Biogeography, ecology and conservation of *Erebia oeme* (Hübner) in the Carpathians (Lepidoptera: Nymphalidae: Satyrinae)

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Abstract. The European endemic *Erebia oeme* (Hübner [1804]) (Lepidoptera: Nymphalidae: Satyrinae) is discovered in the Carpathian Chain, from where it was considered to be absent. The single population found is situated in the southern part of the Romanian Carpathians (Retezat Mountains), where it flies sympatrically and synchronically with *Erebia medusa* ([Denis & Schiffermüller] 1775). The similar external morphology of these two species probably caused *E. oeme* to be overlooked in the Carpathians, leading to an unexpected information gap in the otherwise thoroughly studied European continent. The morphology of the Romanian specimens is compared to populations from the rest of the species' range and to *E. medusa*. In addition, we tested DNA barcoding as a method to discriminate between these species and confirmed that it represents an effective identification tool for the taxa involved. The habitat of *E. oeme*, adults of both sexes and their genitalia are illustrated in comparison with *E. medusa*. Based on the study of several collections, we show that *E. oeme* is likely to be extremely local in the Carpathians and provide arguments to consider the species as vulnerable in Romania.

Résumé. Biogéographie, écologie et conservation d'*Erebia oeme* (Hübner) dans les Carpathes (Lepidoptera : Nymphalidae : Satyrinae). L'espèce endémique Européenne, *Erebia oeme* (Hübner [1804]) (Lepidoptera: Nymphalidae: Satyrinae), a été découverte dans la Chaîne des Carpates d'où elle était considérée comme absente. La seule population trouvée se situe dans la partie méridionale des Carpates Roumaines (Massif du Retezat), où l'espèce est sympatrique et synchronique avec *Erebia medusa* ([Denis & Schiffermüller] 1775). La similarité morphologique externe entre ces deux espèces est probablement la raison pour laquelle *E. oeme* a été méconnu dans les Carpates ce qui a laissé un déficit d'information inattendu au niveau du continent Européen ayant fait l'objet d'études approfondies. La morphologie des spécimens Roumains est comparée aux populations du reste de l'aire de répartition de cette espèce et avec *E. medusa*. D'autre part nous avons testé le codage à barres de l'ADN comme méthode pour identifier ces deux espèces et avons confirmé que ceci est en effet un outil efficace d'identification pour les taxons concernés. L'habitat d' *E. oeme*, les adultes des deux sexes et leurs organes génitaux sont illustrés en comparaison à *E. medusa*. Basé sur l'étude de différentes collections, nous démontrons que *E. oeme* est probablement très local dans les Carpates et nous apportons des arguments pour considérer l'espèce comme vulnérable en Roumanie.

Keywords: Distribution, DNA barcoding, butterfly, Romania, taxonomy.

Erebia oeme (Hübner 1804) (Lepidoptera: Nymphalidae: Satyrinae) is a European species present in the Pyrenees, Massif Central, Jura, Alps, Dinaric Alps, Balkans and Rhodopes (Tolman & Lewington 1997; Lafranchis 2000; Kudrna 2002; Sonderegger 2005) (fig. 1). Although some old Slovakian and Romanian records exist, these proved to represent confusions with the externally similar *Erebia medusa* ([Denis & Schiffermüller] 1775) and thus *E. oeme* is currently considered absent from the Carpathian chain.

Old records from the Slovakian Carpathians referred to various sites in the Tatra Mountains (Aigner-Abafi *et al.* 1896; Von der Goltz 1937; Hrubý 1964) and Branisko Mountain (eastern Slovakia) (Aigner-Abafi *et al.* 1896; Warren 1936). However, these records were rejected by subsequent studies: Hrubý (1964) questioned the reliability of the data from Branisko; Moucha (1972) and Reiprich & Okáli (1989) discarded the presence of *E. oeme* in Slovakia by invalidating the records from the Low Tatra. In addition, Moucha (1972) stated that

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the specimens from the Silbernag collection stored in the National History Museum in Prague were actually *E. medusa*. Moreover, the presence of *E. oeme* in these parts of the Carpathians was questioned by Higgins & Riley (1970) and Tolman & Lewington (1997). More recently, Varga (2002) mentioned that old records from Branisko Mountain (in coll. Dahlström from the Hungarian Natural History Museum) were never confirmed and concluded that these specimens were probably incorrectly labeled. Slamka (2004 & *pers. com*. 2009) concluded that *E. oeme* was not present in Slovakia by treating the records from Branisko as doubtful and invalidating old reports from the Tatras which were actually *Erebia medusa* f. *slovakiana* Warren 1936. In the Romanian Carpathians, the presence of *E. oeme* was cited several times (Caradja 1895; Fleck 1900; Hormuzachi 1902; Salay 1910), always based on material collected by E. Fleck at the end of the nineteenth century in Bucegi Mountains (surroundings of Azuga). Popescu-Gorj (1963) examined specimens identified as *E. oeme* from Fleck's collection at "Grigore Antipa" National History Museum in Bucharest. He found that all these specimens were actually *Erebia medusa brigobana* Fruhstorfer 1917. Popescu-Gorj also mentioned that he never found (in the field, in museums or private collections) any specimen of *E. oeme* from the Romanian Carpathians, concluding that this species is not present in Romania. We also examined the *Erebia* specimens from the collection "E. Fleck" at the National Museum of Natural History "Grigore Antipa", Bucharest. Among the *Erebia* species collected in the surroundings of Azuga we found four specimens of *E. medusa* (3 males, 1 female) but no *E. oeme*, confirming the conclusion of Popescu-Gorj (1963).

Rákosy *et al.* (2003) excluded the presence in Romania of several *Erebia* species, including *E. oeme*. The arguments invoked for all the excluded *Erebia* species were distributional incompatibilities and the lack of relevant material. Recently, Székely (2008) included *E. oeme* among the species that were erroneously recorded from Romania.

As the old citations of *E. oeme* from both the Slovakian and Romanian Carpathians have been discarded and no new records have emerged from more recent surveys in these regions, the species is by most authors currently regarded as absent from the Carpathian Chain (Karsholt & Razowski 1996; Kudrna 2002; Varga 2002; Lafranchis 2004, 2007; Sonderegger 2005, but see Tolman & Lewington 1997, 2008).

