

Research articleSubmitted: October 2nd, 2018 - Accepted: December 5th, 2018 - Published: December 31st, 2018**The importance of beech forests as reservoirs of moth diversity in Mediterranean Basin (Lepidoptera)**Marco INFUSINO^{1,*}, Stefano SCALERCIO¹¹ Council for Agricultural Research and Economics, Research Centre for Forestry and Wood (CREA-FL) - Contrada Li Rocchi, 87036 Rende (CS), Italy - marco.infusino@crea.gov.it; stefano.scalercio@crea.gov.it

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Abstract

Study analyzes the macrolepidoptera assemblages in beech woodlands of the Orsomarso Mountains (Pollino National Park, Southern Italy) to assess the role of beech forests in preserving diversity in Mediterranean Basin. Research was run between 2015 and 2016 in 15 stands representative of the main successional stages of forest maturation, placed between 990 and 1,475 meters of elevation. Monthly-based sampling was performed using UV-LED light traps. A total of 33,957 individuals belonging to 410 species was collected. The community is rich and the most abundant and characteristic species (*Eilema lurideola*, *Operophtera fagata*, *Campaea margaritata*) are almost all trophically linked to broadleaves or lichens. The community structure appears fairly constant and recognizable in all stands over the two years of sampling. Young beech forests hosted the greatest number of species compared to other forest maturation stages, though the difference is small. The greatest differences in the community structure are found in the clearings, where generalist and/or related to the herbaceous layer species are mostly represented. Biogeographically widely distributed species prevail, 87% of them having European or Asian-European distribution. There are a number of species of faunistic interest, among which *Perizoma juracolaria*, *Chelis maculosa*, *Tiliacea citrigo*, *Poecilocampa populi*, *Triphosa dubitata*, *Sideridis reticulata*, *Nebula senectaria*, including 13 Italian endemics such as *Coenotephria antonii*. Populations of many species show significant genetic diversities compared to other European populations. The Orsomarso Mountains beech forests represent an important biodiversity reservoir, even at the genetic level, and show a good degree of naturalness.

Key words: community, Italy, Natura 2000, fauna, DNA barcoding, *Fagus sylvatica*.**Introduction**

In Europe, beech forests have a key role in the conservation of biodiversity (Annex 1, Habitats Directive, 92/43/EEC) and their conservation value is even greater in the South, where beech forests are ecologically and biogeographically older than those in Central and Northern Europe (Walentowski et al. 2014). For the Mediterranean Basin there are few studies on the relationships between biodiversity and beech forest attributes (Negro et al. 2013; Redolfi De Zan et al. 2014), although they harbour unique species assemblages including endemics and relict species (Walentowski et al. 2014).

The Pollino Massif is the highest mountainous area of the southern Apennines, reaching 2,267 m elevation (Serra Dolcedorme). It marks the administrative border between Basilicata and Calabria, the two southernmost regions of the Italian peninsula. This mountain range is included in the Pollino National Park, the largest protected area in Italy, with its 192,000 ha. The Pollino Massif is structurally contiguous with the Apennines, but it also marks a biogeographical boundary: further to the South, in fact, the

mountain reliefs are of different origin and geological nature (Calabria-Peloritani Terrane) (Amodio-Morelli et al. 1976), and are geographically separated from a wide plain of alluvial origin.

Woodland surface of the Park extends for 86,326 ha, 39.6% of which is occupied by the dominant tree species *Fagus sylvatica*. In Calabria, beech forests cover 77,237 ha, 6.6% of the total forest surface (INFC 2015). Pollino beech forests extend between 900 and 1,900 m a.s.l., with exceptional presence above 2,000 m of elevation too.

Due to its characteristics, the Pollino Park is of great interest in wildlife studies (Brandmayr et al. 2002; Belmonte et al. 2006; Todaro et al. 2007; Di Gristina et al. 2015). In particular, moths offer many possibilities for analysis in these environments, considering the numerous ecological roles they play (Usher & Keiller 1998; Summerville & Crist 2004).

