# Improving the knowledge on Romanian Rhopalocera, including the rediscovery of *Polyommatus amandus* (Schneider, 1792) (Lycaenidae) and an application of DNA-based identification

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**Abstract.** From May 19th to June 8th of 2007, the authors undertook lepidopterological research in 29 localities in Romania, several of which are poorly or totally unstudied from a lepidopterological point of view. 105 taxa were identified, out of which seven receive special attention in the text, with comments on their distribution, ecology and conservation status. *Polyommatus amandus* (Schneider, 1792) (Lycaenidae) is recorded for the first time in Romania after 28 years. We provide the first record from Muntenia for *Cupido* (*Everes*) *decolorata* (Staudinger, 1886) (Lycaenidae), and the third known Romanian location for *Pseudophilotes bavius egea* (Herrich-Schäffer, 1852) (Lycaenidae). Lepidoptera DNA-based identification was used for species identification based on larval stages. This technique confirmed the discovery of *Zerynthia polyxena* ([Denis & Schiffermüller], 1775) (Papilionidae) in southern Dobrogea and its rediscovery in the entire province after 80 years.

## Introduction

Although the Romanian butterfly fauna has been studied for more than 150 years (e.g. Fuss 1850; Franzenau 1852, 1856, 1859; Mann 1866), there are still large gaps regarding the distribution and conservation status of many of the species known to occur within the country's territory. The newest version of the Catalogue of the Romanian Lepidoptera (Rákosy et al. 2003) reflects the knowledge for Romania's main historical regions, which turn out to be very unequally studied, with the greatest gaps in the southern parts of the country. Moreover, a considerable amount of faunistical data was published decades ago and for many specific locations there is no new data available on Lepidoptera, making an objective assessment of Romania's species distribution and conservation status very difficult. This is of particular importance now, as the country joined the European Union and conservation-oriented actions are more accessible (but also more necessary) than ever.

The main goals of the present study are to improve the knowledge regarding the Romanian butterfly fauna and to point out the natural capital of several of the country's areas, which are little known to European biologists. Special attention is given to seven taxa considered of particular importance, including original distribution data and aspects related to their conservation status. In addition, Lepidoptera DNA-based identification is used as a means of precise species identification during larval development.

**Abbreviations**

| | |
|---|---|
| bp | base pairs |
| CI | consistency Index |
| COI | cytochrome oxidase subunit I |
| COII | cytochrome oxidase subunit II |
| DNA | deoxyribonucleic acid |
| dNTP | deoxynucleoside triphosphate |
| HPLC | high performance liquid chromatography |
| MP | maximum parsimony |
| PCR | polymerase chain reaction |
| RI | retention Index |
| RV | Roger Vila |
| TBR | tree bisection reconnection |
| TL | tree length |
| tRNA-leu | leucine-transfer ribonucleic acid |
| VD | Vlad Dincă |

## Material and methods

**Collecting.** The field research was undertaken in the interval May 19th – June 8th of 2007 and covered various regions of Romania (Fig. 1). Most of the collecting was made using insect nets, but in several cases we also looked for preimaginal stages. When considered necessary, we preserved material for DNA studies, in which case the insect body was kept in 100% ethanol vials, and the wings inside glassine envelopes as reference. All samples are stored in RV lab's DNA and Tissues Collection at Universitat Autònoma de Barcelona.

Several specimens of *Carcharodus*, *Pyrgus*, *Pieris, Leptidea* and *Melitaea* where identified through genitalia examination. The systematics used follows that of the Catalogue of the Romanian Lepidoptera (Rákosy et al. 2003), with the exception of the misspelled genus name *Plebeius* Kluk, 1780 that we replaced with the correct *Plebejus* according to Bálint et al. (2001).

**Specimen sequencing.** Total genomic DNA was extracted using Chelex 100 resin, 100-200 mesh, sodium form (Biorad), following the protocol of Walsh, Metzger and Higuchi (1991). The following samples were extracted: *Zerynthia polyxena* ([Denis & Schiffermüller], 1775), Romania, Constanţa County, Canaraua Fetei, 22.v.2007, RVcoll.07-D023 (collected as last instar larva); *Z. polyxena*, Romania, Buzău County, Dănciuleşti, 20.v.2007, RVcoll.07-D396 (collected as last instar larva); *Allancastria cerisyi ferdinandi* Stichel, 1907, Romania, Constanţa County, Canaraua Fetei, 23.v.2007, RVcoll.07-D031 (collected as adult).

A 650 bp fragment at the 5' end of the mitochondrial gene cytocrome oxidase subunit I (COI) was amplified by polymerase chain reaction using the primers LCO 1490 (5' -GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al. 1994) and Nancy (5' -CCCGGTAAAATTAAAATATAAACTTC-3') (Simon et al. 1994). Double-stranded DNA was amplified in 25 µl volume reactions: 17.65 µl ultra pure (HPLC quality) water, 2.5 µl 10X buffer, 1 µl 100mM $MgCl_2$, 0.25 µl 100 mM dNTP, 1.2 µl of each primer (10 mM) and 0.2 µl Taq DNA Polymerase (Bioron, GmbH). The typical thermal