In this study we report the first confirmed population of *E. oeme* in the Carpathian Chain. Given the similar habitus of *E. oeme* and *E. medusa*, we performed comparisons of both external and internal characters that were often reported to be useful in discriminating between the two taxa: wing pattern (Higgins & Riley 1970; Lafranchis 2000; Varga 2002),

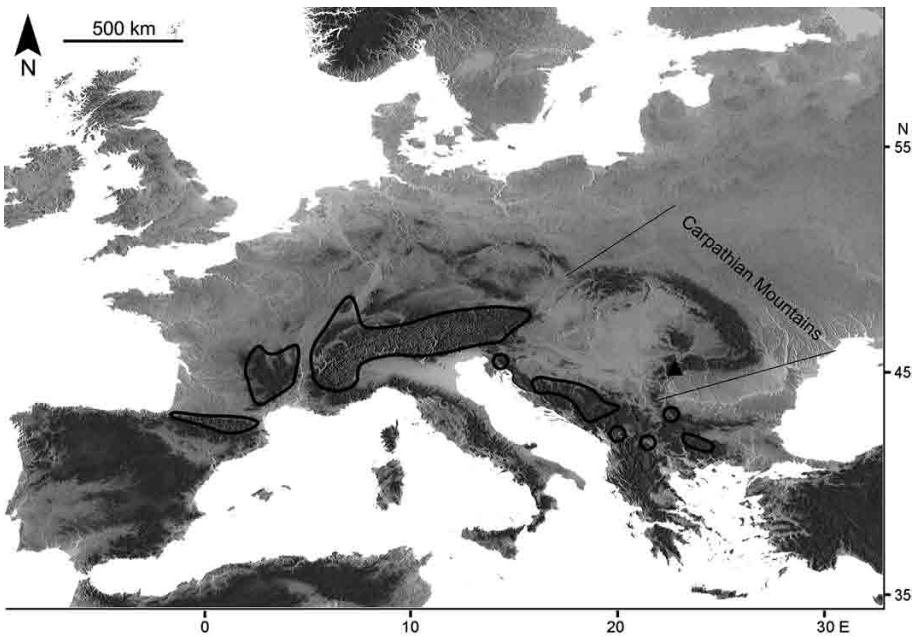

Figure 1
General distribution of *Erebia oeme* in Europe. The triangle marks the position of the Carpathian population (Retezat Mountains).

antennal tip (Higgins & Riley 1970; LSPN 1987; Tolman & Lewington 1997, 2008; Lafranchis 2000; Varga 2002; Lafranchis 2004; Sonderegger 2005), male genitalia (Higgins 1975; Sonderegger 2005) and female genitalia (Sonderegger 2005). The external similarity of the two species makes them candidates that could benefit from DNA-based identifications such as DNA barcoding (Hebert *et al*. 2003). We assessed the ability of this technique to discriminate between the two species and tested its congruence with morphology-based techniques.

Material and methods

Collecting

Material. *Erebia oeme*. **Romania**: 2♂♂, Retezat Mountains, Scorota Valley (Hunedoara county), 1550m, 7.VIII.2006, prep. genit. 528/Dincă, 21.VII.2008, prep. genit. 642/Dincă; 4♀♀, Retezat Mountains, Scorota Valley (Hunedoara county), 1500–1550m, 21.VII.2008, prep. genit. 656, 657, 658, 667/Dincă.

The specimens of *E. oeme* were collected using the insect net during two field trips to the Retezat Mountains in 2006 and 2008 (tab. 1). Although tab. 1 refers to the specimens sequenced for the study, a much richer comparative material of *E. oeme* and *E. medusa* collected by the authors in various parts of Europe was examined, especially for wing pattern and antennal tip comparisons. All the samples that are stored in Roger Vila's DNA and Tissue collection (Spain), have the entire body in tubes with 100% ethanol and the wings inside glassine envelopes as reference. For the prepared specimens stored in V. Dincă's collection (Spain), only one leg was used for DNA sequencing. In this case, each specimen sequenced is labeled with a unique sample ID attached to the insect's pin (tab. 1).

Genitalia were processed as follows: maceration in 10% potassium hydroxide, dissection and cleaning under a stereomicroscope and storage in tubes with glycerin. Genitalia photos were taken in a thin layer of distilled water under a Carl Zeiss Stemi 2000-C stereomicroscope equipped with a DeltaPix Invenio 3S digital camera. The female genitalia were photographed while being slightly pressed under a cover slip. Whole specimen and antennae photos were taken by using either a Nikon D40x (AF-S Nikkor 18–55mm GII ED with three Prinz lens attachments) or a Nikon D70 (AF Micro Nikkor 60mm) digital reflex camera.

COI amplification

DNA was extracted from a single leg removed from each voucher specimen employing a glass fiber protocol (Ivanova *et al*. 2006). All polymerase chain reactions (PCR) and DNA sequencing were carried out following standard DNA barcoding procedures for Lepidoptera as described previously (Hajibabaei *et al*. 2005; deWaard *et al*. 2008). The primers LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') were used to amplify the target 658-bp fragment of COI. Sequences were obtained by using an ABI3730 (bidirectional read) sequencer following manufacturer's recommendations.

Data analysis

Five specimens of *E. oeme* collected in Romania (Retezat Mts.) and thirteen of *E. medusa* from various parts of Romania were included in the analyses (tab. 1). Up to two sequences per species available in GenBank by 1st of March 2009 and overlapping to our COI barcodes were included in the data set. A total of 24 GenBank COI sequences were used, originally from the studies

Table 1. Samples of *Erebia oeme* and *E. medusa* sequenced for DNA barcoding.

Sample ID	Taxon	Date	Collection site	Alt. (m)	Lat.	Long.	Stored in
RVcoll.06-V691	*Erebia oeme*	07.VIII.2006	Scorota Valley (Retezat Mts.)	1550	45°17'56" N	22°53'36" E	Coll. R. Vila
RVcoll.08-M631	*Erebia oeme*	21.VII.2008	Scorota Valley (Retezat Mts.)	1550	45°17'56" N	22°53'36" E	Coll. R. Vila
RVcoll.08-M623	*Erebia oeme*	21.VII.2008	Scorota Valley (Retezat Mts.)	1550	45°17'56" N	22°53'36" E	Coll. V. Dincă
RVcoll.08-M626	*Erebia oeme*	21.VII.2008	Scorota Valley (Retezat Mts.)	1550	45°17'56" N	22°53'36" E	Coll. V. Dincă
RVcoll.08-M624	*Erebia oeme*	21.VII.2008	Scorota Valley (Retezat Mts.)	1550	45°17'56" N	22°53'36" E	Coll. V. Dincă
RVcoll.08-M625	*Erebia medusa*	21.VII.2008	Scorota Valley (Retezat Mts.)	1300	45°17'12" N	22°53'35" E	Coll. V. Dincă
RVcoll.08-M615	*Erebia medusa*	20.VII.2008	Capra chalet (Făgăraş Mts.)	1630	45°35'06" N	24°37'43" E	Coll. R. Vila
RVcoll.07-D282	*Erebia medusa*	27.V.2007	Dumbrava Vadului (Braşov)	495	45°46'37" N	25°06'53" E	Coll. R. Vila
RVcoll.06-N019	*Erebia medusa*	21.VII.2006	near CiucaşPeak (Ciucaş Mts.)	1700	45°30'59" N	25°56'10" E	Coll. R. Vila
RVcoll.06-N015	*Erebia medusa*	21.VII.2006	near Ciucaş Peak (Ciucaş Mts.)	1560	45°30'30" N	25°57'02" E	Coll. R. Vila
RVcoll.08-M341	*Erebia medusa*	01.VI.2008	E of Gheorgheni (Harghita)	900	46°44'34" N	25°39'52" E	Coll. R. Vila
RVcoll.08-H025	*Erebia medusa*	12.VII.2008	Corongiş (Rodnei Mts.)	1500	47°30' N	24°51' E	Coll. R. Vila
RVcoll.07-C150	*Erebia medusa*	03.VII.2007	Corongiş (Rodnei Mts.)	1600	47°30' N	24°51' E	Coll. R. Vila
RVcoll.07-C306	*Erebia medusa*	03.VI.2007	Rădăuţi (Suceava)	450	47°50' N	25°55' E	Coll. R. Vila
RVcoll.08-M522	*Erebia medusa*	05.VII.2008	Molhaşuri Izbuce (Apuseni Mts.)	1200	46°35'31" N	22°45'41" E	Coll. R. Vila
RVcoll.06-K622	*Erebia medusa*	23.V.2006	Baciu forest (Cluj-Napoca)	530	46°48' N	23°30' E	Coll. R. Vila
RVcoll.06-K618	*Erebia medusa*	23.V.2006	Baciu forest (Cluj-Napoca)	530	46°48' N	23°30' E	Coll. R. Vila
RVcoll.07-C112	*Erebia medusa*	30.IV.2007	Gheorgheni (Cluj-Napoca)	600	46°43'27" N	23°38'55" E	Coll. R. Vila
RVcoll.08-M541	*Brintesia circe*	07.VII.2008	Pecinişca (Caraş-Severin)	500	44°51'46" N	22°25'23" E	Coll. R. Vila