Knowledge about the Lepidoptera of Pollino mainly concerns the Basilicata side and was often drawn from occasional research (Parenzan 1977, 1979, 1988, 1994; Marini & Russo 1980; Maffei, 1984; Parenzan & Scalercio 1996; Parenzan et al. 1999; Parenzan & Porcelli 2006;

Scalercio 2014), while regular monitoring has only been dedicated to the highest peaks of the massif (Scalercio 2009; Scalercio et al. 2014). These studies demonstrated the biogeographic interest of this area as several species with relict and disjunct populations were discovered but, in most cases, studies refer to open grasslands.

In studies concerning the assessment of the conservation status of forest environments, Lepidoptera are considered good indicators (Summerville et al. 2003), although often saproxylic beetles, better known and easily sampled, are used (Badano et al. 2016; Mazzei et al. 2017). The latter, however, provide only partial information on the environment, being essentially associated to the mass of dead wood, while the Lepidoptera show a greater number of links with the entire ecological network (Summerville et al. 2001). Knowledge on the Lepidoptera of beech forest is fragmented and it has never been a specific subject under study. In addition to the articles mentioned above, the report by Parenzan et al. (2005) and the faunistic contributions by Teobaldelli (1994) and Dapporto et al. (2004), covering a variety of environments among which beech forest in Central Italy, should be considered. The only community study that involved the beech forests is that of Scalercio et al. (2008) which, however, was made near a wide wet pasture on the edge of a beech forest in the Sila Massif (Calabria, Italy).

This research aims to provide new data on the macro moth fauna of beech forests in the Mediterranean Basin and, more in detail, to analyze the composition of the macro moth assemblages, underlying the importance of this habitat for the conservation of biodiversity at a continental scale.

Material and Methods

Study area

The study area, Orsomarso Mountains, is almost entirely comprised in the south-western part of the Pollino National Park. These mountains are separated from the Pollino Chain from the Campotenese Plain and represent the wildest part of the Park (Fig. 1). According to Barbati et al. (2014), the beech forest studied can be classified as Apennine-Corsican mountainous beech forest (EFTs - Type level 7.3), and it appears after the typical thermophilic plant association *Aquifolium-fagetum* (Piovesan et al. 2005) (Fig. 2).

The geological substrate is mainly calcareous. The morphology of the landscape, shaped by frequent karst phenomena, is characterized by a succession of mountains, plains, dolines, caves and gorges, offering a great variety of microclimates depending on the exposure of the slopes. Generally the climate is wetter on west-facing slopes. Up to 500 meters the Mediterranean climate dominates, higher up, a cool temperate climate prevails, while above 1,000 meters the climate is typically montane: winter is stiff and snowy, while summer is cool and windy, with some afternoon thunderstorms. Precipitation exceeds 2,000 mm per year.

Sampling methods

Moths were sampled by UV-LED traps (emission peak 398 nm, light angle per LED 120°, ~ 15 W) positioned at approximately 1.30 meters above the ground and powered by a 12V battery (Infusino et al. 2017a).

