

Host-plant relationships of 29 Mediterranean Lepidoptera species in forested ecosystems unveiled by DNA Barcoding (Insecta: Lepidoptera)

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Abstract

A total of 45 Lepidoptera larvae were collected in ethanol from 16 target plant species in southern Italy, requiring a few hours of field work only. Identification was performed by DNA barcoding unveiling host-plant relationships for 29 Lepidoptera species. The authors propose a larger-scale approach using this rapid and efficient method and encourage entomologists to join the team.

KEY WORDS: Insecta, Lepidoptera, caterpillar, feeding behavior, tree species, larval identification, Italy.

Relación de las plantas nutricias de 29 especies de Lepidoptera mediterráneas en ecosistemas forestales revelados por el Código de barras de ADN (Insecta: Lepidoptera)

Resumen

Se colectaron un total de 45 larvas de Lepidoptera en etanol de 16 especies de plantas en el sur de Italia, requiriendo sólo unas pocas horas de trabajo de campo. La identificación fue llevada a cabo con el sistema de código de barras de ADN revelando la relación con las plantas nutricias de 29 especies de Lepidoptera. Los autores proponen un enfoque a mayor escala usando este método rápido y eficaz y animando a otros entomólogos a sumarse al equipo. PALABRAS CLAVE: Insecta, Lepidoptera, oruga, comportamiento alimenticio, árbol de especies, identificación larvaria, Italia.

Introduction

Available literature on host-plant relationships of Mediterranean Lepidoptera is very scarce and feeding biology of many species is still unknown. Only a very few authentic, original data are published for host-plant relationships of Mediterranean Lepidoptera and most of them concern pest species, such as *Tuta absoluta* (Meyrick, 1917) (Gelechiidae) (PORTAKALDALI *et al.*, 2013), or caterpillars found on plants of economic importance such as *Euzophera bigella* (Zeller, 1848) (Pyralidae) on *Olea europaea* L. (SIMOGLU *et al.*, 2012), *Lymantria dispar* (Linnaeus, 1758) (Lymantriidae) on *Quercus suber* L. (PROTA, 1970), *Cynaeda perspectalis* (Walker, 1859) (Crambidae) on *Buxus* (KRUGER, 2008), and others. Books like those of GÓMEZ DE AIZPURUA (1985-1992, 2002-2012) on Iberian moth larvae report a lot of host-plant data, but in most cases it remains unclear if the data come from observations in nature or not. It is most likely that the vast majority comes from artificial conditions during rearing. The situation is still worse for southern Italian Lepidoptera, but an exception is the paper devoted to the lepidopteran fauna of the *Quercus suber* L. in Sardinia showing a large number of species

feeding on this tree (PROTA, 1970). We estimate that for >90% of the putatively 4000 southern Italian Lepidoptera species (approx. 2500 Macrolepidoptera species are mentioned in PARENZAN & PORCELLI 2006; 2008 for the Italian fauna) no authentic feeding records from nature are published for the study area. We furthermore estimate that for >50% of these species no authentic feeding record from nature is reported for the whole of their distribution areas.

Few original data concern species of non-economic importance, e.g. several Microlepidoptera species which are often reared from larvae. *Caryocolum siculum* (Gelechiidae) was described rearing larvae on *Gypsophila arrostii* Guss. (Caryophyllaceae) (BELLA, 2008), *Coleophora sardiniae* was described rearing larvae on seeds of *Genista corsica* (Loisel.) DC. (Labiatae) (BALDIZZONE, 1983).

Not only in old papers devoted to European caterpillars and their food-plants (e.g. ROÜAST, 1883, 1884; HOFMANN, 1983) but also in large recent book series, such as Microlepidoptera of Europe, Lepidoptera of Europe (LERAUT, 2006-2014) and Noctuidae Europaeae it is usually impossible to trace original observations and separate them from copied and unreliable, secondary literature citations. In the book series "The Geometrid Moths of Europe" (HAUSMANN, 2001, 2004; MIRONOV, 2003; HAUSMANN & VIIDALEPP, 2012; SKOU & SIHVONEN, 2015) as well as in the "Schmetterlinge Baden-Württembergs (EBERT 1991-2005, ed.) however, there is a consistent system allowing discrimination between original (feeding) observations in nature, copied literature citations (usually copied without examining their plausibility) and data from rearing, potentially altered by the artificial conditions.