of Vila & Björklund (2004), Peña *et al.* (2006) and Nakatani *et al.* (2007). No COI sequences were available for *E. medusa*, while only two were available for *E. oeme* (both of the specimens from the French Pyrenees). *Brintesia circe* (Fabricius 1775) was used as root for the resulting tree.

Sequences were edited and aligned with Sequencher 4.7 (Genecodes Corporation, Ann Arbor, MI). The Kimura 2-parameter model of base substitution (Kimura 1980) was used to calculate genetic distances in MEGA 4 software (Tamura *et al.* 2007). MEGA 4 was also used to produce the neighbour-joining (NJ) tree and to perform bootstrap analysis (1000 replicates) (Felsenstein 1985).

Sequence information was uploaded in the Barcode of Life Data System (www.barcodinglife.org) along with an image and collateral information for each voucher specimen. The detailed specimen records and sequence information, including trace files, are available in the EOEME project file on BOLD. All sequences were also submitted to GenBank (Accession numbers FJ938179 to FJ938196).

Results

Erebia oeme (fig. 3h, i, k, l) was collected in Scorota Valley, situated in the upper mountainous part of the southern calcareous Retezat (Meridional Carpathians), on the western side of the Piule massif (fig. 2). This area lies within the borders of the oldest Romanian National Park and Biosphere Reserve, the Retezat National Park.

In addition, in order to test the possible presence of *E. oeme* in other localities, we examined hundreds of specimens of *E. medusa* from the collections of the National Museum of Natural History "Grigore Antipa" in Bucharest (coll. A. Ostrogovich, E. Fleck, A. Popescu-Gorj). However, we found no specimen of *E. oeme* among the numerous specimens of *E. medusa* from various parts of the Romanian Carpathians, including the Retezat Mountains.

Identification

Erebia oeme is considered to be a species that is fairly difficult to identify because of its morphological similarity to certain specimens of *E. medusa* (e.g. LSPN 1987; Lafranchis 2000; Varga 2002; Slamka 2004; Sonderegger 2005). The difficulty in providing an absolute key for the differentiation between the two species derives from the considerable variability of wing patterns in both *E. oeme* and *E. medusa* (examples in fig. 3). The characters mentioned most frequently as diagnostic are:

(1) The antennal tip (especially its underside), which is black in *E. oeme* and brownish in *E. medusa* (Higgins & Riley 1970; LSPN 1987; Tolman & Lewington 1997, 2008; Lafranchis 2000; Varga 2002; Lafranchis 2004; Sonderegger 2005).

(2) The ocelli of the hind wing that have a brighter white center in *E. oeme* (Higgins & Riley 1970; Lafranchis 2000; Varga 2002).

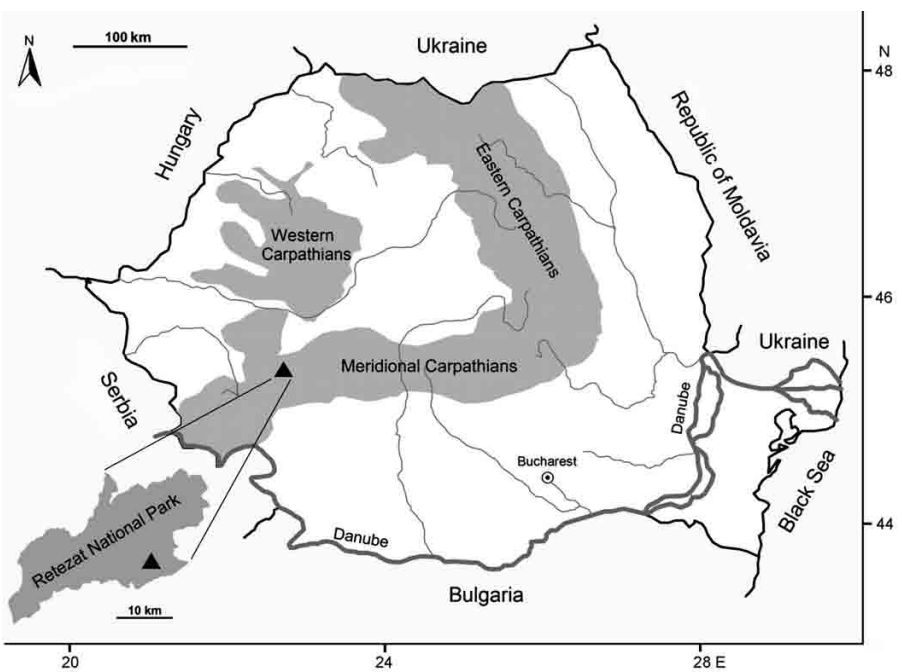

Figure 2
Location of the newly discovered population of *Erebia oeme* in the Carpathians. Lower left corner illustrates the position of the site (black triangle) within the Retezat National Park.