We identified 15 sampling stands grouped in triplets

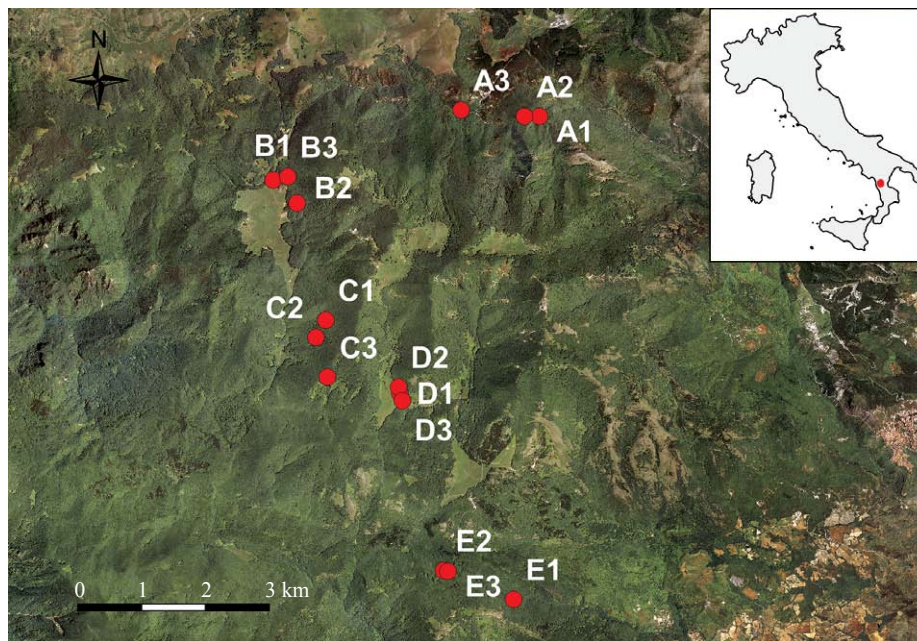


Fig. 1 – Study area. Location of sampling sites.



Fig. 2 – Study area. Typical aspect of the Orsomarso Mountains beech forests.

between 990 and 1,475 meters of elevation (Fig. 1; Table 1). Any triplet included a gradient of woodland maturity, that is: (1) mature and uneven aged beech stands; (2) young beech forests/mature coppices converted to high forests; (3) clearings and pastures surrounded by a forest matrix. Sampling was performed one night per month from May to November 2015 and from April to October 2016, during nights favorable to moth activity (no wind, temperatures not too low). The experimental design was planned to investigate three sites during the first year ($A_{1,2,3}$, $B_{1,2,3}$, $C_{1,2,3}$), and two new sites during the second year ($D_{1,2,3}$, $E_{1,2,3}$), replicating $C_{1,2,3}$ (Table 1). Nine traps worked contemporaneously.

Chorology has been drawn from Hellmann & Parenzan (2010), Bertaccini et al. (1995; 1997) and Scalercio & Infusino (2006).

Evenness index was calculated using Excel 2007.

In the list of species (see supplementary material), the families follow the taxonomic order by Parenzan & Porcelli (2006) modified and updated according to subsequent changes in the higher classification of Noctuoidea, while species within the single families are listed in alphabetical order. Nomenclature and family assignments follow the most updated European Checklist (Karsholt & Nieuwekerken 2013). For any species we recorded the number of individuals collected in each stand, the total abundance and the phenology indicated as the month of sampling.

The specimens were determined on the basis of the wing pattern or, in doubtful cases, by examination of genitalia. Moreover, some specimens, belonging to 29 species of faunistic importance or randomly selected among others

(Table 2), have been processed for barcoding analysis, according to the procedures indicated by the Canadian Centre for DNA Barcoding (CCDB) for sequencing the mitochondrial 5' cytochrome oxidase subunit 1 gene (COI), fragment length 658 bp (Hajibabaei et al. 2006). These data were utilised to confirm morphological identifications and to explore genetic diversification of local population. Specimens were deposited in the Lepidoptera collection of the Research Centre for Forestry and Wood (CREA-FL), Office of Rende.

Results

During the samplings, 33,957 individuals belonging to the 410 species listed below were collected (see supplementary material). The “head” of the community is well characterized: the 15 most abundant species represent about the 70% of the total, almost all of them trophically linked to broadleaved trees (especially beech) or lichens. The relative abundances of the species are distributed rather evenly, with the exception of the first three species (*Eilema lurideola*, *Operophtera fagata*, *Campaea margaritata*), which alone constitute 44% of the whole assemblage (Fig. 3). However, this data is strongly influenced by the exceptional massive capture of *E. lurideola* in July 2015 in stands $A_{1,2,3}$, mainly due to the peak of adult emergence of this species, and the synergic effect of high nocturnal temperatures and topography of the sites (for more information see Greco et al. 2017). Excluding this unusual event, for example by aligning the figures for *E. lurideola*

Table 1 – Detailed data about the location of sampled stands.