Availability of affordable data is of great importance for forestry, agriculture and ecological research mainly from a conservation point of view, but they could also be important to design environment-friendly control strategies against pests and defoliators.

The classical method to identify larvae is to rear them in captivity until the emergence of the adult and identify it through morphology. This method is time consuming and the rearing success may be quite low, especially if the hostplant is not easily available where the rearing takes place. Identification of lepidopteran larvae was successfully carried out by DNA barcoding (COI 5') (MILLER *et al.*, 2007; EMERY *et al.*, 2009; GOSSNER & HAUSMANN, 2009), permitting an easy, cheap and rapid identification of species. MILLER *et al.* (2007) and MATHESON *et al.* (2008) investigated and ascertained relationships between plants and caterpillars by a method based on the DNA identification of the larval gut content, an effective but expensive and time-consuming approach, especially in larger surveys. Identification through DNA barcoding is possible even from dry skins after moulting and from empty pupal exuviae after hatching of the moths (own, original, unpublished data). In southern Italy, SCALERCIO *et al.* (2014) identified through DNA barcoding a larva of the endemic *Hydriomena sanfilensis* (Stauder, 1915) (Geometridae) feeding on *Rosa canina* L., the first record of a host-plant for this species. Currently, there are large-scale projects devoted to identification larvae along with their host-plants in Papua New Guinea (MILLER *et al.*, 2013) and Costa Rica (JANZEN & HALLWACHS, 2015). Both are based on an integrative approach combining morphology, rearing and molecular techniques.

The aim of this paper was to establish host-plant relationships of caterpillars based on the identification of larvae collected on their foodplants by DNA barcoding. In this approach we assume (and in several cases observed) that the collected larvae feed on the plants on which they stay. This assumption is based on the behaviour of larvae usually resting on feeding plants during their development. Exceptions are larvae of certain species which abandon their host-plants searching for a hidden resting place during daytime and mature larvae leaving their food-plant in the last days before pupation looking for a suitable pupation site, sometimes far from their feeding plants. Only during this short timespan it is possible to collect larvae on plants different from the typical host-plant.

Material and methods

In this paper we report the results of 12 days of fieldwork in 2013 and 2014 (with a total of 21 working hours of a single person) collecting in southern Italy (Calabria and southern Basilicata regions, Table 1). Larvae were beaten from plants or, sometimes, directly collected on them. Sampling from

trees was performed by spreading a white sheet below the tree branches and beating their tips in order to avoid sampling of feeders on epiphytic lichens and mosses. Successively the sheets were inspected and larvae preserved in absolute alcohol. Larvae from herbaceous plants were sampled by beating or sweeping with a robust net in order to sample larvae with the net before falling down. In both cases we carefully made sure that collected specimens came from the target plant species. This was ascertained by choosing target plants isolated from other plant species. Few larvae were directly collected on feeding plants while they were feeding.

Table I.– Collecting localities.

ID	Region	Province	Municipality	Exact site	Altitude	Latitude, Longitude
1	Calabria	Cosenza	Rende	Contrada Li Rocchi	200 m	39.3671, 16.2317
2	Calabria	Cosenza	Cosenza	Fosso Cucolo	550 m	39.2577, 16.2857
3	Calabria	Cosenza	San Lucido	Passo Crocetta	955 m	39.3284, 16.1069
4	Basilicata	Potenza	Trecchina	Piano dei Peri	280 m	39.9913, 15.1938
5	Basilicata	Potenza	Valle del Noce	Moor near Rivello	585 m	40.0833, 15.7281
6	Calabria	Crotone	Cotronei	Vallone di Calusia	120 m	39.1761, 16.8344
7	Calabria	Cosenza	Mendicino	Monte Cocuzzo	1100 m	39.2244, 16.1548
8	Calabria	Cosenza	Rogliano	Monte Gabriele	705 m	39.1781, 16.3218
9	Basilicata	Potenza	Lagonegro	Monte Sirino NE	1380 m	40.1588, 15.8392
10	Calabria	Cosenza	Praia a Mare	P.a.M. Centro	30 m	39.8935, 15.7834

44 larvae were submitted for DNA barcoding. One additional larva was reared to the adult stage and its pupal exuvia was submitted for DNA barcoding. Vouchers are stored at the Bavarian State Collection of Zoology, Germany.