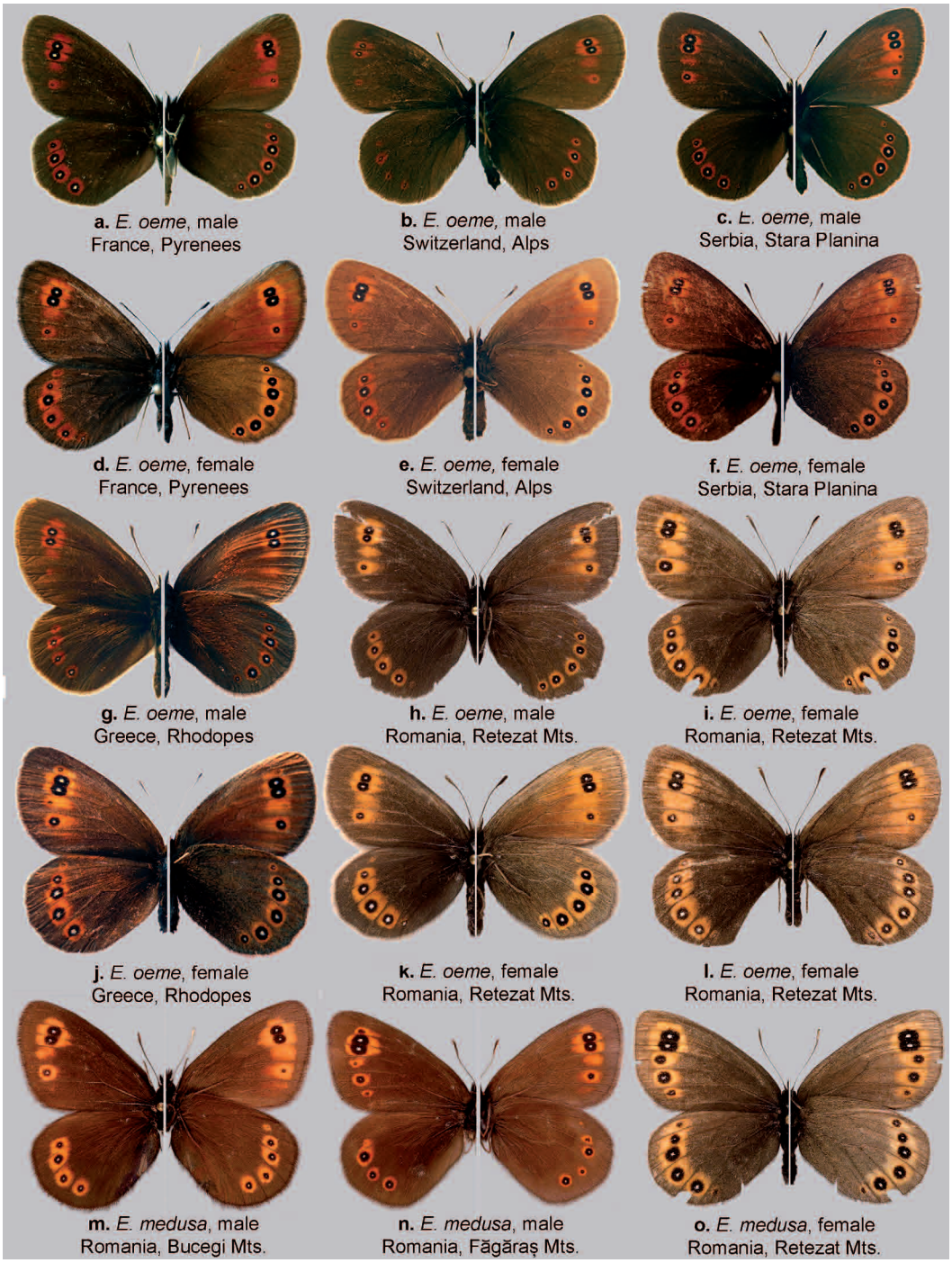

Figure 3
Dorsal (left pair of wings) and ventral (right pair of wings) view of adults belonging to different European populations of *Erebia oeme* and Romanian Carpathian populations of *E. medusa*. ***Erebia oeme***: **a,** ♂, France, Pyrenees, Etang Lanoux, 11.VII.2003; **b,** ♂, Switzerland, Alps, Rawyl Wallis, 2.VIII.1980; **c,** ♂, Serbia, Stara Planina, Babin Zub, 11.VII.2006; **d,** ♀, France, Pyrenees, Etang Lanoux, 11.VII.2003; **e,** ♀, Switzerland, Alps, Rawyl Wallis, 2.VIII.1980; **f,** ♀, Serbia, Stara Planina, Golema Reka, 10.VII.2006; **g,** ♂, Greece, Rhodopes, Elatia forest, 18.VII.1998; **h,** ♂, Romania, Carpathians, Retezat Mts., Scorota Valley, 21.VII.2008, prep. genit. 642/Dincă; **i,** ♀, Romania, Carpathians, Retezat Mts., Scorota Valley, 21.VII.2008, prep. genit. 658/Dincă; **j,** ♀, Greece, Rhodopes, Elatia forest, 18.VII.1998; **k,** ♀, Romania, Carpathians, Retezat Mts., Scorota Valley, 21.VII.2008, prep. genit. 656/Dincă ; **l,** ♀, Romania, Carpathians, Retezat Mts., Scorota Valley, 21.VII.2008, prep. genit. 657/Dincă. ***Erebia medusa***: **m,** ♂, Romania, Carpathians, Bucegi Mts., Cota 2000, 10.VII.2004, prep. genit. 550/Dincă; **n,** ♂, Romania, Carpathians, Făgăraş Mts., below Bâlea lake, 22.VII.2004, prep. genit. 552/Dincă; **o,** ♀, Romania, Carpathians, Retezat Mts., Scorota Valley, 21.VII.2008, prep. genit. 659/Dincă. Photos a-g, j: S. Cuvelier; h, i, k, l, o: R. Vila; m, n: V. Dincă

(3) The colour of the underside of the wings, which often bears yellowish tones in *E. oeme* (especially females), while in *E. medusa* it is generally uniformly brown, similarly to its upperside (Higgins & Riley 1970; Lafranchis 2000; Varga 2002).

Some of these features do seem to successfully differentiate the species at particular localities, but the overall variability of *E. oeme* and *E. medusa* generates exceptions to these "rules". The colour of the antennal tip is often invoked as the most stable of these differentiating characters, but it is also subject to some variability (examples in fig. 4), meaning that identifications should not be made only on this character. Moreover, this difference is often difficult to evaluate in collection specimens, where the antennal tip may be deformed or even damaged (e.g. antennal tip with partially lost black scales in fig. 4c). Taking into account all of the "diagnostic" external features will provide better resolution, but if the habitus shows intermediate characters between *E. oeme* and *E. medusa*, genitalia examination (fig. 5, 6) or DNA barcoding (fig. 7) is recommended (see DNA-based identification chapter).

Male genitalia

The male genitalia of *E. oeme* cannot be mistaken with those of any other *Erebia* species. The terminal part of the valva is fusiform and slightly curved, ending up with a small (but obvious) sharp tooth (fig. 5a-g). The phallus is slender in the medial part (fig. 5i). These features clearly separate *E. oeme* from the externally similar *E. medusa*. The valva of the latter has a thicker terminal part and ends up relatively blunt, with a series of tiny teeth (fig. 5h), that are reported to be variable in number (6–15) (Sonderegger 2005). The phallus of *E. medusa* is comparatively less slender in the medial part (fig. 5j). Although the male genitalia of *E. oeme* shows some variability (e.g. in the width of the proximal part of the valva or the curvature of the terminal fusiform part) (fig. 5a-g), the overall aspect is homogenous and allows a correct identification. As mentioned by Sonderegger (2005), the sharp aspect of the valva tip may even allow for field identification of male adults and their separation from *E. medusa*, where the valva tip has a blunt aspect.