Stand	Locality	Longitude Est (EPSG:4265)	Latitude North (EPSG:4265)	Elevation (m)	Year of sampling
A1	Serrapaolo	16.090377	39.820183	990	2015
A2	Serrapaolo	16.087639	39.820174	1010	2015
A3	Serra Ambruna	16.076070	39.821070	1035	2015
B1	Bocca di Novacco	16.041719	39.811224	1315	2015
B2	Bruscata	16.046084	39.807978	1370	2015
B3	Bocca di Novacco	16.044414	39.811704	1339	2015
C1	Timpone della Magara	16.051344	39.791553	1465	2015, 2016
C2	Timpone della Magara	16.049549	39.789142	1460	2015, 2016
C3	Timpone della Magara	16.051644	39.783554	1475	2015, 2016
D1	Piano del Minatore	16.064845	39.781264	1433	2016
D2	Piano del Minatore	16.064646	39.782148	1440	2016
D3	Piano del Minatore	16.065325	39.780223	1419	2016
E1	Carraci	16.085653	39.752315	1270	2016
E2	Piano del Faggio	16.072849	39.756353	1357	2016
E3	Piano del Faggio	16.073649	39.756253	1366	2016

in those stands to the average of the other sites, the overall value of the Evenness Index is rather high ($E_{SH} = 0.70$).

Regarding the chorology, widespread species are prevalent, representing about 87% of the assemblage (60% with Holarctic or Asian-European distribution, 27% with European distribution), while Mediterranean species sum up to only 7% of the total. In quantitative terms, the prevalence of individuals of widely distributed species is even more pronounced, exceeding 95% of the total (Fig. 4).

The monthly frequency of the sampling does not allow an accurate evaluation of the phenology, however some considerations can be made. Most species fly between June and August (65%), peaking in July. Autumn species, present between September and November, represent about 14%, while only 10% of the species is active at the beginning of spring, between April and May, many of which overwintering as larva or adult. For the remaining species it is not possible to define a clear phenology. Bivoltine species are few (10%).

Some specimens were subjected to barcoding analysis. Therefore, the genetic data of 29 species are available (Table 2). Of these, 7 show a divergence between 1% and 3% compared to other European populations: *Coenotephria ablutaria*, *Drymonia dodonaea*, *Hyloicus pinastri*, *Nyctola revayana*, *Perizoma juracolaria*, *Trichiura crataegi*, and *Triphosa dubitata*.

Discussion and conclusions

The assemblage of macro-Heterocera hosted by the beech forest of Orsomarso Mountains is very rich in species. Its

structure appears fairly constant and recognizable in all stands and in the two years of sampling, with the important exception of *Operophtera fagata* (see supplementary material). This species, trophically linked to beech, is on the whole one of the species most characterizing the assemblage, but it is definitely less present in the sites A_{1,2,3} and in all the sites sampled in 2016. The sites A_{1,2,3} are positioned at lower altitudes, where the beech tree, although still predominant, cohabits with other broadleaved trees, on which *O. fagata* does not feed. The difference in abundance between the two years is essentially due to a meteorological event: a late frost in April 2016 which caused the complete loss of beech foliage in a range between 1,400 and 1,600 altitude meters (Greco et al. 2018a). As a result, the primary food source for *O. fagata* larvae disappeared, causing the collapse of the population.

Young beech forests are the ones that have shown the greatest number of species, even if the differences with the other stages of woodland maturity are small. The greater differences in the community structure are found in the clearings (see supplementary material). Even if surrounded by forest, they show their own characteristics: first of all, the generalist species (e.g. *Alcis repandata*, *Gymnoscelis rufifasciata*,) and those related to herbaceous vegetation (e.g. *Agrotis cinerea*, *Mythimna conigera*, *Dasypolia templi*, *Athetis pallustris*) are more represented.

Remarkable is the presence of 13 Italian sub-endemic species: *Hydriomena sanfilensis*, *Coenotephria antonii* (South-Apennine), *Hemistola siciliana*, *Idaea mutilata*, *Hylaea mediterranea* (South-Apennine-Sicilian), *Xanthorhoe vidanoi* (Apennine-Sicilian), *Itame sparsaria*, *Megalycinia serraria*, *Lycia florentina* (Apennine), *Clematha-*

Table 1 – List of species subjected to DNA Barcoding analysis. For each species are reported: the Barcode Index Number (BIN) generated by BOLD, minimum and maximum genetic distance (in percentage) and relative geographical origin of the specimen to which it is referred, number of the samples subjected to Barcoding analysis (N), information related to biogeographically consistent clustering.