Tissue sampling was carried out by using scissors and pincers which were carefully cleaned in 100% alcohol in order to avoid contamination of samples. Tissues (one vertically cut segment) were transferred to a lysis plate under ca 0.5 ml 100% alcohol.

Tissue samples were submitted according to the standard procedures of the Canadian Centre for DNA Barcoding (CCDB) for sequencing the mitochondrial 5' cytochrome oxidase gene, subunit I (COI), the standard marker for the identification of most animals. LepF1 and LepR1 were the primers used for PCR and sequencing (HAJIBABAEI *et al.*, 2006). Sequences were blasted against the complete sequence database of BOLD data systems (RATNASINGHAM & HEBERT, 2007) in order to investigate the closest matches using the BOLD Identification Engine (http://www.boldsystems.org/index.php/IDS_OpenIdEngine).

For nomenclature of moth names we follow the Fauna Europaea (KARSHOLT & VAN NIEUKERKEN, 2013).

Plant identification was based on morphology in most cases and carried out in the field. In some cases we collected leaves, fruits or blossoms and had the species identified by botanists specialized in the flora of southern Italy, of the Museo di Storia Naturale della Calabria e Orto Botanico, Italy. For nomenclature of plant names we follow the Flora Europaea (available online at <http://rbg-web2.rbge.org.uk/FE/fe.html>).

Results and Discussion

A total of 45 larvae were collected on 16 target plant species. 32 larvae belonging to 27 species were identified at species level by DNA barcoding (Tables II, III). Four additional larvae belonging to two further species could be identified to species level based on larval morphology, one to genus level. Altogether we publish host-plant relationships for 29 species, here. 13 larvae (out of 45 larvae submitted to DNA barcoding = 29%) failed to yield DNA sequences, probably because the absolute alcohol content was excessively diluted by larval fluids in too small vials, especially in the case of large caterpillars.

Table II.– List of 36 identified larvae belonging to 29 species. Abbreviations: Id. = method of identification: b = larva identified by DNA barcoding (COI 5', then blasted on BOLD); rb = larva reared and adult or exuvia submitted to DNA barcoding; m = morphological identification of larva; Loc. = locality code: see Table I.