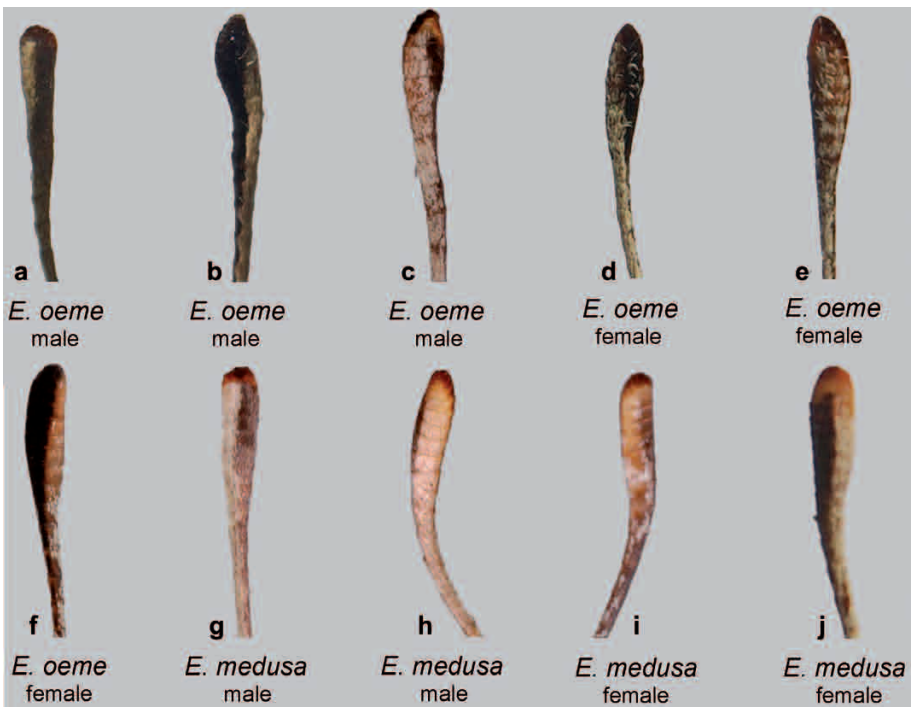

Figure 4
Examples of antennal tip appearance of *Erebia oeme* and *E. medusa* from different European populations. ***Erebia oeme*: a,** ♂, Switzerland, Alps, Rawyl Wallis (dorso-lateral view); **b,** ♂, Greece, Elatia forest (lateral view); **c,** ♂, Romania, Carpathians, Retezat Mts., Scorota Valley (ventral view); **d,** ♀, France, Pyrenees, Etang Lanoux (lateral view); **e,** ♀, Serbia, Golema Reka (ventral view); **f,** ♀, Romania, Carpathians (Retezat Mts., Scorota Valley) (lateral view). ***Erebia medusa*: g,** ♂, Romania, Carpathians, Bucegi Mts., Cota 2000 (ventral view); **h,** ♂, Romania, Carpathians, Făgăraş Mts., below Bâlea lake (ventral view); **i,** ♀, Romania, Carpathians, Retezat Mts., Scorota Valley (ventral view); **j,** ♀, France, Mont Revard (lateral view). Photos a, b, d, e, j: S. Cuvelier; c, f-i: V. Dincă.

Female genitalia

The female genitalia of *E. oeme* have a rather complex three-dimensional structure that makes illustrations fairly variable according to the method of preparation and image capture. An accurate and detailed description of the female genitalia of both *E. oeme* and *E. medusa* was provided by Sonderegger (2005). Therefore, we only point out some of the features that, in our opinion, allow a correct identification and that are also easier to observe under the stereomicroscope. The terminology