* Species to which the BOLD System assigns more BIN.

** Species that show clusters with a potential taxonomic interest, but related to geographical areas different than those treated in this research.

Species	BIN	Min. distance Country	Max. distance Country	N	Clustering
<i>Charissa variegata</i>	BOLD:AAC1039	0.76% - Sicily (IT)	2.61% - Greece	1	NO
<i>Coenotephria ablutaria</i>	BOLD:ACF5573*	0.46% - France	3.77% - Greece	3	YES
<i>Coenotephria antonii</i>	BOLD:ADB7125	3.98% - Abruzzi (IT)	3.98% - Abruzzi (IT)	1	YES
<i>Drymonia dodoneaea</i>	BOLD:ADH0342*	1.68% - Czech Rep.	2.14% - Austria	2	YES
<i>Epirrhoe alternata</i>	BOLD:ACE4142	0.15% - Sardinia (IT)	1.07% - Germany	1	NO
<i>Eupithecia dodoneata</i>	BOLD:AAC1636	0.17% - Germany	1.16% - Germany	1	NO
<i>Eupithecia innotata</i>	BOLD:AAB8929	0.15% - Germany	0.61% - Germany	1	NO
<i>Gymnoscelis rufifasciata</i>	BOLD:AAA7404*	0.16% - United Kingdom	4.53% - Spain	1	YES**
<i>Hoplodrina superstes</i>	BOLD:AAK5525	0.15% - Macedonia	0.76% - France	2	NO
<i>Hyloicus pinastri</i>	BOLD:AAA8863	1.38% - Cen.-North Europe	1.68% - Turkey	1	YES
<i>Lithophane socia</i>	BOLD:AAE6607	0.15% - Switzerland	0.78% - Germany	1	NO
<i>Nebula senectaria</i>	BOLD:AAC6527	0.15% - Abruzzi (IT)	1.01% - Turkey	1	NO
<i>Nothocasis rosariae</i>	BOLD:AAM2755	0.31% - Basilicata (IT)	1.77% - Greece	6	YES
<i>Nycteola columbana</i>	BOLD:AAB8994	0.15% - Spain	0.46% - Spain	1	NO
<i>Nycteola revayana</i>	BOLD:AAB8993	0.15% - Abruzzi (IT)	1.53% - Austria	2	YES
<i>Perizoma juracolaria</i>	BOLD:AAD4226	0.31% - Abruzzi (IT)	0.61% - Lazio (IT)	1	YES
<i>Phlogophora meticulosa</i>	BOLD:AAB7358	No difference between the European populations		1	NO
<i>Ptilophora variabilis</i>	BOLD:AAL6676	Endemic: Genetic data available only for Calabria		4	-
<i>Puengeleria capreolaria</i>	BOLD:AAD9519	0.15% - Germany	1.99% - Germany	1	NO
<i>Scotopteryx angularia</i>	BOLD:AAC9097	0.15% - France	0.31% - France	1	NO
<i>Scotopteryx moeniata</i>	BOLD:AAE1660*	0.52% - Germany	2.29% - Sudtirol (IT)	1	YES**
<i>Selidosema brunnearia</i>	BOLD:ACE5955	0.31% - Sicily	0.76% - Denmark	1	NO
<i>Shargacucullia lychnitis</i>	BOLD:ABY4109	0.15% - Denmark	0.46% - Austria	1	NO
<i>Shargacucullia thapsiphaga</i>	BOLD:ABY4819	0% - Greece	0.15% - Austria	1	NO
<i>Sideridis reticulata</i>	BOLD:AAD4204*	0.15% - Cen.-North Europe	1.99% - Georgia	1	YES**
<i>Tephronia sepiaria</i>	BOLD:AAD2603	0.15% - Spain	0.31% - Austria	2	NO
<i>Tiliacea cypreago</i>	BOLD:ADJ6365*	2.60% - Bulgaria	2.60% Bulgaria	2	NO
<i>Trichiura crataegi</i>	BOLD:AAB4489	0.65% - France	1.53% - Finland	2	NO
<i>Triphosa dubitata</i>	BOLD:AAD3995*	0.51% - Germany	2.78% - Georgia	1	YES