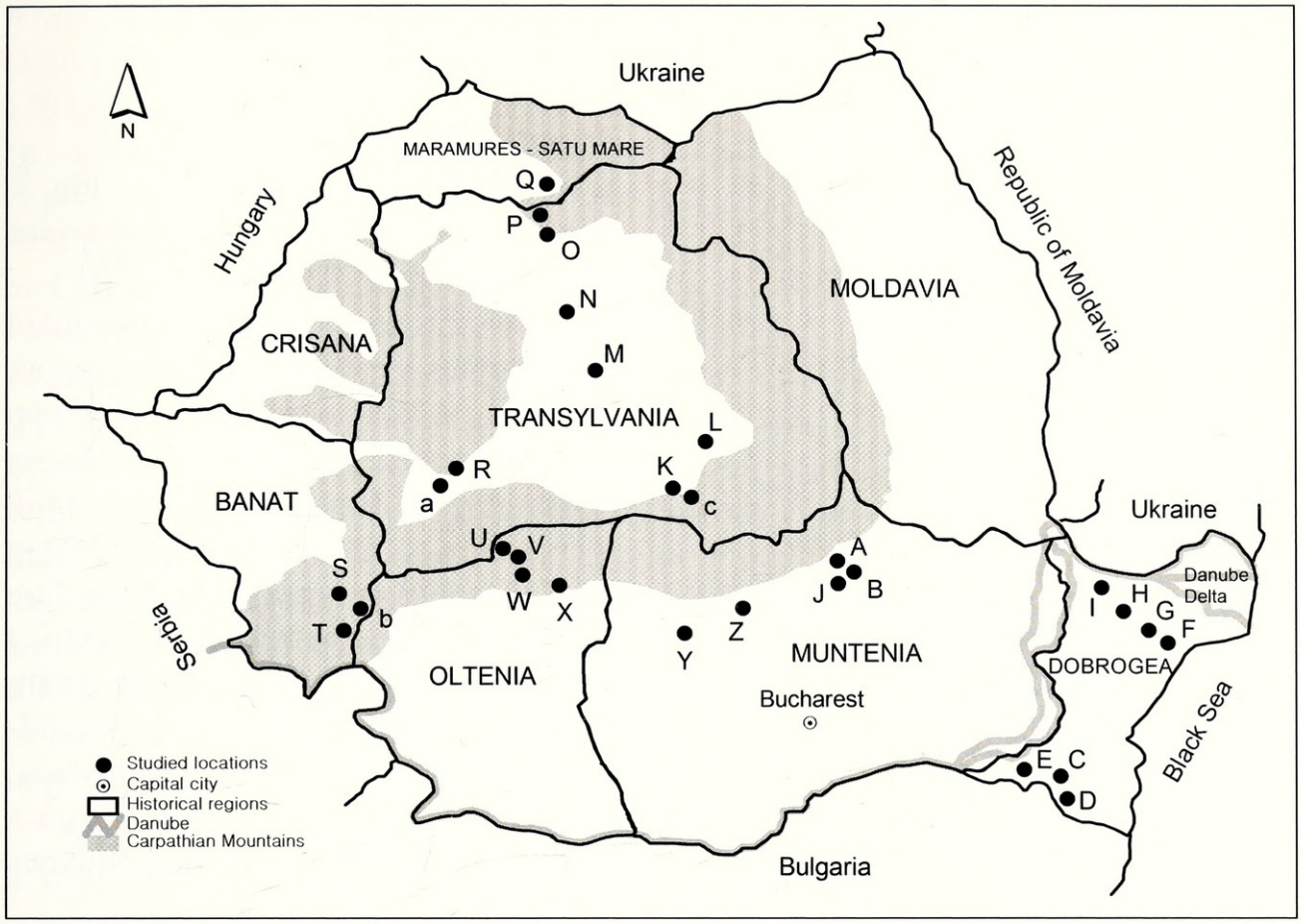


**Fig. 1**. Studied locations from May 19th to June 8th of 2007. Letters for each locality refer to those in Appendix 1.

cycling profile, carried in a PTC-100 Peltier Thermal Cycler, was 95 °C for 60 seconds, 44 °C for 60 seconds and 72 °C for 90 seconds, for 35 cycles. PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea).

**Sequence analysis.** The sequences were edited and aligned using Sequencher 4.2 (Genecodes Corporation, Ann Arbor, MI) and MacClade 4.05 (Maddison & Maddison 1992). The fragments were of equal length and alignments unambiguous. Primer sequences were cropped and missing data and ambiguities were designated by the letter "N". All sequences were submitted to GenBank (codes EU667423 to EU667425).

Available GenBank COI sequences overlapping with the fragment sequenced by us and belonging to *Zerynthia* (DQ351039, AF170870), *Allancastria* (AF170869, DQ351040 to DQ351043) and *Buthanitis* (DQ351036 to DQ351038) were included in our analysis. For details regarding these samples, refer to Nazari et al. (2007). The three *Buthanitis* taxa were selected as outgroup and as root for our analysis, based on the results of Nazari et al. (2007). The phylogenetic analyses of the DNA data was conducted in PAUP* 4.0b10 (Swofford 2001) under the maximum parsimony (MP) criterion. Heuristic searches were conducted for MP analysis with all characters equally weighted and under the TBR swapping algorithm with 10000 random addition sequences. The parsimony hypothesis was evaluated by bootstrap analysis (Felsenstein 1985) with 1000 pseudo replicates under the same parameters as the initial parsimony searches, except for using 1000 random addition sequences.

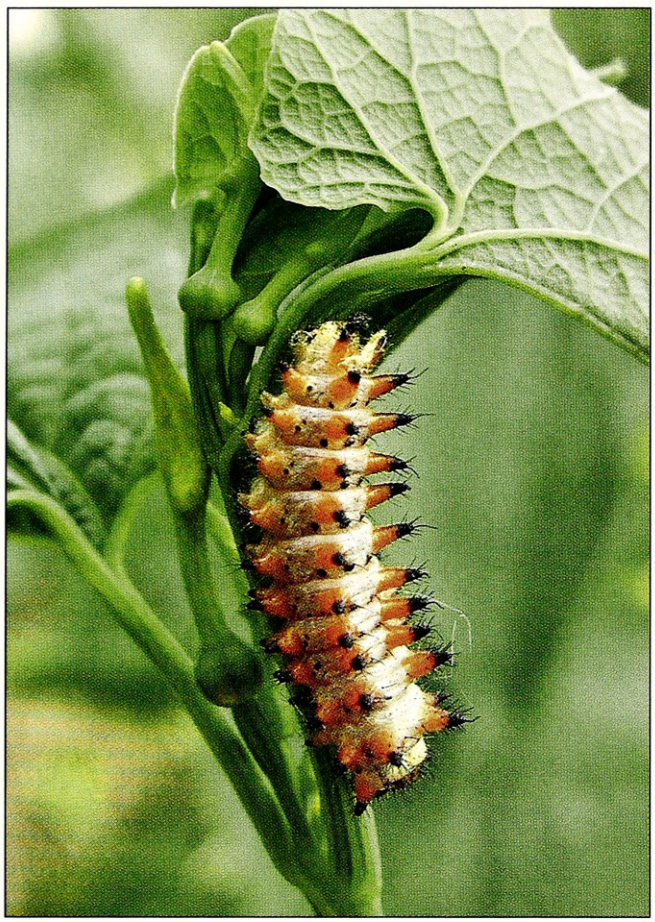

**Fig. 2**. Last instar larva of *Z. polyxena* on *Aristolochia clematitis* at Canaraua Fetei, 22.v.2007. Photo V. Dincă.

## Results and discussion

### *Zerynthia polyxena* ([Denis & Schiffermüller], 1775) Fig. 2

Material. 5 terminal instar larvae, **Romania**, Constanţa county, Canaraua Fetei, 22.v.2007.

Although this species has been recorded from various localities distributed across Romania (Rákosy et al. 2003), only two very old records exist from Dobrogea (south-eastern Romania) (Mann 1866 –surroundings of Tulcea– and Fiebig 1927 –locality not specified). Therefore it was considered to be possibly extinct in this region (Rákosy & Wieser 2000). Moreover, *Z. polyxena* was never recorded from the southern part of the province, which is relatively well studied from a lepidopterological point of view (Rákosy & Székely 1996).

During the spring of 2007, we managed to find several larvae in southern Dobrogea (Canaraua Fetei) (Fig. 2), which we tentatively attributed to *Z. polyxena*. The larvae were found feeding on *Aristolochia clematitis* L. (Aristolochiaceae) (which is well represented in the area) and were collected in order to be reared. A few days after collecting, one larva manifested signs of disease and therefore it was preserved in 100% ethanol. The others reached the stage of prepupa or pupa without problems and had to be transported by plane from Romania to the laboratory in Spain where the rearing process was to be continued. During the flight, the luggage containing the larvae was severely damaged and the recipient with the larvae was lost.