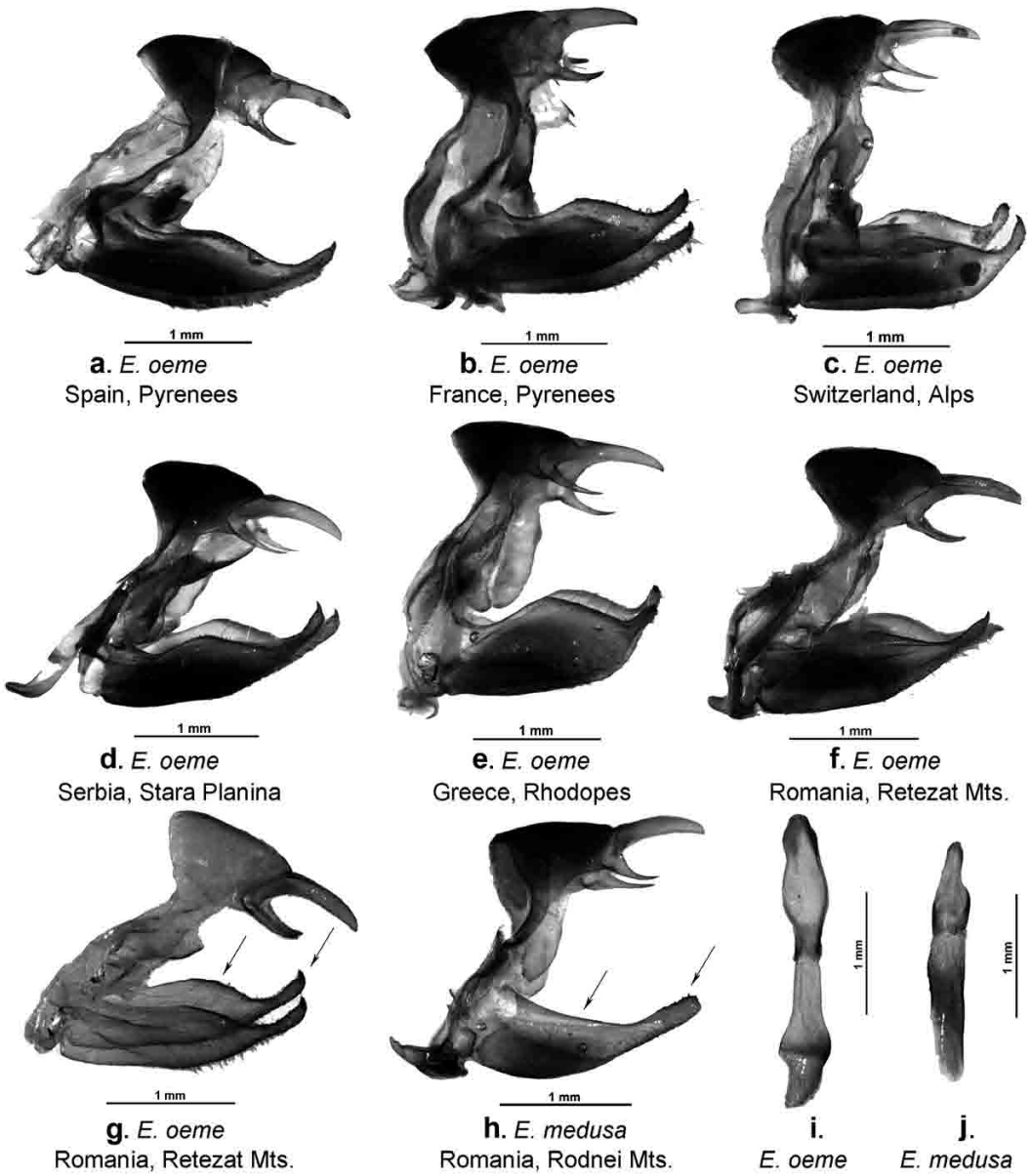

Figure 5
Male genitalia of *Erebia oeme* and *E. medusa* specimens from different European populations. **a,** Lateral view of male genitalia of *Erebia oeme*, Spain, Vall d'Aran, 30.VI.2007, prep. genit. 663/Dincă; **b,** Lateral view of male genitalia of *Erebia oeme*, France, Etang Lanoux, 11.VII.2003, prep. genit. 704/Dincă; **c,** Lateral view of male genitalia of *Erebia oeme*, Switzerland, Rawyl Wallis, 2.VIII.1980, prep. genit. 685/Dincă; **d,** Lateral view of male genitalia of *Erebia oeme*, Serbia, Golema Reka, 11.VII.2006, prep. genit. 687/Dincă; **e,** Lateral view of male genitalia of *Erebia oeme*, Greece, Elatia forest, 18.VII.1998, prep. genit. 688/Dincă; **f,** Lateral view of male genitalia of *Erebia oeme*, Romania, Scorota Valley (Retezat Mts.), 21.VII.2008, prep. genit. 642/Dincă; **g,** Lateral view of male genitalia of *Erebia oeme*, Romania, Scorota Valley (Retezat Mts.), 7.VIII.2006, prep. genit. 528/Dincă; **h,** Lateral view of male genitalia of *Erebia medusa*, Romania, Izvorul Bistriței (Rodnei Mts.), 25.VII.2005, prep. genit. 643/Dincă; **i,** Phallus of *Erebia oeme*, Romania, Scorota Valley (Retezat Mts.), 21.VII.2008, prep. genit. 642/Dincă; **j,** Phallus of *Erebia medusa*, Romania, Izvorul Bistriței (Rodnei Mts.), 25.VII.2005 prep. genit. 643/Dincă.

used in the descriptions of the female genitalia follows Sonderegger (2005), adapted from German.

In the case of *E. oeme* (fig. 6a, b), the lateral folds (LF) of the postvaginal wing (W) are well sclerified and prominent, creating the impression of two "shoulders" (more obvious without pressing the genitalia). By contrast, in *E. medusa* (fig. 6d, e), the lateral folds are weakly sclerified and not so prominent. The flap (F) is more developed in *E. oeme* than in *E. medusa*. The membrane (Mb) is approximately round in *E. oeme*, while in *E. medusa* it is oval. Even without a very good genitalia preparation, the sclerified and prominent lateral folds (LF) and the round membrane (Mb) should be easily observed and allow the identification of a female of *E. oeme*. Another element, which we have not found mentioned anywhere in literature, is the length of the signum of the corpus bursae, which is substantially longer in *E. medusa* (0.98–1.2 mm) compared to *E. oeme* (0.62–0.67 mm) (fig. 6c, f).

DNA-based identification

We tested the effectiveness of DNA-based methods in discriminating between *E. oeme* and *E. medusa*. We have used DNA barcoding because, while it is a relatively new approach, it has proven useful in discriminating between some closely related species (Hebert *et al*. 2003; Dinca & Vila 2008) and COI barcodes are relatively well represented in GenBank.

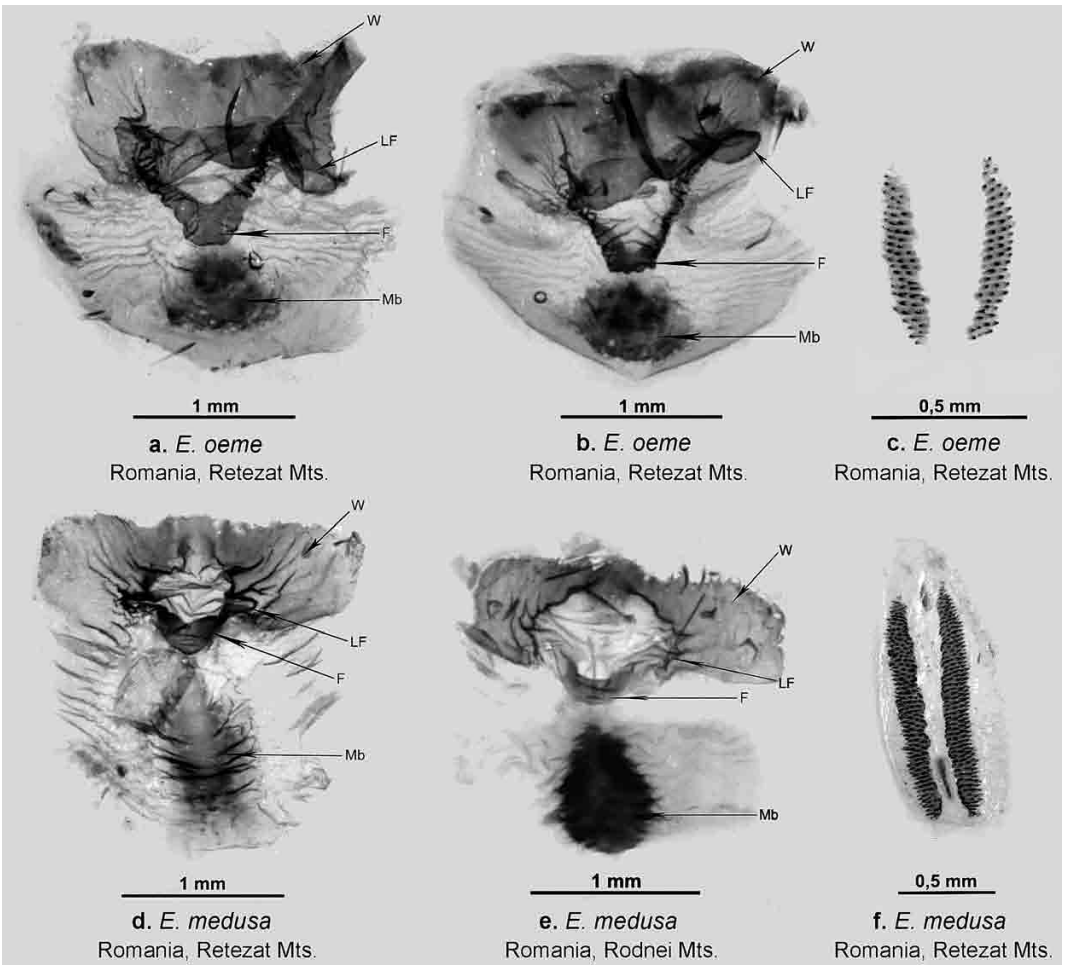

Figure 6
Female genitalia of *Erebia oeme* and *E. medusa* and their diagnostic characters. **a,** Female genitalia of *Erebia oeme*, Romania, Scorota Valley (Retezat Mts.), 21.VII.2008, prep. genit. 658/Dincă; **b,** Female genitalia of *Erebia oeme*, Romania, Scorota Valley (Retezat Mts.), 21.VII.2008, prep. genit. 667/Dincă; **c,** Signum of the female genitalia of *Erebia oeme*, Romania, Scorota Valley (Retezat Mts.), 21.VII.2008, prep. genit. 657/Dincă; **d,** Female genitalia of *Erebia medusa*, Romania, Scorota Valley (Retezat Mts.), 21.VII.2008, prep. genit. 659/Dincă; **e,** Female genitalia of *Erebia medusa*, Romania, Corongiş (Rodnei Mts.), 12.VII.2008, prep. genit. 689/Dincă; **f,** Signum of the female genitalia of *Erebia medusa*, Romania, Scorota Valley (Retezat Mts.), 21.VII.2008, prep. genit. 659/Dincă.

