in Portugal (Maravalhas 2003). These findings indicate that the region has a high natural value with conservation importance and with an environment that has a great potential concerning the provision of ecosystem services.

The rarefaction curves trend as well as results shown by the sampling completeness indicate that there are still additional species to be discovered, especially along the Vale do Corgo and Marão trials.

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