The area of Canaraua Fetei is well known for being one of the few locations in Romania where *A. cerisyi ferdinandi* flies (Rákosy & Székely 1996), a species with quite variable larvae, sometimes fairly similar to *Z. polyxena*, which also uses *A. clematitis* as larval food plant. Furthermore, the lack of adult specimens might cast doubt on the record, especially in the eyes of some Romanian lepidopterists for whom the famous area of Canaraua Fetei is a "classical" place for *A. cerisyi*, but certainly not one for *Z. polyxena*. As a matter of fact, doubts on the veracity of our record were expressed by some colleagues who bred larvae of *A. cerisyi* from Canaraua Fetei and noticed that some are similar to those of *Z. polyxena*. Therefore, in order to confirm our record, we decided to use DNA-based methods as a tool for precise species identification. This was possible because of the larva preserved in ethanol (RVcoll.07-D023), for which a 650 bp COI mitochondrial fragment was sequenced and compared to those of a sympatric *A. cerisyi*

*ferdinandi* (RVcoll.07-D031) and a Romanian specimen of *Z. polyxena* (RVcoll.07-D396) from one of the closest known populations (Buzău county, Dănciuleşti – about 150 km far). The putative *Z. polyxena* sample from Dobrogea presented an uncorrected "p" distance of only 0,15% (1 base-pair difference in a 650 bp fragment) compared to *Z. polyxena* from Dănciuleşti. On the other hand, the distance with the sympatric *A. cerisyi ferdinandi* was 6,92% (45 differences in 650 bp). Since a phylogenetic tree based on COI sequences that includes all the species of *Allancastria* and *Zerynthia* from the Palaearctic region exists in literature (Nazari et al. 2007), we included our sequences in the COI dataset and reanalysed it with Maximum Parsimony. The resulting tree (Fig. 3) has the same topology as that of Nazari et al. (2007). The Romanian *A. cerisyi ferdinandi* sample pairs with the other representative of the same species with good support. Both the Romanian *Z. polyxena* and the enigmatic Rvcoll.07-D023 sample form a strongly supported clade with *Z. polyxena* from Russia. These results confirm the identification of the larva from Canaraua Fetei as *Z. polyxena* and demonstrate the usefulness of our DNA-based approach within this group of taxa.

This result represents the first record of *Z. polyxena* from southern Dobrogea, as well as its rediscovery in the entire province after 80 years. With the current data, it is virtually impossible to know whether the species has been surviving in south-western Dobrogea for a long time or if it has recently colonized the area. The possibility of a permanent population is in our opinion reasonable because:

- although old, previous records from Dobrogea do exist (Mann 1866, Fiebig 1927);
- its historical presence in Dobrogea would logically complete its distribution in Eastern Europe as it is mentioned as widespread from the Republic of Moldavia and most of Ukraine (both bordering Romania in northern Dobrogea) (Tshikolovets 2003), as well as from Bulgaria where the nearest records to southern Dobrogea are located less than 70 km away (Abadjiev 2001);
- the larval food plant is well represented in parts of southern Dobrogea;
- *Allancastria cerisyi* and *Z. polyxena* sometimes fly in the same locations without excluding each other (e.g. Belasitsa and Skakavitsa in Bulgaria) (Abadjiev 2001).

Canaraua Fetei is a protected area consisting mainly of xerophilous *Quercus* forest (*Q. cerris* L., *Q. pedunculiflora* Koch) and clearings, plus several karstic areas which shelter a characteristic flora and fauna. Many of these taxa are of great conservation significance at national and European levels.

*Zerynthia polyxena* is protected by law in Romania and is considered as endangered in the Romanian Red List for butterflies (Rákosy 2003). This adds a new taxon to the list of protected butterfly species known from Canaraua Fetei: *Pyrgus sidae* (Esper, 1784), *A. cerisyi ferdinandi*, *Euchloe ausonia* (Hübner, 1804), *Apatura metis* Freyer, 1829, *Lycaena dispar rutila* (Werneburg, 1864) and *Pseudophilotes bavius egea* (Herrich-Schäffer, 1852) (Rákosy & Székely 1996).

During the field trip, we recorded two new populations of *Z. polyxena* in Muntenia (Valea Mare – Dâmboviţa county, and Dănciuleşti – Buzău county) (Appendix 2), a