Taxonomy (larva)	Id.	Host-plant	Loc.	Date
NOCTUIDAE				
<i>Orthosia miniosa</i> (Denis & Schiffermüller, 1775)	b	<i>Quercus pubescens</i> Willd.	2	12-V-2014
<i>Orthosia miniosa</i> (Denis & Schiffermüller, 1775)	b	<i>Arbutus unedo</i> L.	2	12-V-2014
<i>Orthosia cerasi</i> (Fabricius, 1775)	b	<i>Arbutus unedo</i> L.	2	12-V-2014
<i>Orthosia cerasi</i> (Fabricius, 1775)	m	<i>Arbutus unedo</i> L.	2	12-V-2014
<i>Orthosia gothica</i> (Linnaeus, 1758)	b	<i>Ranunculus acris</i> L.	2	28-IV-2014
<i>Autographa gamma</i> (Linnaeus, 1758)	b	<i>Ocimum basilicum</i> L.	4	24-VIII-2014
<i>Autographa gamma</i> (Linnaeus, 1758)	b	<i>Calamintha nepeta</i> (L.) Savi	6	29-IX-2014
<i>Pyrrhia umbra</i> (Hufnagel, 1766)	b	<i>Calamintha nepeta</i> (L.) Savi	7	3-X-2014
EREBIDAE				
<i>Dysgonia algira</i> (Linnaeus, 1767)	rb	<i>Punica granatum</i> L.	10	7-VIII-2013
<i>Clytie illunaris</i> (Hübner, 1813)	b	<i>Tamarix gallica</i> L.	6	29-IX-2014
<i>Calliteara pudibunda</i> (Linnaeus, 1758)	m	<i>Fagus sylvatica</i> L.	3	6-X-2013
<i>Orgyia antiqua</i> (Linnaeus, 1758)	b	<i>Erica arborea</i>	5	20-VIII-2014
NOTODONTIDAE				
<i>Phalera bucephala</i> (Linnaeus, 1758)	m	<i>Quercus pubescens</i> Willd.	1	3-X-2013
GEOMETRIDAE				
<i>Phaetogramma etruscaria</i> (Zeller, 1849)	b	<i>Populus nigra</i> L.	1	7-X-2013
<i>Cyclophora suppunctaria</i> (Zeller, 1847)	b	<i>Quercus pubescens</i> Willd.	1	11-IX-2013
<i>Cyclophora ruficiliaria</i> (Herrich-Schäffer 1855)	b	<i>Quercus pubescens</i> Willd.	5	20-VIII-2014
<i>Hydriomena sanfilensis</i> (Stauder, 1815)	b	<i>Rosa canina</i> L.	9	29-VIII-2013
<i>Eupithecia semigraphata</i> Bruand, 1850	b	<i>Calamintha nepeta</i> (L.) Savi	7	3-X-2014
<i>Eupithecia semigraphata</i> Bruand, 1850	b	<i>Calamintha nepeta</i> (L.) Savi	7	3-X-2014
<i>Eupithecia semigraphata</i> Bruand, 1850	b	<i>Calamintha nepeta</i> (L.) Savi	7	3-X-2014
<i>Eupithecia semigraphata</i> Bruand, 1850	m	<i>Calamintha nepeta</i> (L.) Savi	7	3-X-2014
<i>Stegania trimaculata</i> (de Villers, 1789)	b	<i>Salix alba</i> L.	1	9-IX-2013
<i>Stegania trimaculata</i> (de Villers, 1789)	b	<i>Salix alba</i> L.	1	9-IX-2013
<i>Opisthograptis luteolata</i> (Linnaeus, 1758)	b	<i>Prunus cocomilia</i> Ten.	9	29-VIII-2013
<i>Cabera pusaria</i> (Linnaeus, 1758)	b	<i>Alnus glutinosa</i> (L.) Gaertn.	9	29-VIII-2013
<i>Pachynemia hippocastanaria</i> (Hübner, 1799)	b	<i>Erica arborea</i> L.	5	20-VIII-2014
<i>Agriopsis leucophaearia</i> (Denis & Schiffermüller, 1775)	b	<i>Quercus pubescens</i> Willd.	2	12-V-2014
<i>Biston betularia</i> (Linnaeus, 1758)	b	<i>Prunus cocomilia</i> Ten.	9	29-VIII-2013
<i>Lycia hirtaria</i> (Clerck, 1759)	b	<i>Quercus pubescens</i> Willd.	2	12-V-2014
<i>Hypomecis punctinalis</i> (Scopoli, 1763)	b	<i>Erica arborea</i> L.	5	20-VIII-2014
<i>Ematurga atomaria</i> (Linnaeus, 1758)	b	<i>Erica arborea</i> L.	5	20-VIII-2014
DREPANIDAE				
<i>Watsonalla</i> cfr. <i>cultraria</i> (Fabricius, 1775)	rb	<i>Fagus sylvatica</i> L.	3	12-X-2013
CRAMBIDAE				
<i>Uresiphita gilvata</i> (Fabricius, 1794)	b	<i>Spartium junceum</i> L.	4	24-VIII-2014
TORTRICIDAE				
<i>Ditula angustiorana</i> (Haworth, 1811)	b	<i>Quercus pubescens</i> Willd.	2	12-V-2014
PTEROPHORIDAE				
<i>Stangeia siceliota</i> (Zeller, 1847)	b	<i>Conyza canadensis</i> (L.) Cronquist	1	3-X-2013
LYCAENIDAE				
<i>Satyrium ilicis</i> (Esper, 1779)	b	<i>Quercus pubescens</i> Willd.	2	12-V-2014

For largely polyphagous species, such as *Autographa gamma* and *Dysgonia algira*, the presented records are not surprising and probably already published somewhere in literature. For little known species and for those feeding on just a few plants, it is easier to overview the published records in literature. The following records are of particular interest.

The pterophorid *Stangeia siceliota* was recorded on *Conyza canadensis* for the first time. Other known host-plants are *Cistus* sp., *Sanguisorba* sp., *Inula viscosa* L., *Ononis natrix* L. (GIELIS, 1996).