The majority of the high bootstrap supports correspond to terminal nodes of the tree (fig. 7). This is in concordance with the fast evolving rate of mitochondrial markers such as COI that are usually not suitable for the recovery of ancient relationships, but are often useful in resolving more recent splits. For example, in our tree, closely related taxa such as *Erebia palarica* (Chapman 1905) and *Erebia meolans* (de Prunner 1798) or as *Erebia euryale* (Esper [1805]) and *Erebia ligea* (L. 1758) are recovered as sister taxa with good support.

As for *E. oeme* and *E. medusa*, the NJ tree recovers both taxa as two strongly supported (bootstrap values of 100) clades (fig. 7). The minimum interspecific pairwise distance between *E. medusa* and *E. oeme* is 8.2%, while the maximum intraspecific distance is 0.3% for *E. medusa* and 1.2% for *E. oeme*. Although the sampling for *E. medusa* and *E. oeme* does not cover all the species' distribution, the sequences included in the analyses cover the extremes of the range of *E. oeme* and a large part of the distribution of *E. medusa* in the Carpathians. The 13 sequenced specimens of *E. medusa* were collected both at low (ca. 400–500 m) and high (over 1600 m) altitudes and sampling sites were separated by up to 400 km (tab. 1). The large gap between intraspecific and interspecific divergence for

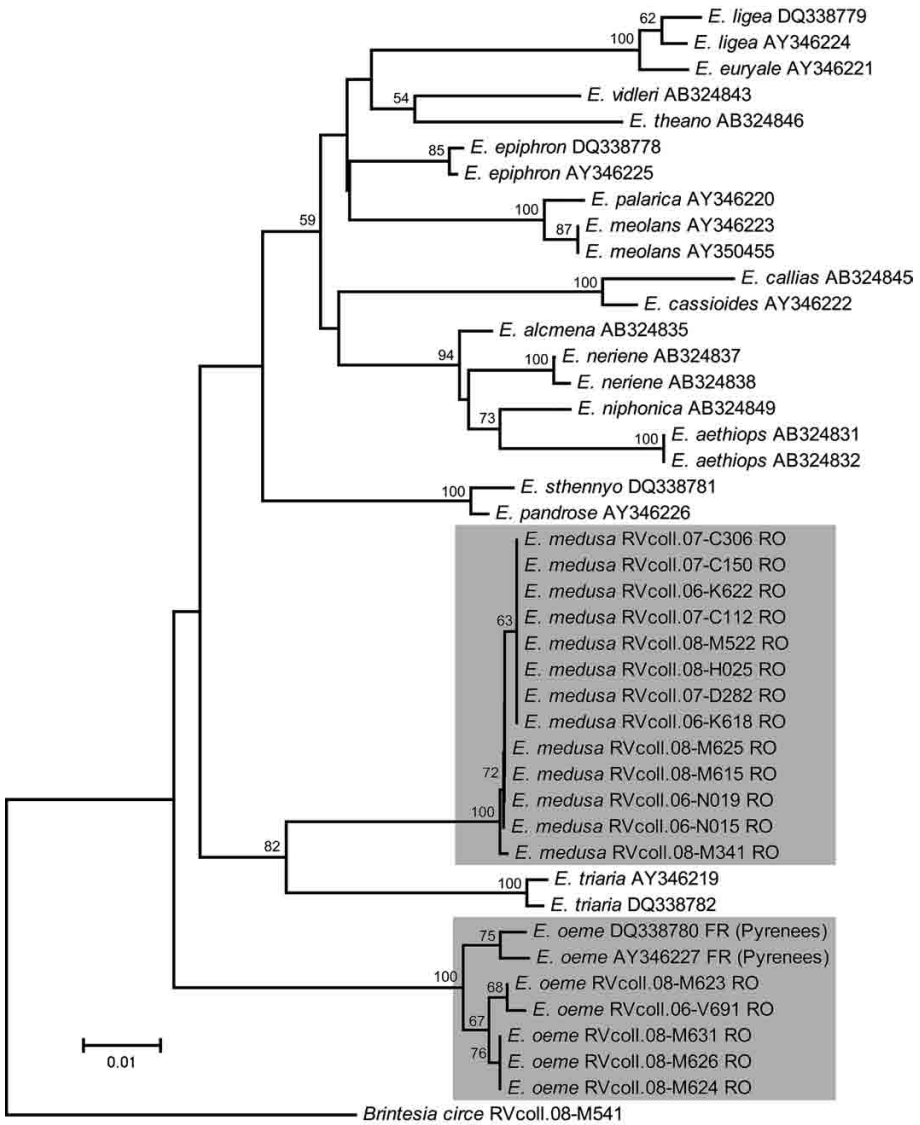

Figure 7
NJ tree based on K2P distances including COI barcodes of *Erebia oeme* and *E. medusa* and up to two overlapping sequences of all other *Erebia* species available in GenBank. Bootstrap values (> 50) are shown above recovered branches. RO – Romania, FR – France.

the two species strongly suggests that, at least in the Carpathians, no shared barcodes should be expected, making DNA barcoding a reliable tool for the correct discrimination between the two taxa.

Discussion

Erebia oeme has never been recorded from the Retezat Mountains (Popescu-Gorj 1963; Rákosy 1997), although these represent some of the best studied sites in the Romanian Carpathians (e.g. Diószeghy 1930, 1934; Căpuşe & Kovács 1987; König 1959, 1963, 1969; Burnaz & König 1984; Rákosy 1992, 1993, 1997).

Our data provide the first certain record of *E. oeme* for the entire Carpathian Chain. The nearest populations of *E. oeme* are the ones from eastern Serbia and north-western Bulgaria (Stara Planina) (Abadjiev 2001; Kudrna 2002; Abadjiev & Beshkov 2007), about 200 km south from the Retezat Mountains (fig. 1).