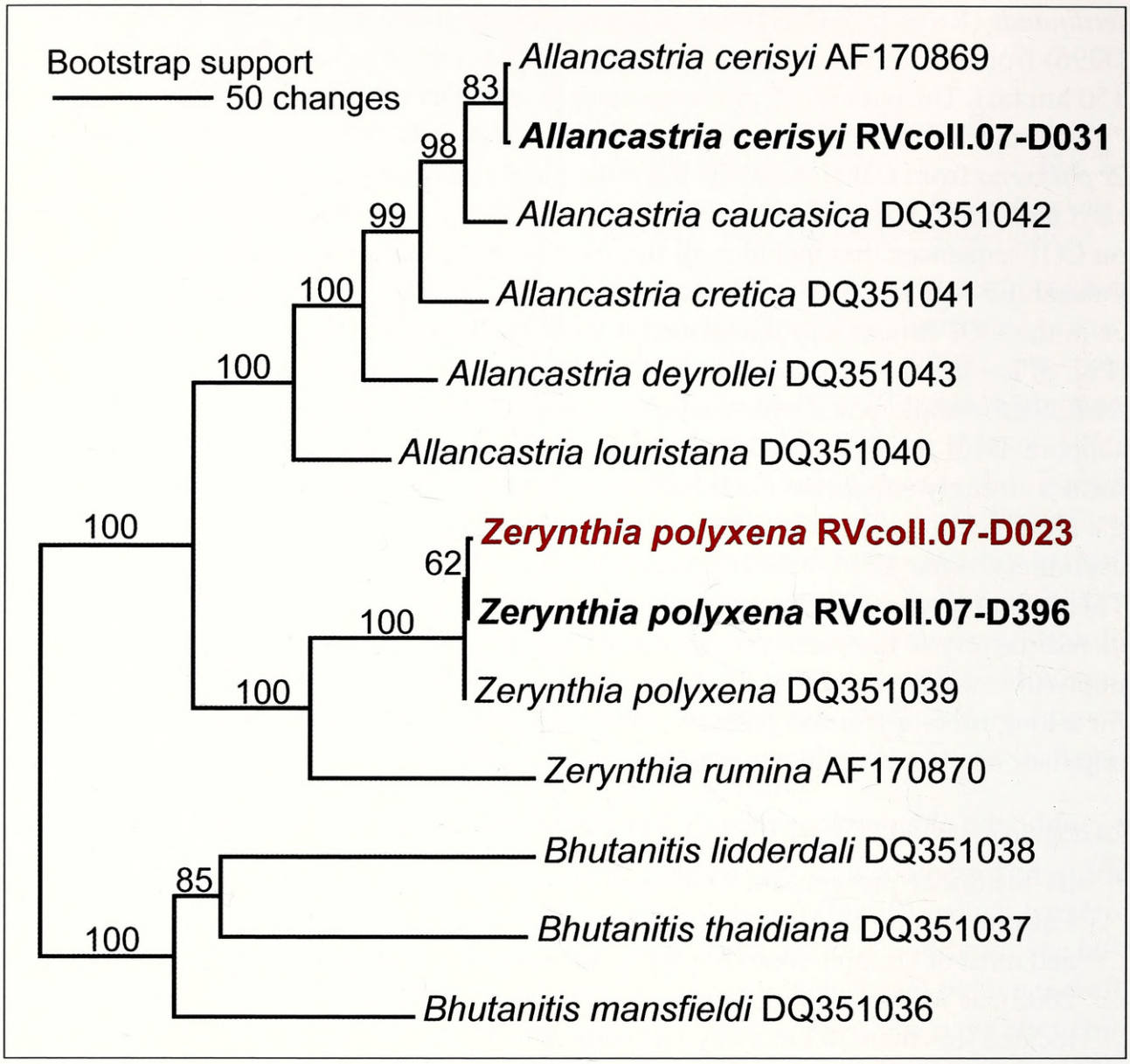


**Fig. 3**. Maximum parsimony (MP) tree of *Allancastria* and *Zerynthia*, with *Bhutanitis* as outgroup, inferred from 2306 bp of COI+tRNA-leu+COII (only 650 bp of COI for the Romanian samples). One best tree, TL = 685, CI = 0.746 and RI = 0.715. In red, the sample collected in southern Dobrogea; in bold, samples from Romania used as comparison. Bootstrap values are shown above recovered branches.

region from where the species has few and rather obsolete records (Niculescu 1961). These observations together with previous records from the Subcarpathian hills of Muntenia strongly suggest that *Z. polyxena* might be much more widespread than previously suspected in this vast region of the country. The population from Dănciuleşti seems to be particularly vigorous as a rather fast inspection of the many *Aristolochia* present there allowed us to observe dozens of larvae on just a few square meters.

The distribution of *Z. polyxena* is closely linked to the areas where *A. clematitis* occurs. This plant is fairly localized and it is often associated with neglected vineyards or abandoned agricultural fields which may be subject either to natural vegetation succession (habitat closure) or to other economical uses (rehabilitation, construction, etc.). For example, at Dănciuleşti the plant is abundant, but restricted to a recently abandoned

agricultural field that is probably private property and might at any time be completely transformed for various purposes. At Călan and Valea Mare the plants occur just next to the main road (1–2 meters away) next to small ditches, while at Valea Călugărească the adults were observed flying among the railways. All these areas experience high antropic pressures. On the other hand, the plant is generally considered as a weed and therefore people try to eliminate it. As a matter of fact, *Z. polyxena* has probably faced local extinctions (Székely 2005; Goia pers. comm.). Therefore, the status of protected species should not remain only a formality but should be effectively applied in order to preserve at least some of the most vigorous populations known to occur in the country.

***Cupido (Everes) decolorata* (Staudinger, 1886)**

Material. 1♂, **Romania**, Buzău county, Dănciuleşti, 20.v.2007.

While it has been recorded from several localities from the northern parts of the country, usually as rare and local (Rákosy & al. 2003; Dincă pers. obs.), *C. decolorata* is very poorly known from most of the southern regions of Romania, being recorded only from the extreme south of Dobrogea where it seems to be very scarce (Rákosy & Székely 1996). A few females were collected on Istriţa Hill (Buzău county), but their habitus didn't allow for a clear separation between *C. decolorata* and *C. alcetas* (Dincă 2006). In May 2007, we collected a male of this taxon in the Subcarpathian hills of Muntenia, very near to Istriţa Hill. This represents the first certain record from Muntenia.
Although not protected by law, *C. decolorata* is listed in the Red List of Romanian Butterflies (Rákosy 2003) as a vulnerable taxon. This species is probably threatened by various factors generally affecting the places where it occurs: overgrazing, overgrowing of open areas by bushes or trees, land burning. Yet, as is the case in other countries (Beneš et al. 2002), the information available on this species is very scarce, and additional data are needed in order to allow a finer assessment of its conservation status in Romania.

***Pseudophilotes bavius egea* (Herrich-Schäffer, 1852)**

Material. 1♂, **Romania**, Constanţa county, Dumbrăveni forest, 21.v.2007.

On May 21th of 2007, we visited the area of Dumbrăveni forest (Constanţa county, southern Dobrogea). This is a protected area that, although fairly similar to other well known "butterfly hot spots" from southern Dobrogea (Canaraua Fetei, parts of Hagieni forest), has been relatively ignored by lepidopterists. In addition to other localized or protected taxa such as *Apaustis rupicola* (Denis & Schiffermüller, 1775), *Carcharodus orientalis* Reverdin, 1913, *Spialia orbifer* (Hübner, 1823), *Parnassius mnemosyne* (Linnaeus, 1758), *Lycaena dispar rutila* and *L. thersamon* (Esper, 1784), we collected a specimen of *P. bavius egea*.
This taxon was recorded for the first time in southern Dobrogea by Székely (1994) who collected the species at Canaraua Fetei. Two years later Rákosy & Székely (1996)

added another locality for this taxon in southern Dobrogea (Şipote). Therefore, our citation represents the third locality for this taxon in Romania. The populations occurring in southern Dobrogea were considered to belong to ssp. *egea*, previously known only from the Asian part of Turkey (Rákosy & Székely 1996; Jutzeler et al. 1997).
In the Red List of Romanian Rhopalocera (Rákosy 2003), *P. bavius egea* is considered endangered, and it is protected in the country. Although it is possible that it is present at other localities from the same area, it is clearly a very local taxon associated to unaltered steppic areas which in Dobrogea are highly threatened by grazing and agriculture. Further studies are urgently needed in order to improve the knowledge on its distribution in Romania and to allow the identification of the best areas that should be correctly managed in order to assure its survival.

***Polyommatus amandus* (Schneider, 1792)** **Fig. 4**

Material. 1♂, **Romania**, Tulcea county, Babadag forest, 24.v.2007.

On May 24 and 25 2007, we made day and night time lepidopterological collecting in the area of Babadag forest (Tulcea county, northern Dobrogea). Among several species that are considered to be rare and/or considerably local in Romania such as *Pyrgus sidae*, *Spialia orbifer*, *Carcharodus orientalis*, etc., we collected one very fresh specimen of *P. amandus* (Fig. 4).
Taking into consideration the literature data, this is the first record of *P. amandus* in Dobrogea in the last 78 years (Caradja 1929) and the first in Romania after a gap of almost three decades (Bálint 1980; Székely 1996, 2005). The specimen was collected in a forest clearing with shrubs and relatively high (apparently lightly grazed) vegetation.