Hypomecis punctinalis (Geometridae, Ennominae) was found on *Erica arborea* for the first time. Other known hostplants are *Quercus*, *Tilia*, *Betula*, *Salix*, *Genista* and *Lonicera* (REDONDO *et al.*, 2009), furthermore *Larix*, *Carpinus*, *Alnus*, *Berberis*, *Sorbus*, *Rubus*, *Prunus*, *Acer*, *Frangula*, *Cornus* and *Vaccinium* (EBERT, 2003).

Phaiogramma etruscaria (Geometridae, Geometrinae) was found on *Populus nigra* and thus on Salicaceae for the first time. HAUSMANN (2001) characterized this species as polyphagous with a certain preference for Umbelliferae (Apiaceae), listing 14 species of herbs and shrubs, but also *Quercus*.

Stegania trimaculata (Geometridae, Ennominae) was found on *Salix alba* for the first time in nature, a plant which had been accepted only in captivity, so far (RUNGS, 1981). Usually it feeds on *Populus* and *Alnus* (FLAMIGNI *et al.*, 2007).

A very young larva of *Orthosia gothica* (Noctuidae, Noctuinae) was collected on a plant of *Ranunculus acris*, where it was observed feeding on the petals. This plant seems to be listed as a host-plant for this moth species for the first time here, though caterpillars are known to be polyphagous on several tree species and more rarely "on various herbs" (e.g. FORSTER & WOHLFAHRT, 1980).

Furthermore, in this paper we publish the COI sequences of *Clytie illunaris*, *Uresiphita gilvata* and *Stangeia siceliota* for the first time.

26 out of 32 sequences blasted in BOLD received a 100% match with existing sequences on BOLD, whilst there was a small divergence of just one basepair in four other sequences and a smaller match (98.83%) for one sequence (Table III). These diverging sequences belong to *Clytie illunaris*, *Eupithecia semigraphata* and *Watsonalla* cfr. *cultraria* which resulted to be very interesting species genetically.

Clytie illunaris (Erebidae) shows at least three different haplotypes in the Mediterranean. One haplotype occurs in France and Spain, one in Israel, and two belonging to the same genetic lineage in southern Italy. Our barcoded larva just differed by one basepair from an existing Calabrian barcode on BOLD (collection specimen) and thus confirmed the presence of the diverging lineage in Calabria.

Eupithecia semigraphata (Geometridae) exhibits a high genetic diversity in its European range, especially in southern Italy where it shows at least 13 haplotypes grouping to three major lineages at larger distances. Some haplotypes seem to be restricted to Sicily and Calabria. At one of our collecting sites we found three haplotypes (BC ZSM Lep 86015/86017/86019) belonging to two different *E. semigraphata*-BINs (BOLD:AAB6662/ACS6376) at comparatively large distances of 1.1-1.9%. These syntopic haplotypes were found on the same *Calamintha nepeta* plant, suggesting that different females laid their eggs on a single plant. It might be worth testing, however, whether polymorphisms in mitochondrial genes can lead to polymorphic descendants of one single female in this species. Vulnerable plants attracting egg-laying females might be a more likely explanation than the occurrence of three mitochondrial lineages in a single egg-laying female that reached fixation in its offspring.

Perhaps the most interesting case taxonomically concerns *Watsonalla* cfr. *cultraria* (Drepanidae). The neighbor-joining tree generated from BOLD database shows specimens morphologically identified as *W. cultraria* placed within two different clusters. One includes specimens from the Netherlands to Macedonia, and includes the Basilicata region of southern Italy, the other (at a distance of 1.1%) includes *Watsonalla* specimens identified either as *W. uncinula* (Spain; perhaps misidentified) or *W. cultraria* (Tunisia and Calabria region). Apart from these two clusters there is a third cluster including *W. uncinula* from southern Italy and France, at distances of 1.1-1.9% from the first two and making paraphyletic the two *W. cultraria* clusters in some but not all tree constructions. We can hypothesize the existence of a third taxon in Spain, North Africa and southernmost peninsular Italy, but this question deserves further study.