da calberlai (Alpine-Apennine-Sicilian), *Perizoma juracolaria* and *Stilbia faillae* (Alpine-Apennine), to which we must add *Ptilophora variabilis*, from recent research considered as *bona species* replacing *P. plumigera* along the Apennines (Infusino et al. 2018).

There are many species of faunistic interest only recently reported in Calabria (*Perizoma juracolaria*, *Chelis maculosa*, *Tiliacea citrigo* and *Poecilocampa populi*) (Infusino et al. 2016; Greco et al. 2018b) that had been previously reported for the Pollino Massif but only for the Basilicata side; in addition, also *Coenotephria antonii*,

Triphosa dubitata and *Sideridis reticulata* were found for the first time in Calabria, while for *Nebula senectaria*, the Orsomarso Mountains are the only known locality for central-southern Italy (Infusino et al. 2017b). In particular, the specimen of *C. antonii* we found is the only known male of this species (Infusino et al. 2017b).

The finding in many stations of *Euplagia quadripunctaria*, species included in Annexes II and IV of the Habitats Directive, should be noted.

The species composition of Macrolepidoptera Heterocera of the Orsomarso Mountains is quite rich and well

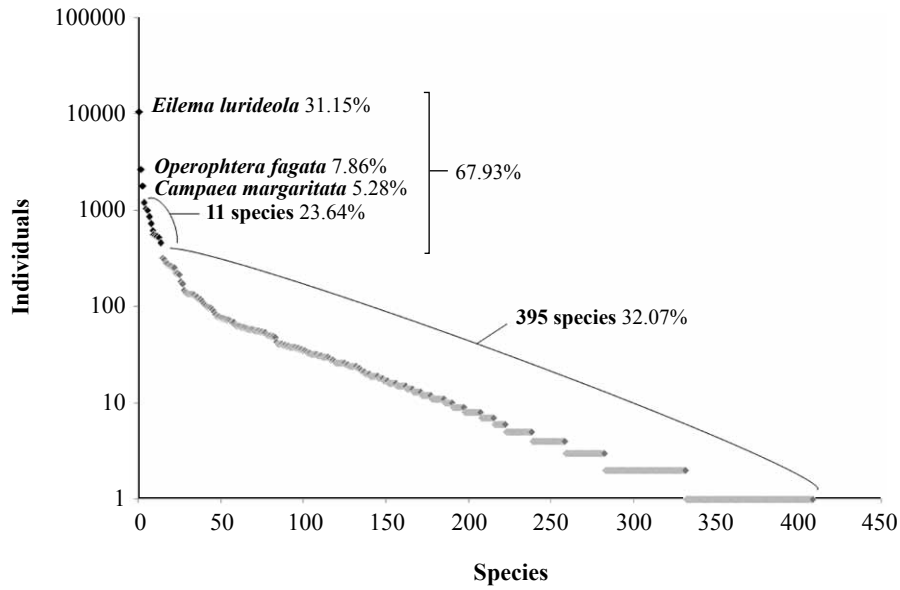


Fig. 3 – Community structure. Relative abundances are expressed in a logarithmic scale.

characterized. The clearings show their own composition and showed a biodiversity comparable to those of forested stands. Actually, where pasture is less intense (as in C3 site), the number of species is similar or slightly greater than in the corresponding wooded sites: this is because these clearings, some of which are of considerable extension, are mostly of natural origin, due to the action of the karst phenomena that characterize the geomorphology of this mountainous area.

The sites of the A triplet are of particular ecological and conservation importance. The particular topographic conformation of this area, a valley, makes it a perfect cor-

ridor that connects the beech forest with the mixed thermophilous woods, typical of lower elevation. The result is a great species richness that benefits too from species such as *Tiliacea cypreago*, *P. populi*, *C. antonii* and *Triphosa dubitata*.