**Fig. 4**. *Polyommatus amandus*, Babadag forest (Tulcea county), 24.v.2007. Photo V. Dincă.

It is interesting that the first record of *P. amandus* from Dobrogea comes from the surroundings of Ciucurova (Mann 1866), an area situated about 20 km west of Babadag. In fact, analyzing the vegetation map of the region, it becomes obvious that the two localities are linked by habitats that are similar to the ones from Babadag.
*Polyommatus amandus* is one of the least known species in Romania. The overall distribution of this species covers relatively uniformly the country's territory, but usually with large gaps among localities (Fig. 5) and with very few records per site. Interestingly, the species is reported as widespread in several countries bordering Romania such as Ukraine, Republic of Molda-

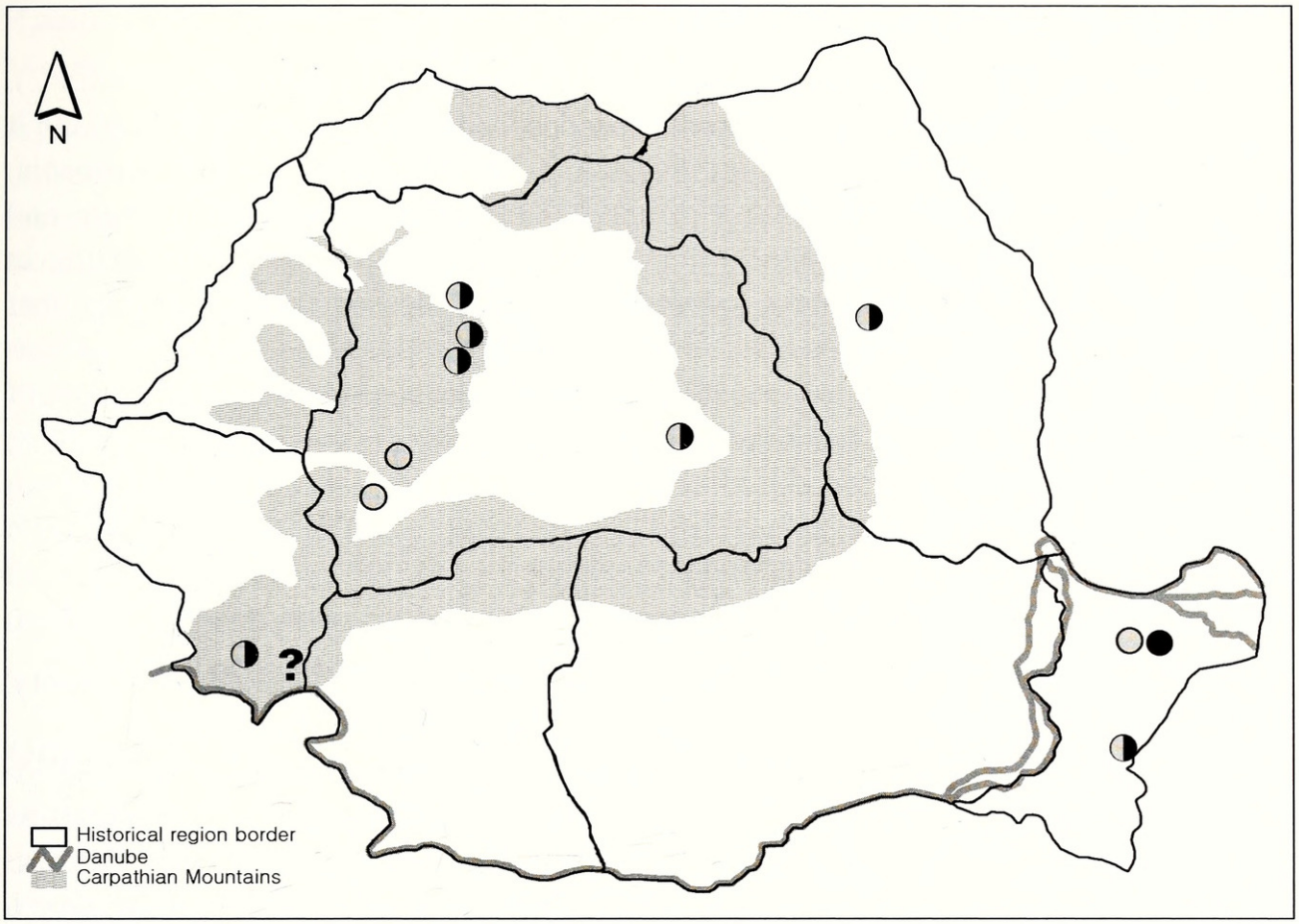


**Fig. 5**. Records of *P. amandus* from Romania. Grey dots = collected before 1900 (Fuss 1850, Mann 1866, Czekelius 1898); Black-and-grey dots = collected between 1900-1980 (Caradja 1929, Popescu-Gorj 1964, König 1975, Nadolschi & Şugăr 1975, Bálint 1980, Rákosy 1988, Burnaz 1993, Stănescu 1995, Székely 1996, Rákosy 2002); Black dot = collected after 1980 (this paper); Question mark = doubtful record (Rebel 1911).

via (Tshikolovets 2003), and Bulgaria (Abadjiev 2001). Moreover, some of the populations previously reported in the literature seem to have gone extinct due to unknown causes (Székely 2005, Goia & Dincă 2008). One of the most suggestive examples is represented by the population from Fânaţele Clujului (Cluj-Napoca, Transylvania), where the species was fairly common more than seven decades ago. The species seems to have totally disappeared from the area, although it was flying inside a protected area that has suffered little antropic impact and still today is very rich in butterflies, sheltering almost 100 species on a few hectares (Goia & Dincă 2008).

In the Red List of Romanian Rhopalocera (Rákosy 2003), *P. amandus* is listed as an endangered species, with populations ranging from data deficient to endangered at a regional scale. Further research is strongly needed in order to clarify its distribution, to assess its habitat preferences, and to find explanations for its rarity and decline. As the species is protected by law in Romania and Babadag forest is a protected area, there is a good justification for directed studies that should aim at studying and safeguarding this probable population.

***Lycaena dispar rutila* (Werneburg, 1864)**

During less than three weeks, we observed this taxon at 13 localities (Appendix 2). *Lycaena dispar rutila* has numerous literature citations from all over the country and it is probably the most widespread butterfly present in the Habitats Directive in Romania. However, large populations surviving in optimal habitats are considerably rare and many of the citations for this taxon refer to rather modest populations occurring in small areas. It may often be found in humid spots such as tiny flooded patches near streams, small humid depressions and ditches. Due to the fragility of many of its current habitats, *L. dispar rutila* is correctly considered as vulnerable in the Red List of Romanian Rhopalocera (Rákosy 2003).

***Nymphalis xanthomelas* (Esper, 1781)**

M a t e r i a l . 1♂, **Romania**, Braşov county, Dumbrava Vadului, 6.vi.2007 (ex. larva).

We found this species in the botanical reserve of Dumbrava Vadului (Braşov county, Transylvania). Another larva was observed pupating in the same place.

This species has a poorly understood distribution in Romania, where it is considered as critically endangered (Rákosy 2003). Although it prefers the same habitats as *N. polychloros*, it seems to be rarer and more local than the former. *Nymphalis xanthomelas* could have often remained unnoticed due to its similarity with *N. polychloros*. Nevertheless, other than local apparent regression (König 1972) or probable population fluctuations (Goia & Dincă 2008), another possible explanation for its rarity could be offered by its occasional migratory behaviour (Tolman & Lewington 1997; Beneš et al. 2002) and by its presence close to the western range limit. Given the precarious state of knowledge regarding this taxon, we would consider it as data deficient rather than critically endangered.