Table III.– List of larvae submitted to DNA barcoding, in alphabetic order of the species name. For each sample the genetic similarity with conspecific sequences on BOLD is given, along with sample ID, fragment length, GenBank Accession number, and BIN.

Species	Family	Similarity	Sample ID	Fragment Length	Gen Bank	BIN
<i>Agriopsis leucophaearia</i>	Geometridae	100%	BC ZSM Lep 84839	658 bp	KU379758	BOLD: AAC5604
<i>Amata sp.</i>	Erebidae	-	BC ZSM Lep 84830	0	-	-
<i>Autographa gamma</i>	Noctuidae	100%	BC ZSM Lep 86014	658 bp	KU379764	BOLD: AAB4345
<i>Autographa gamma</i>	Noctuidae	100%	BC ZSM Lep 84848	658 bp	KU379771	BOLD: AAB4345
<i>Biston betularia</i>	Geometridae	100%	BC ZSM Lep 73513	658 bp	KU379757	BOLD: ABY9367
<i>Cabera pusaria</i>	Geometridae	100%	BC ZSM Lep 73515	649 bp	KU379783	BOLD: AAA9589
<i>Clytie illunaris</i>	Erebidae	99,85%	BC ZSM Lep 86016	658 bp	KU379777	BOLD: AAK5589
<i>Cyclophora ruficiliaria</i>	Geometridae	100%	BC ZSM Lep 84846	608 bp	KU379753	BOLD: ABX4957
<i>Cyclophora suppunctaria</i>	Geometridae	100%	BC ZSM Lep 80849	658 bp	KU379762	-
<i>Dasychira pudibunda</i>	Erebidae	-	BC ZSM Lep 80851	0	-	-
<i>Ditula angustiorana</i>	Tortricidae	100%	BC ZSM Lep 84831	658 bp	KU379774	BOLD: AAA8699
<i>Ematurga atomaria</i>	Geometridae	100%	BC ZSM Lep 84843	658 bp	KU379755	BOLD: AAC1877
<i>Eupithecia semigraphata</i>	Geometridae	99,85%	BC ZSM Lep 86015	658 bp	KU379756	BOLD: AAB6662
<i>Eupithecia semigraphata</i>	Geometridae	98,83%	BC ZSM Lep 86017	658 bp	KU379759	BOLD: AAB6662
<i>Eupithecia semigraphata</i>	Geometridae	99,85%	BC ZSM Lep 86019	657 bp	KU379782	BOLD: AAB6662
<i>Eupithecia semigraphata</i>	Geometridae	-	BC ZSM Lep 86018	0	-	-
<i>Hydriomena sanfilensis</i>	Geometridae	100%	BC ZSM Lep 73516	658 bp	KU379772	BOLD: AAB8306
<i>Hypomecis punctinalis</i>	Geometridae	100%	BC ZSM Lep 84845	658 bp	KU379765	BOLD: AAB1059
<i>Lycia hirtaria</i>	Geometridae	100%	BC ZSM Lep 84829	658 bp	KU379763	BOLD: ACF3346
<i>Lymantriinae</i>	Erebidae	-	BC ZSM Lep 80846	0	-	-
<i>Morphospecies_1</i>	Lepidoptera	-	BC ZSM Lep 84834	0	-	-
<i>Morphospecies_2</i>	Lepidoptera	-	BC ZSM Lep 84836	0	-	-
<i>Morphospecies_3</i>	Lepidoptera	-	BC ZSM Lep 84837	0	-	-
<i>Morphospecies_4</i>	Lepidoptera	-	BC ZSM Lep 84838	0	-	-
<i>Morphospecies_5</i>	Geometridae	-	BC ZSM Lep 84844	0	-	-
<i>Morphospecies_6</i>	Lepidoptera	-	BC ZSM Lep 86013	0	-	-
<i>Morphospecies_7</i>	Lepidoptera	-	BC ZSM Lep 80848	0	-	-
<i>Orgyia antiqua</i>	Erebidae	100%	BC ZSM Lep 84841	658 bp	KU379781	BOLD: AAA6432
<i>Opisthograptis luteolata</i>	Geometridae	100%	BC ZSM Lep 73514	658 bp	KU379754	BOLD: AAA9865
<i>Orthosia cerasi</i>	Noctuidae	-	BC ZSM Lep 84832	0	-	-
<i>Orthosia cerasi</i>	Noctuidae	100%	BC ZSM Lep 84835	658 bp	KU379767	BOLD: ACL8047
<i>Orthosia gothica</i>	Noctuidae	100%	BC ZSM Lep 84840	658 bp	KU379760	BOLD: AAB6211
<i>Orthosia miniosa</i>	Noctuidae	100%	BC ZSM Lep 84828	658 bp	KU379776	BOLD: AAE5344
<i>Orthosia miniosa</i>	Noctuidae	100%	BC ZSM Lep 84833	658 bp	KU379775	BOLD: AAE5344
<i>Pachycnemia hippocastanaria</i>	Geometridae	100%	BC ZSM Lep 84842	658 bp	KU379770	BOLD: AAB9094
<i>Phaetogramma etruscaria</i>	Geometridae	100%	BC ZSM Lep 80845	658 bp	KU379778	BOLD: ABY4065
<i>Phalera bucephala</i>	Notodontidae	-	BC ZSM Lep 80850	0	-	-
<i>Pyrrhia umbra</i>	Noctuidae	100%	BC ZSM Lep 86012	658 bp	KU379766	BOLD: AAC7527
<i>Satyrium ilicis</i>	Lycanidae	100%	BC ZSM Lep 84827	658 bp	KU379779	BOLD: AAB6628
<i>Stangeia siceliota</i>	Pterophoridae	100%	BC ZSM Lep 80844	658 bp	KU379761	BOLD: AAV8864
<i>Stegania trimaculata</i>	Geometridae	100%	BC ZSM Lep 80842	658 bp	KU379768	BOLD: AAC2483
<i>Stegania trimaculata</i>	Geometridae	100%	BC ZSM Lep 80843	658 bp	KU379773	BOLD: AAC2483
<i>Uresiphita gilvata</i>	Crambidae	100%	BC ZSM Lep 84847	658 bp	KU379767	BOLD: ACF5204
<i>Watsonalla cfr. cultraria</i>	Drepanidae	99,82%	BC ZSM Lep 80847	572 bp	KU379780	BOLD: AAE9886

Conclusions

The method used in this paper allows for the collection of many data in a few hours of field and laboratory work. The identification success was low (67.5% in this paper) compared to other studies (77.0% in GOSSNER & HAUSMANN, 2009; 97.3% in MILLER *et al.*, 2013), but higher than the rates usually obtained by the time-consuming rearing of larvae to adults in captivity. The identification success can be increased considerably by storing the larvae in larger quantities of absolute alcohol adequate to their dimensions in order to avoid an excessive dilution.

Former critics of identification by DNA barcoding argued that species identification is hampered by missing “barcode gaps” between interspecific COI distances and intraspecific variation (WIEMERS & FIEDLER, 2007). Many recent studies (HAJIBABAEI *et al.*, 2006; DINCA *et al.*, 2011; HAUSMANN *et al.*, 2011; MUTANEN *et al.*, 2013; HUEMER *et al.*, 2014) showed, however, that small or missing barcode gaps are very rare (and well known) in European Lepidoptera, revealing DNA barcoding as a promising and reliable method for species identification of preimaginal stages. A major publication listing and analyzing cases of non-monophyly (barcode-sharing and barcode-splits) in European Lepidoptera is close to publication. Comprehensive DNA reference libraries of Lepidoptera are now available for a few countries of Central and Northern Europe (HAUSMANN *et al.*, 2011; HUEMER *et al.*, 2014). At a European level approximately 55% of the lepidopteran species have DNA barcodes at present (unpublished from own analysis of data in BOLD database). Although several species are widespread in Europe, Mediterranean countries host a peculiar fauna and DNA reference libraries for Mediterranean Lepidoptera need to be further improved.

Our approach of DNA-identification of caterpillars collected directly from their foodplants could help to fill many gaps in the knowledge of host-plant relationships of Mediterranean Lepidoptera. Furthermore we will be able to update (verify/reject) old data concerning common and widespread species which, in literature, often were transferred from other European areas without explicitly stating this. We propose to start a large-scale project on larval host-plants of Mediterranean Lepidoptera using our approach. For that purpose we encourage all interested entomologists to join our team.

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