The lack of bibliographic documentation does not allow a direct comparison of fauna with other similar environments. In Scalercio et al. (2008), the community of Lepidoptera of Monte Curcio (Sila Massif) was performed with a different methodology and was focused in an area at the edge of beech forest. In fact, in the community of Monte Curcio species related to herbaceous environ-

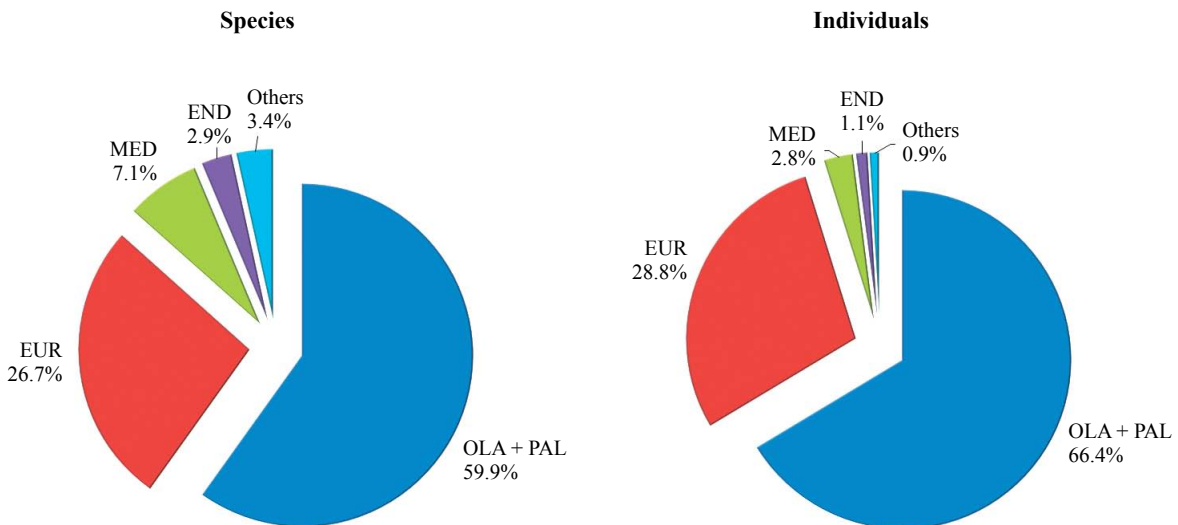


Fig. 4 – Chorological analysis of the community. Percentage breakdown of the number of species (on the left) and individuals (on the right) based on the main chorological categories. OLA+PAL: species with a wide Holarctic distribution (Holarctic, Palearctic, Asian-European); EUR: species with European distribution; MED: species with Mediterranean gravitation; END: Italian endemic species.

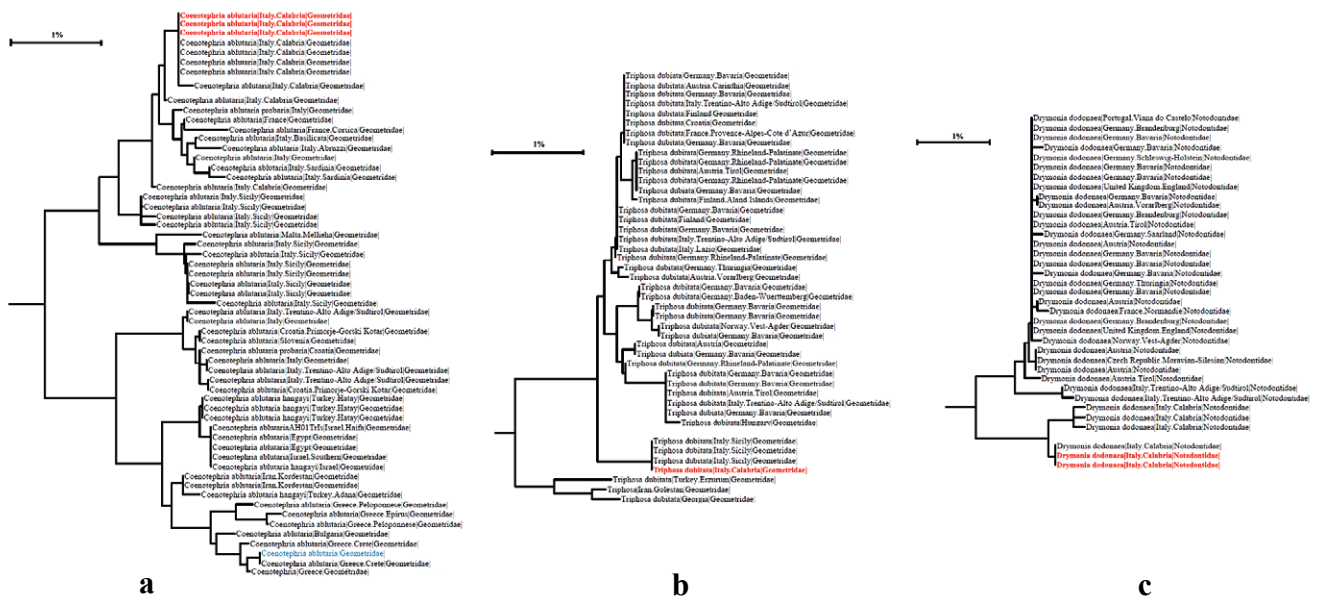


Fig. 5 – Neighbor joining trees (Kimura 2-parameter distance model for COI-5P marker) of species covered in this paper built using sequences deposited in BOLD. **a**, *Coenoteprhia ablutaria*, **b**, *Thiphsosa dubitata*, **c**, *Drymonia dodonaea*. In red the specimens subjected to barcoding analysis in this study, in blue the species of unknown country.

ments, such as *Agrotis cinerea*, *A. segetum*, *A. clavis*, *Peridroma saucia* and *Tholera decimalis* are more represented, while among the strictly forest species characterizing the community, only *O. fagata*, *Watsonalla cultraria*, and *Colocasia coryli* show relative abundance comparable in both woods. The distribution of the chorotypes is similar in both sites, with the difference that in the community of Monte Curcio the species with a wide Palearctic distribution are mostly represented, thus weakening the European component.

Barcoding analysis has shown some peculiar genetic lineages that can also have taxonomic relevance, as shown by recent papers about *Nothocasis rosariae* and the *Ptilophora* species group (Scalercio et al. 2016; Infusino et al. 2018). This is the case, for example, of *C. ablutaria*, *T. dubitata* and *D. dodonaea*, for which the biomolecular data show a relatively high genetic distance from northern populations. In addition, the specimens from the Pollino/Orsomarso area form a cluster separate with respect to other populations, suggesting the need of taxonomic studies for these populations (Fig. 5). Also for other species, *G. ruffasciata*, *Scotapteryx moeniata* and *S. reticulata*, genetic information suggests taxonomic insights but, in these cases, the interpretation appears more complex and involves biogeographical areas different than those treated in this research. The barcoding analysis of the other species, although not bearing on taxonomic relevance, highlights a certain degree of genetic distance of these populations. Of the 29 species subjected to molecular investigations, 7 showed evident genetic divergence. Extending the data at the whole species sample, potentially, 20-30% of the species could present a distinct genetic footprint, dem-

onstrating the high biodiversity value of this forest type in the Mediterranean Basin. The barcoding analysis is used almost exclusively to deepen taxonomic studies, but they can provide new biogeographic keys to interpretation, or valuable information on the isolation degree of the populations (Dapporto et al. 2017). This information can be important for the conservation of biodiversity and the development of wildlife management plans (Vodá et al. 2016).

The Orsomarso Mountains show a good degree of naturalness. Grazing, which is the main disturbance factor, is generally limited to the largest grassland areas and is not particularly intense. This area of the Pollino National Park represents, therefore, an important reservoir of biodiversity, even at the genetic level, not yet exhaustively investigated.

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