A considerable number of taxa of national or European interest were previously recorded from the botanical reserve of Dumbrava Vadului (IUCN category IV) (Székely & al. 2000): *Lycaena helle* (Denis & Schiffermüller, 1775), *L. dispar rutila*, *Maculinea teleius* (Bergsträsser, 1779), *M. alcon* ([Denis & Schiffermüller], 1775), *Euphydryas aurinia* (Rottemburg, 1775), and *Argynnis laodice* (Pallas, 1771). According to the previously published data (Székely & al. 2000) and to our personal observations, the *L. helle* population in this area is probably the largest known in Romania. For these reasons, we reaffirm the proposal made by Székely & al. (2000) that the reserve of Dumbrava Vadului should extend its status to a botanical and zoological one, which would facilitate the implementation of an effective management plan that would ensure the survival of the protected species mentioned above. The main disturbing factor that could severely affect the butterfly communities in the area is represented by natural vegetation successions leading to habitat closure by shrubs and trees.

### *Apatura metis* Freyer, 1829

Material. 1♂, **Romania**, Dobrogea, Constanţa County, Canaraua Fetei, 22.v.2007.

Although it has been previously recorded form the area of Canaraua Fetei (Rákosy & Székely 1996), the species was considered univoltine in southern Dobrogea, being recorded exclusively between June 25th and July 14th. Although definitive conclusions cannot be drawn based on a single specimen, the collecting date suggests that the species has probably two broods in southern Dobrogea, similarly to the populations along the Danube and in the Danube Delta.

The species is protected and listed as vulnerable at the national scale in the Red List of Romanian Rhopalocera (Rákosy 2003). Although mainly restricted to areas along the Danube, the species should be safe within the Danube Delta Biosphere Reserve. Nevertheless, some of the populations still occurring along the Danube's shores might face extinction due to the various types of human activities related to navigation and the improvement of the banks.

## Other taxa

During May 19-June 8 of 2007, we visited 29 Romanian locations and identified 105 butterfly taxa, meaning more than 55% of the country's fauna. Three species, namely *Pyrgus armoricanus* (Oberthür, 1910), *Thymelicus sylvestris* (Poda, 1761) and *Cupido minimus* (Fuessly, 1775) are recorded for the first time from Oltenia. These species are probably fairly widespread and abundant in this region; such a lack of data stands as a proof for the great necessity of Lepidoptera studies in many parts of southern Romania (especially Oltenia). This region, although generally not targeted by autochthonous lepidopterists, may shelter populations of local, rare or endangered species that need to be studied. For *Carcharodus orientalis*, currently known in Romania only from Dobrogea and the Danube Delta (Rákosy & Varga 2001), two new localities are reported from the same region (Babadag and Dumbrăveni forests). Both records are based on male genitalia examination, which is the only reliable method for distinguishing between these two taxa. The exact distributions of *C. orientalis* and *C. floccifera* (Zeller, 1847) in Romania still remain very poorly known due to their external similarity.

Besides the species we paid special attention to in this paper, it is worth mentioning several other taxa recorded on this trip (see appendix 2) that are fairly localized in Romania, and/or are of European interest: *Muschampia cribrellum* (Eversmann, 1841), *Pyrgus sidae*, *Allancastria cerisyi ferdinandi*, *Parnassius mnemosyne*, *Euchloe ausonia*, *Colias chrysotheme* (Esper, 1781), *Maculinea arion* (Linnaeus, 1758), *Plebejus sephirus* Frivaldsky, 1835, *Brenthis ino* (Rottemburg, 1775), *Euphydryas aurinia* (Rottemburg, 1775), and *Kirinia roxelana* (Cramer, 1777). Most of these species can survive only in good quality habitats and stand as proof of the (still) high degree of

preservation of the biodiversity of many areas of Romania. Nevertheless, the future of the Romanian "biodiversity hotspots" is now on the edge as the country joined the European Union and has the possibility to make vital socio-economical choices that might have profound effects on its natural capital. An analysis of such potential effects on Lepidoptera is provided by Schmitt & Rákosy (2007) and for various taxa groups by Cremene et al. (2005) and Baur et al. (2006).

## Conclusions

- Butterfly DNA-based identification was used in order to provide precise species identification based on larval stages. This technique proved to be excellent for the taxa studied. The case provides an example of how classical identification methods may be effectively combined with molecular techniques.
- Distributional data is improved for 105 butterfly taxa, given the fact that many regions from Romania are still poorly studied from a lepidopterological point of view and local information regarding many taxa is either obsolete or needs reconfirmation.
- *Polyommatus amandus* is recorded for the first time in Romania in the last 28 years and in Dobrogea after 78 years. The decline suffered by this species in Romania remains without a clear explanation.
- *Zerynthia polyxena* is recorded for the first time from southern Dobrogea. This record also represents the rediscovery of the species in the whole province after 80 years.
- A new locality for *Pseudophilotes bavius egea* in southern Dobrogea is reported. This is the third known locality for this taxon in Romania.
- Three species (*Pyrgus armoricanus, Thymelicus sylvestris, Cupido minimus*) are recorded for the first time from Oltenia and one (*Cupido decolorata*) from Muntenia.
- Based on the collection of one specimen of *Apatura metis* in southern Dobrogea in May, the existence of two broods in this region is considered to be probable.

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# Appendices

**Appendix 1**. Localities visited during May 19 – June 8 of 2007 (the symbols refer to the localities in Fig. 1 and Appendix 2).

| Date | Locality | County | Alt. (m) | Symbol |
|---|---|---|---|---|
| 19. v. 2007 | Ciolanu | Buzău | 255 | **A** |
| 20. v. 2007 | Dănciuleşti | Buzău | 360 | **B** |
| 21. v. 2007 | Şipote | Constanţa | 90 | **C** |
| 21. v. 2007 | Dumbrăveni forest | Constanţa | 80 | **D** |
| 22. v. 2007 | Canaraua Fetei, Băneasa | Constanţa | 15 | **E** |
| 23. v. 2007 | Canaraua Fetei, Băneasa | Constanţa | 15 | **E** |
| 24. v. 2007 | Enisala | Tulcea | 40 | **F** |
| 24. v. 2007 | Babadag forest | Tulcea | 115 | **G** |
| 25. v. 2007 | Babadag forest | Tulcea | 115 | **G** |
| 25. v. 2007 | Horia | Tulcea | 140 | **H** |
| 25. v. 2007 | Greci (Măcin mountains) | Tulcea | 90–200 | **I** |
| 26. v. 2007 | Breaza (Istriţa hill) | Buzău | 340–730 | **J** |
| 27. v. 2007 | Dumbrava Vadului (Vad) | Braşov | 495 | **K** |
| 27. v. 2007 | Perşani | Braşov | 500 | **c** |
| 28. v. 2007 | Dumbrava Vadului (Vad) | Braşov | 495 | **K** |
| 28. v. 2007 | Racoş | Braşov | 460 | **L** |
| 29. v. 2007 | Iernut | Mureş | 355 | **M** |
| 30. v. 2007 | Suatu | Cluj | 380 | **N** |
| 01. vi. 2007 | Câţcău | Cluj | 255 | **O** |
| 01. vi. 2007 | Cheile Babei | Maramureş | 265 | **P** |
| 03. vi. 2007 | Rohia village | Maramureş | 360 | **Q** |
| 04. vi. 2007 | Spini | Hunedoara | 240 | **R** |
| 04. vi. 2007 | Călan | Hunedoara | 230 | **a** |
| 05. vi. 2007 | Teregova | Caraş-Severin | 385 | **S** |
| 05. vi. 2007 | Pecinişca | Caraş-Severin | 220–300 | **T** |
| 06. vi. 2007 | Pecinişca | Caraş-Severin | 220–300 | **T** |
| 06. vi. 2007 | 8 km W of Godeanu | Caraş-Severin | 380 | **b** |
| 07. vi. 2007 | 11 km S of Rânca | Gorj | 940 | **U** |
| 07. vi. 2007 | Baia de Fier | Gorj | 640 | **V** |
| 07. vi. 2007 | 3 km W of Ciupercenii de Olteţ | Gorj | 500 | **W** |
| 07. vi. 2007 | Bârzeşti (Otăsău river valley) | Vâlcea | 515 | **X** |
| 08. vi. 2007 | Valea Mare | Dâmboviţa | 225 | **Y** |
| 08. vi. 2007 | 4 km W of I. L. Caragiale | Dâmboviţa | 270 | **Z** |