The past failure to detect *E. oeme* in the Carpathian Chain probably reflects both its external similarity to *E. medusa*, and its apparent rarity and localization in these mountains. Confusion with *E. medusa* seems possible as this species is not only widespread and common in Central and Eastern Europe, but displays considerable morphological variability (e.g. Higgins & Riley 1970; LSPN 1987; Tolman & Lewington 1997; Lafranchis 2000; Varga 2002; Sonderegger 2005). In the Romanian Carpathians, *E. medusa* is widespread and common, flying from 300 m to over 2000 m (Popescu-Gorj 1950; Székely 2008; Dincă *pers. obs.*). In the Retezat Mountains *E. medusa* occurs in various (more or less humid) habitat types between 800–2150 m (Popescu-Gorj 1950; Rákosy 1997). On 21st of July 2008, we collected a female of *E. medusa* (fig. 3o) in Scorota Valley, at an altitude of ca. 1300 m, in a coniferous forest clearing that was just 200 m lower and one kilometer distant from the site where *E. oeme* was discovered. Thus, *E. medusa* and *E. oeme* are likely to be sympatric in some parts of Retezat Mountains and confusions may have been made concerning their identification. On the other hand, the absence of specimens of *E. oeme* from several large Romanian collections suggests that the species is very local in the Carpathian Chain. It should also be taken into account that, within the Carpathian Chain, several taxa such as *Erebia cassioides* (Reiner & Hochenwarth 1792) and *Coenonympha rhodopensis* Elwes 1900 do not occur north of the Retezat Mountains. Such taxa are present in the Balkans and might have dispersed north crossing the Danube and reaching Retezat through the calcareous Cerna river valley.

Taxonomical aspects

Erebia oeme and the externally similar *E. medusa* are fairly variable butterflies for which many subspecies and forms have been described. Some authors consider that *E. oeme* presents a morphological cline across Europe, manifested in the number and size of the ocelli and the extension of the postdiscal orange band on the upperside of the wings (Higgins & Riley 1970; Higgins 1975; Varga 2002). Indeed, the variation in these characters roughly follows a longitudinal cline, with the most developed wing pattern being present in some Balkanic populations. However, the species shows significant variability at a much more local scale (Fernández-Rubio 1991; Sonderegger 2005) so that a clear clinal trend is rather difficult to observe. Moreover, there is also noticeable variability of wing pattern and wingspan linked to altitudinal and humidity gradients (e.g. Sonderegger 2005).

Erebia medusa raises similar problems. Many described subspecies are difficult to separate as their morphological variability often overlaps (Varga 2002). Concerning the Romanian Carpathians, several subspecies and forms of *E. medusa* have been described, which have been revised by different authors with different results (e. g. Popescu-Gorj 1950; Varga 2002). Two subspecies are generally accepted, *E. medusa medusa* ([Denis & Schiffermüller] 1775) and *E. medusa psodea* (Hübner [1804]) (Varga 2002; Rákosy *et al.* 2003; Székely 2008). However, both display considerable variability in Romania (Varga 2002; Dincă *pers. obs.*) and have insufficiently documented distribution limits in the Carpathians. Molecular phylogeography studies might give clues concerning the subspecific structure of *E. medusa* at European scale (Schmitt 1999; Schmitt & Seitz 2001; Hammouti 2006; Schmitt *et al.* 2007), but no recent revision using an integrative approach (combining morphological and molecular data) has been published. It is difficult to assess the subspecific status of the *E. oeme* population from Retezat because a large series is not available. Nevertheless, the prominence of the postdiscal orange band and the well developed ocelli (especially in females) (fig. 3h, i, k, l) make the Retezat population closest to the ones occurring in the eastern Alps and the Balkans, which are usually considered to belong to the subspecies *spodia* Staudinger 1871 (Tolman & Lewington 1997, 2008; Varga 2002).

Habitat, biology and conservation of *Erebia oeme* in the Carpathians

The population of *E. oeme* in the Scorota Valley (Retezat Mountains) occurs on a south-facing slope (ca.

1550 m) corresponding to the transition between the coniferous forest and mesophilous subalpine meadows (fig. 8). The adults flew mostly along the forest border, seeming to prefer the areas of tall grasses along a small stream, where they fed on yellow Asteraceae and *Thymus* flowers. Other taxa relatively common in the same habitat include *Parnassius mnemosyne* (L. 1758), *Aricia artaxerxes* (Fabricius 1793), *Erebia euryale* (Esper 1805), *Erebia epiphron* (Knoch 1783), and *Coenonympha rhodopensis*.

The association of *E. oeme* to damp or wet grasslands is well documented in literature, the species generally being considered as mesophilous to hygrophilous (Abadjiev 1993; Tolman & Lewington 1997, 2008; Varga 2002; Sonderegger 2005). Nevertheless, there are some cases where *E. oeme* is associated with drier biotopes (LSPN 1987).

The biology of *E. oeme* in the Romanian Carpathians is currently unknown. In the rest of its range, the larvae feed on various Cyperaceae and Poaceae such as *Carex*, *Poa*, *Molinia*, *Briza* as well as *Festuca* and *Luzula* (Higgins & Riley 1970; LSPN 1987; Fernández-Rubio 1991; Tolman & Lewington 1997, 2008; Lafranchis 2000; Sonderegger 2005; Lafranchis 2007).

Most of these plants are common in the Carpathians, including the Retezat Mountains (Oprea 2005), so food plant is unlikely to constitute a limiting factor for the potential distribution of *E. oeme* in the Carpathians.

Most of the specimens (males and females) we collected in the Retezat Mountains were rather worn, suggesting that, in Scorota Valley, *E. oeme* reaches the peak of its flight period during the first half of July. Taking into account the altitude where we collected the butterfly (1500-1550 m), these data correlate well with the phenology of *E. oeme* outside the Carpathian chain, where it is generally reported to fly between end of June – mid August, according to altitude and local climatic conditions (Higgins & Riley 1970; LSPN 1987; Fernández-Rubio 1991; Abadjiev 1993; Tolman & Lewington 1997, 2008; Coutsis & Ghavalás 2001; Sonderegger 2005).

In the Red Data Book of European Butterflies (Van Swaay & Warren 1999) *E. oeme* is not listed as threatened in Europe, but it is considered a species of conservation concern due to its European endemic status. It was reported as "most likely vulnerable"

Figure 8
Habitat of *Erebia oeme* in Scorota Valley (Retezat Mts., Meridional Carpathians), 1550 m, 21.VII.2008. Photo V. Dincă

in Bulgaria (Abadjiev 1993) and under significant anthropogenic pressure in the lower parts (800–1500 m) of the Alps (LSPN 1987). Moreover, *E. oeme* was included among the target species of the Prime Butterfly Areas in Bulgaria (Abadjiev & Beshkov 2007).

According to the Climatic Risk Atlas of European Butterflies (Settele *et al*. 2008) *E. oeme* is a species under climate change risk. The modeled distribution of climatic niche for *E. oeme* indicates suitable conditions for this species in some parts of the Carpathians (Slovakia – the Tatra, Romania – parts of the Western and Meridional Carpathians). However, under the "no dispersal" assumption, the species is expected to suffer up to 39% loss of its climatic niche by 2050 and 67% by 2080. Two of the three applied scenarios (BAMBU and GRAS) indicate a total loss of suitable climatic niche from the Carpathian Chain by 2080 (Settele *et al*. 2008). Therefore, although the population from the Scorota Valley lies within the Retezat National Park, it might be affected by factors that are beyond local management measures. On the other hand, it is worth mentioning that exactly in the area where the butterflies fly there is a sheep camp that produces a rather high grazing pressure. Given (1) the very limited known distribution of *E. oeme* in the Carpathians, (2) overgrazing and (3) ominous prognostics by the Climatic Risk Atlas of European Butterflies, we consider the species as vulnerable in Romania.

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