**Appendix 2**. Checklist of the species recorded during May 19th - June 8th 2007 (locality symbols correspond to the ones used in Appendix 1). In a few cases, species-level identification was not possible, either because the material was not collected or because the taxa involved lack reliable diagnostic morphological features. Notes: (1) The collected specimen is a female and is difficult to clearly assign it either to *C. alcetas* or *C. decolorata*; (2) *Maculinea alcon* sensu lato (including *rebeli*), based on several recent studies (e.g. Als et al. 2004); (3) *Melitaea phoebe* sensu lato, because of the difficult imago identification and data deficient Romanian distribution of *Melitaea punica telona* Oberthür, 1876 (Rákosy & Varga 2001); (4) Following Rákosy (1999), who considers that the taxon *H. volgensis delattini* is present in all Dobrogea, including Măcin Mountains.

| **Taxon/Locality symbols** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **J** | **K** | **L** | **M** | **N** | **O** | **P** | **Q** | **R** | **S** | **T** | **U** | **V** | **W** | **X** | **Y** | **Z** | **a** | **b** | **c** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Hesperiidae** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Erynnis tages* | | | | | | | x | | | x | x | x | | | | | | | | | | | | | | | | | |
| *Carcharodus alceae* | | | x | x | x | | | | | | | | | | | | | | | | | | | | | | | | |
| *Carcharodus floccifera* | | | | | | | | | | | x | | | | | | | | | | | | | | | | | | |
| *Carcharodus orientalis* | | | | x | | | x | | x | | | | | | | | | | | | | | | | | | | | |
| *Spialia orbifer* | | | | x | | | x | | | | | | | | | | | | | | | | | | | | | | |
| *Muschampia cribrellum* | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | |
| *Pyrgus carthami* | | | | | | | | | | | | | x | x | | | | | | | | | | | | | | | |
| *Pyrgs sidae* | | | x | | x | | x | | | | | | | x | | | | | | | | | | | | | | | |
| *Pyrgus malvae* | | | x | x | x | | | | x | x | x | | | | | | x | | | | | | | | | | | | |
| *Pyrgus armoricanus* | | | x | x | x | | x | | | | x | x | | | x | | x | | | | | | x | x | | | | | |
| *Carterocephalus palaemon* | | | | | | | | | | | x | | | | | | x | | x | | | | | | | | | | |
| *Thymelicus sylvestris* | | | | | | | | | | | | | | | | | | x | | | | | x | | | | | | |
| *Ochlodes venatus faunus* | | x | | x | | | | | | x | x | x | | | | | x | | x | | | | | | x | | | | |
| **Papilionidae** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Zerynthia polyxena* | | x | | | x | | | | | x | | | | | | | | | | | | | | | x | | x | | |
| *Allancastria cerisyi ferdinandi* | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | |
| *Parnassius mnemosyne wagneri* | | | | x | x | | | x | | | | | | | | | | | | | | | | | | | | | |
| *Iphiclides podalirius* | | | | | | | | | | x | | | | | | | | | | | | | | | | | | | |
| *Papilio machaon* | | | | | | | | | | | x | | | | | | | | | | | | | | x | x | | | |

**Appendix 2**. Continuation.

| Taxon/Locality symbols | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | a | b | c |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pieridae** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Leptidea sinapis* | x | | | x | | | | | | x | | | | | | | | | | x | | | x | | x | | | | |
| *Leptidea sinapis/reali* | | | | | | | | | | | | x | x | | x | x | x | | | | | | | x | | | | | |
| *Anthocharis cardamines* | | | | x | x | | | | | x | x | x | | | | | | | | | | | | x | | | | | |
| *Euchloe ausonia taurica* | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | |
| *Aporia crataegi* | | | | | | | | | | | | | | | x | | | | | | | | | | | | | | |
| *Pieris brassicae* | | | | | | | | | | | | x | | | x | | | | | x | | | | | | | | | |
| *Pieris mannii* | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | |
| *Pieris rapae* | | x | | x | x | | | | | x | x | x | x | x | | | | | x | x | x | | x | x | x | | | | |
| *Pieris napi/balcana* | x | | | | x | | | | x | x | x | x | x | x | | | x | x | x | x | | x | x | x | x | x | | | |
| *Pontia edusa* | | | | x | x | | | | | x | x | x | | x | | | | | | x | | | | x | x | x | | | |
| *Colias croceus* | | | x | x | x | | | | | | | x | | | | | | | x | x | | | | | | | | | |
| *Colias chrysotheme* | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | |
| *Colias hyale/alfacariensis* | | | x | | x | | | | | x | | x | | | x | | | | | x | | | | | | | | | |
| *Gonepteryx rhamni* | | | | | x | | | | | | x | | | | | | | | | | | | | | | | | | |
| **Lycaenidae** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Hamearis lucina* | x | | | | | | | | | x | | | | | | | | | | | | | | | | | | | |
| *Lycaena phlaeas* | | | | x | x | | | | x | | | | | | | | | | | | | | x | x | x | | | | |
| *Lycaena helle* | | | | | | | | | | | x | | | | | | | | | | | | | | | | | | |
| *Lycaena dispar rutila* | | | | x | x | | | | | x | x | x | | | | x | x | | x | x | | | x | x | | x | | | x |
| *Lycaena virgaureae* | | | | | | | | | | | | | | | | | x | | x | | | | | | | | | | |
| *Lycaena tityrus* | | | | | | | | | | | x | x | | | | | | | x | | | | | | | | | | |
| *Lycaena alciphron* | | | | | | | | | | | x | x | | | x | | x | | x | x | | | | x | | | | | |
| *Lycaena thersamon* | | | | x | x | | | | | | | | | | | | | | | | | | | | | | | | |
| *Callophrys rubi* | | | x | | | | | | | x | x | x | | x | x | | | | | | | | x | | | | | | |

**Appendix 2**. Continuation.

| Taxon/Locality symbols | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | a | b | c |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Satyrium pruni* | | | | | | | | | | | | x | | | | | | | x | | | | | | | | | | |
| *Satyrium ilicis* | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | |
| *Satyrium acaciae* | | | | | | | | | | | | | x | x | | | | x | | x | | x | | | x | x | | | |
| *Cupido minimus* | | | | | | | | | | | | | | | | | x | | x | x | | | x | | | | | | |
| *Cupido* (*Everes*) *argiades* | | | | | | | | | | | | | | | | | | x | | x | | | x | | x | | | | |
| *Cupido* (*Everes*) *decolorata* | | x | | | | | | | | | | x | | | | | | | | | | | | | | | | | |
| *Cupido* (*Everes*) *alcetas* / *decolorata* (1) | | | | | | | | | | x | | | | | | | | | | | | | | | | | | | |
| *Celastrina argiolus* | | | | | | | | | | | | | | | | | | x | x | | | | x | x | x | x | | | |
| *Pseudophilotes schiffermuelleri* | | x | x | x | x | | x | | | x | | | | | | | | | | | | | | | | | | | |
| *Pseudophilotes bavius egea* | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Scolitantides orion lariana* | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | |
| *Glaucopsyche alexis* | | | | | x | | x | | | x | x | x | x | x | x | x | | x | x | | | | | | | | | | |
| *Maculinea arion* | | | | | | | | | | | | | | x | | x | x | | | | | | | | | | | | |
| *Maculinea alcon* (2) | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | |
| *Plebejus sephirus* | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | |
| *Plebejus argus* | | x | x | x | x | | | | | x | x | x | x | x | x | x | x | | | | | x | | | x | | | | |
| *Plebejus idas/argyrognomon* | | | | | | | | | | | | x | x | | | x | x | | | | | | | | | | | | |
| *Aricia agestis* | | | x | x | x | x | x | | x | | | x | | | | | x | | | | | | | | | | | | |
| *Polyommatus* (*Cyaniris*) *semiargus* | | | | | | | | | | | | x | | | | | x | | x | x | | | | | | | | | |
| *Polyommatus amandus* | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | |
| *Polyommatus thersites* | | | | x | | x | | | | x | | x | x | | | | | | | | | | | | | | | | |
| *Polyommatus icarus* | x | | x | x | x | | x | | | x | x | x | x | x | x | x | x | x | x | x | x | x | | x | | x | | | |
| *Polyommatus bellargus* | | | x | x | | | x | | | x | | x | | x | | x | x | | | | | x | | | | | | | |

**Appendix 2**. Continuation.

| Taxon/Locality symbols | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | a | b | c |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nymphalidae** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Argynnis paphia* | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | |
| *Argynnis pandora* | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | |
| *Argynnis aglaja* | | | | | | | | | | | | | | | | x | x | | | | x | | | | | | | | |
| *Argynnis adippe* | | | | | | | | | | | | | | | | x | | | x | x | | | | | | | | | |
| *Argynnis niobe* | | | | | | | x | | | | | | | | | x | | | | | | | | | | | | | |
| *Issoria lathonia* | | | | x | x | | | | | x | x | | | | | | x | | | | x | x | | x | | | | | |
| *Brenthis ino* | | | | | | | | | | | | x | | | | | | | | | | | | | | | | | |
| *Brenthis daphne* | | | | | | | | | | | | | | | | | | | x | | | | | | | x | | | |
| *Brenthis hecate* | | | | | | | | | | | | x | | x | x | | x | | | x | | | | | | | | | |
| *Boloria (Clossiana) euphrosyne* | | | | | | | | | | x | x | | | | | x | | | | | | | | | | | | | |
| *Boloria (Clossiana) selene* | | | | | | | | | | | x | x | | | x | x | x | x | x | | | | x | | | x | | | |
| *Boloria (Clossiana) dia* | | | | | | | | | | | | | | | | x | | x | x | x | | x | | x | x | x | | | |
| *Vanessa atalanta* | | | x | | x | | | | | x | | x | | | | | | | x | | | | | | | | | | |
| *Vanessa cardui* | | | | x | x | | | x | | | | | | x | x | | | | x | | x | | | x | | x | | | |
| *Aglais urticae* | | | | | | | | | | | | x | | | | | x | | | | | | | | | | | | |
| *Polygonia c-album* | | | | | | | | | | | | | | | | | | x | | | | | x | x | | | | | |
| *Araschnia levana* | | | | | | | | | | | | | | | | | x | x | x | | | | | x | | | | | |
| *Nymphalis polychloros* | | | | | | | | | | | | | | | | | | | | | | | x | | | | | | |
| *Nymphalis xanthomelas* | | | | | | | | | | | x | | | | | | | | | | | | | | | | | | |
| *Euphydryas aurinia* | | | | | | | | | | | x | x | | | | | | | | | | | | | | | | | |
| *Melitaea cinxia* | | | x | x | x | | x | | | | x | x | | | x | | | | | x | | | | | | | | | |
| *Melitaea phoebe* (3) | | x | x | x | x | x | x | | | | x | x | | x | x | x | | | | | | | | | | | | | |
| *Melitaea trivia* | | | | | | | x | | | x | | | | | | x | | | x | | | | | | | | | | |
| *Melitaea diamina* | | | | | | | | | | | | | | | | | x | | | | | | | | | | | | |

**Appendix 2**. Continuation.

| Taxon/Locality symbols | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | a | b | c |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Melitaea athalia* | x | | | | | | | | | | x | x | | x | x | x | x | | x | x | | | x | | | x | | | |
| *Melitaea aurelia* | | | | | | | | | | | | x | x | x | | | | | x | x | | | | | | | | | |
| *Limenitis populi* | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | |
| *Neptis rivularis* | | | | | | | | | | | | | | | | x | | | | | | | | | | | | | |
| *Apatura metis* | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | |
| *Apatura ilia* | | | | | | | | | | | | | | | | | | | x | | | | | | | | | | |
| *Kirinia roxelana* | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | |
| *Pararge aegeria tircis* | | | | | | | | | | x | | | | | | | | | x | x | | | | | | | | | |
| *Lasiommata megera* | | | | x | | | | | x | x | | | | | | | | | | x | | | | | | | | | |
| *Lasiommata maera* | | | x | x | x | | | x | | x | | | | | | x | | | | x | | | | | | | | | |
| *Coenonympha arcania* | | | | | | | | | | | | | | | x | x | x | x | x | x | | | | | | x | | | |
| *Coenonympha glycerion* | | | x | x | x | | x | | | | x | x | x | x | x | x | | x | x | x | x | x | x | x | | | | | |
| *Coenonympha pamphilus* | | | x | x | x | | | | x | x | x | x | x | x | x | x | x | x | | x | x | | x | x | x | | | | |
| *Maniola jurtina* | | | | | x | | | | x | | x | x | x | | x | x | x | x | x | x | x | x | x | x | x | | | | |
| *Erebia medusa psodea* | | | | | | | | | | | x | x | | | | | | | | | | | | | | | | | |
| *Melanargia galathea* | | | | | | | | | | | | | | x | | x | | x | | x | | | | | x | | | | |
| *Hipparchia semele* | | | | | | | | | | | | | | x | | | | | | | | | | | | | | | |
| *Hipparchia volgensis delattini* (4) | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | |