

Pseudo-endemism and cryptic diversity in Lepidoptera – case studies from the Alps and the Abruzzi

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Abstract

The Alps are a hotspot of endemism for Lepidoptera with 230+ species occurring only in this area. In discussing endemism there is, however, a high risk of relying on biased spatial and taxonomic data. Pseudo-endemism may be much more widespread than expected as proved by a week-long excursion to Gran Sasso National Park and Monte Terminillo (Central Italy). Results of this research are five species hitherto only known from the Alps: *Aspilapteryx spectabilis* Huemer, 1994, *Agonopteryx alpigena* (Frey, 1870), *Depressaria incognitella* Heinemann, 1990, *Pammene laserpitiana* Huemer & Erlebach, 1999 and *Epermenia theimeri* Gaedike, 2001; and several additional taxa not yet known from central Italy. Not only is our knowledge of distribution incomplete, alpha-taxonomy of numerous Lepidoptera has been insufficiently explored to date. A transnational barcoding project of all Alpine Lepidoptera within the framework of the International Barcode of Life project (iBOL) is providing strong evidence of much higher levels of cryptic diversity than hitherto known. Examples of probably as yet undescribed Alpine taxa, based on combined molecular and morphological datasets, are demonstrated for the genera *Callisto*, *Eulamprotes* and *Caryocolum*.

Profile

Mountain range

Alps & Abruzzi

Introduction

Butterflies and moths are groups of animals beloved by a great number of people. The beauty of many species and the fascinating metamorphosis may be responsible for a long history of exploration of Lepidoptera in Europe dating back to the Linnean period more than 250 years ago. More than other orders of invertebrates, butterflies and moths have attracted the life-long interest of a large number of amateurs and professional entomologists. As a result of these efforts, species diversity on the European continent is nowadays considered sufficiently well known, at least outside the scientific community. More than 9 000 described species out of 180 000 globally have been recorded so far (Karsholt & Razowski 1996). The Alps, as one of the mega-diverse regions, host an estimated 5 500 species. Major parts of the fauna are more or less widely distributed in Europe and/or Palearctic Asia or even in the Holarctic, whereas endemic taxa restricted to the Alpine Arc or parts of it are less diverse. However, compared with other European ecosystems, the Alps are clearly a hotspot of Lepidoptera in terms of endemism (Huemer 1998). Specific biology and habitat requirements as well as historical glaciation processes strongly influenced the survival of species in unglaciated parts of the Alps (*massifs de refuge* and *nunataks*) or in adjacent refugia and subsequent re-colonization (Holdhaus 1954). Recent distribution patterns of Alpine endemics reflect these influences and the large majority of species is restricted to a fairly small area (Huemer 1998, 2009a).

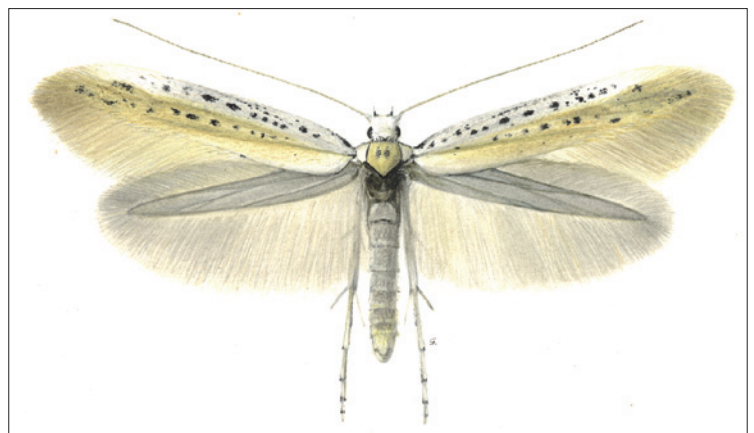


Figure 1 – *Aspilapteryx spectabilis*, hitherto only known from Hobe Tauern National Park (NP), water colour by F. Gregor. © Tiroler Landesmuseum (TLM)

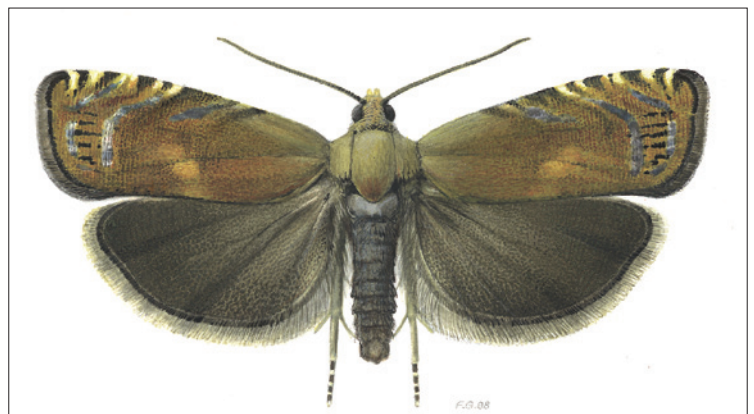


Figure 2 – *Pammene laserpitiana*, initially described from the southern Alps (Monte Baldo, Fennberg), water colour by F. Gregor. © TLM



Figure 3 – Gran Sasso NP, Corno Grande (2912 m), highest peak of the Abruzzi.
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The first endemic species of Lepidoptera from the Alps was already described by Giovanni Antonio Scopoli in his famous *Entomologia Carniolica* (Scopoli 1763). It may be a coincidence that already this taxon, viz. *Crocota niveata* (Geometridae), is based on biased spatial and/or taxonomical data, but it is a typical story of widespread errors in Alpine Lepidopterology. The species was described from a lowland locality in today's Slovenia, a country where it probably never occurred. *C. niveata* is in fact an endemic of the Niedere Tauern in Austria, an area where Scopoli has never collected. An erroneous type locality or alternatively a subsequent misidentification of the type species are possible explanations. Following Scopoli, several lepidopterologists discovered and described new and endemic species from the Alps with peaks in the second half of the 19th and in parts of the 20th century. The most remarkable progress in alpha-taxonomy of Alpine endemics, however, is of recent origin, with almost one-third of the species described during the last two decades (Figure 4). Currently the endemic fauna of Lepidoptera from the Alps covers 230+ species from 32 families (Huemer 1998; unpublished data) but the coverage of diversity still seems incomplete.

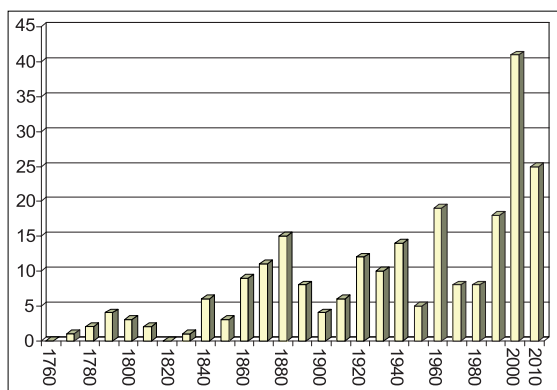


Figure 4 – Numbers of described species of Alpine endemic Lepidoptera per decade.

New endemic species of so-called Microlepidoptera are still described from the Alps almost every year, most recently e.g. *Argyresthia friulii* Huemer, 2010 (Yponomeutidae), *Monocbroa albagonella* Varenne & Nel, 2010 (Gelechiidae), *Monocbroa sperata* Huemer & Karsholt, 2010 (Gelechiidae) and *Dichrorampha tarmanini* Huemer, 2009 (Tortricidae) (Huemer 2009b, 2010; Huemer & Karsholt 2010b; Varenne & Nel 2010). Alpha-taxonomy of Macrolepidoptera is much better analysed, although spectacular unrevised Alpine species groups may be determined even in larger moths and butterflies in future (Huemer & Hausmann 2009). In this paper I will try to demonstrate deficits in spatial and taxonomic data using examples from recent field research outside the Alps in the central Italian Abruzzi (Gran Sasso National Park (NP), Monte Terminillo) (Figures 3 & 7) and extensive barcoding of samples from various, mainly protected, areas of the Alpine Arc.

Material and methods

The material is primarily based on the collections of Alpine Lepidoptera of the Tiroler Landesmuseum Ferdinandeum. Collecting methods varied from time to time but principally covered several standard techniques in entomological research, with a special focus on light trapping (Huemer & Karsholt 2010a). Important parts of voucher material were sampled by the author and colleagues during numerous excursions over the last two decades. Comparative voucher material from the Abruzzi mountains was collected in mid-July 2010. All specimens are spread or set, dried and labelled. Photographs of the adults were taken with an Olympus E3, with a Macro 50 mm 1:2 lens. Genitalia were photographed with an Olympus E1 and an Olympus BH2 microscope. GIS-software BioOffice (<http://www.biooffice.at>) was used to digitalize faunistic data, for data processing and to create maps based exclusively on examined material.

DNA tissue samples from dry legs were sent to the Canadian Center for DNA Barcoding (CCDB, Guelph University, Canada) and DNA extracts are stored at this institution. Handling of tissue as well as data and image submission followed protocols supplied by BOLD (Barcode of Life Data Systems) (Ratnasingham & Hebert 2007). Recovery of mitochondrial cytochrome oxidase I gene (COI) followed automation-friendly standard protocols (Ivanova et al. 2006). A 658 bp region was sequenced, including the 648 bp barcode region described and identified for animals (Hebert et al. 2003). Complete datasets of specimens and barcode sequences are integrated in the project *Lepidoptera of the Alps* (PHLAA, PHLAB) and will be publicly available in BOLD and GenBank from late 2011. Processing and analysis of molecular data follows the analytical tools supplied by BOLD (<http://www.boldsystems.org>). Graphic charts are based on Treegraph 2 (<http://treegraph.bioinfweb.info>).

Results

Pseudo-endemism

Endemics of the Alps are thought to occur only in this mountain area, but such endemism is based on current knowledge of taxonomy and distribution. Obviously these data may be fragmentary and do not necessarily reflect the actual distribution of the species. Taxa where inadequate or uneven surveys have led to a false interpretation of endemism are in fact pseudo-endemics (Cowling 2001). Results from our field research in central Italy support the assumption of widespread pseudo-endemism in Lepidoptera, with records of no fewer than five allegedly Alpine endemics occurring in the Abruzzi:

Aspilapteryx spectabilis Huemer, 1994 (Gracillariidae) (Figures 1 & 5): This species was hitherto only known from the Virgental in East Tyrol (Hohe Tauern NP) (Huemer 1994; 2009). It seems to be restricted to south-facing slopes on limestone at elevations of about 2200 to 2660 m in this area. However, there are large gaps in our knowledge of its biology and habitat preferences, although it is suspected that the larva may be a leaf-miner on an Alpine species of *Plantago*. New record: Italy, L'Aquila, Gran Sasso NP, Campo Imperatore, E Observatorio, 2010 m, 13°34'4"E, 42°26'9"N, 13.7.2010, leg. Huemer.

Agonopteryx alpigena (Frey, 1870) (Depressariidae) (Figure 6): Already described in 1870, this species has been recorded from the entire Alpine Arc, ranging from the French Alps to eastern Austria and from Bavaria to northern Italy and Slovenia. An alleged record from Hungary (Hannemann 1995) remains unconfirmed and is highly unlikely. The larva is restricted to *Laserpitium siler* and feeds on spun leaves of the host plant. New record: Italy, L'Aquila, Gran Sasso NP, Campo Imperatore, ex Miniera di Lignite, 1750 m, 13°42'48"E, 42°25'36"N, 14.7.2010.

Depressaria incognitella Hannemann, 1990 (Depressariidae): This species was hitherto only recorded from few specimens collected in the French, Italian and Swiss Alps (Hannemann 1990; Huisman & Sauter 2001; SwissLepTeam 2010). Nothing has been published about its biology so far. Judging from related species, it may be suspected that the larva feeds on Apiaceae. New record: Italy, L'Aquila, Gran Sasso NP, Campo Imperatore, E Observatorio, 2010 m, 13°34'24"E, 42°26'54"N, 13.7.2010, leg. Huemer.

Pammene laserpitiana Huemer & Erlebach, 1999 (Tortricidae) (Figures 2 & 5): Huemer & Erlebach (1999) described this tortricoid moth from a small corridor of the southern Alps, ranging from Monte Baldo (prov. Verona) to Fennberg (prov. Bolzano). Recently it was also recorded from Slovenia. The type series was bred from seed capsules of *Laserpitium siler*. New records: Italy, L'Aquila, Gran Sasso NP, Campo Imperatore, ex Miniera di Lignite, 1750 m, 13°42'48"E, 42°25'36"N, 14.7.2010; Italy, Rieti, Monte Terminillo N, 1730–1780 m,

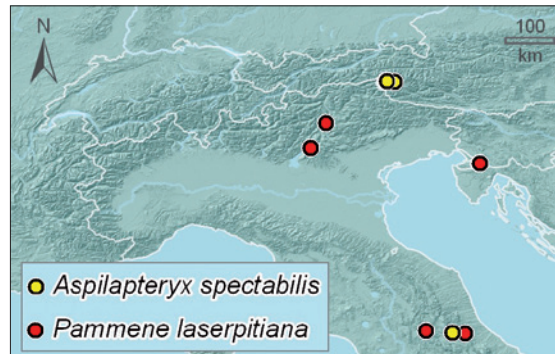


Figure 5 – Distribution pattern of *Aspilapteryx spectabilis* and *Pammene laserpitiana*. © TLM

13°0'0"E, 42°29'0"N, 16.7.2010, leg. Huemer. *Epermenia theimeri* Gaedike, 2001 (Epermeniidae): Gaedike (2001) described this species based on a male and female specimen from Monte Baldo in northern Italy and without any information about the biology. Nothing has been added since the original publication. The new records from Monte Terminillo (Figure 7) in central Italy indicate that *E. theimeri* may be associated with *Laserpitium siler*, which is a common plant at all collecting sites. New record: Italy, Rieti, Monte Terminillo N, 1730–1780 m, 13°0'0"E, 42°29'0"N, 16.7.2010, leg. Huemer.

Apart from these findings, several additional species with limited distribution in European mountain systems are new records to the Gran Sasso NP and central Italy e.g. *Kessleria saxifragae* (Stainton, 1868) (Yponomeutidae), *Aetbes ardezana* (Müller-Rutz, 1922) and *Pelatea klugiana* (Freyer, 1836) (Tortricidae). And last but not least, a number of undescribed species point to considerable research gaps, even in national parks.

Cryptic diversity

Cryptic species complexes are reproductively isolated but morphologically very similar and sometimes virtually identical taxa. Such hidden diversity can only be resolved on a physiological or genetic scale, which



Figure 6 – *Agonopteryx alpigena*, one of three pseudoendemics with trophic linkage to *Laserpitium siler*. © TLM & S. Erlebach



Figure 7 – Monte Terminillo (prov. Rieti), northern slopes. © TLM & M. Mayr

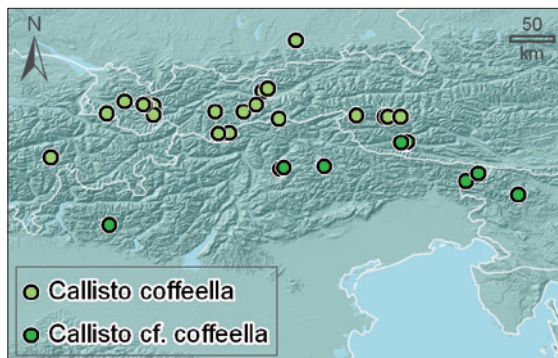


Figure 8 – Distribution pattern of *Callisto coffeella* in the eastern Alps. © TLM

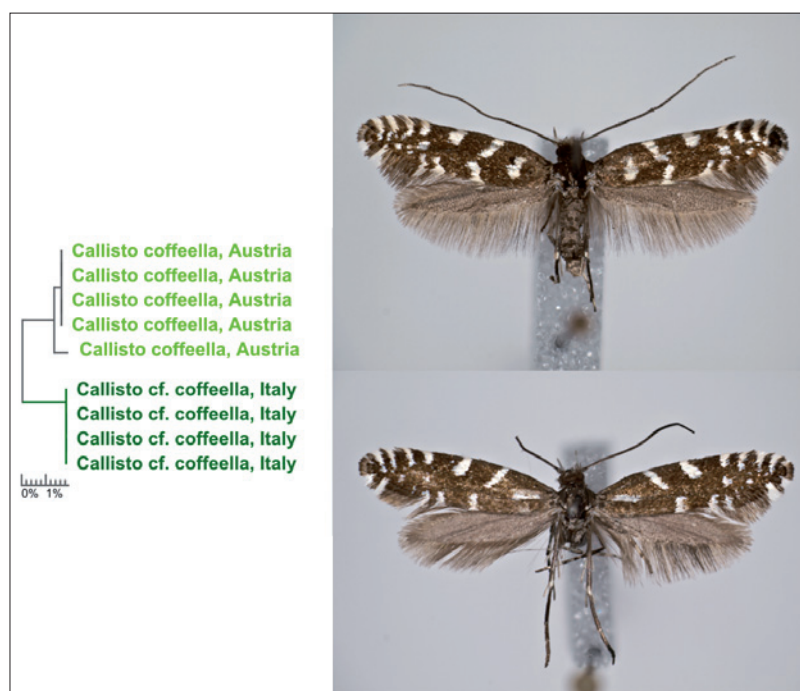


Figure 9 – Genetic distance from the COI barcode fragment and habitus characters of eastern Alpine *Callisto coffeella*. © TLM

sometimes is later reflected by overlooked phenotypical characters. Well known examples in European Lepidoptera are *Colias hyale* (Linnaeus, 1758) and *C. alfacariensis* Ribbe, 1905, which, despite their morphological similarity, are distinctive biological species. However, species delimitation in Lepidoptera is – not least as a consequence of exploration history – still largely based on typological concepts describing morphotaxa rather than biological species (*sensu* Mayr 1970). Recently the increased use of molecular methods in taxonomy and particularly the application of standard gene fragments (barcodes) for species discrimination in Alpine Lepidoptera provide strong evidence of a much higher rate of cryptic diversity and endemism than hitherto known. First and tentative data from our transnational barcoding project, which covers all groups of Lepidoptera from the Alps, confirm recently discovered species complexes, e.g. in high-Alpine genera *Sattleria* and *Sphaleroptera* (Huemer & Karsholt 2010a; Whitebread 2007), but they also prove yet unrecognized cryptic species complexes. Conflicting evidence of genetic data (COI barcode fragment) and morphological characters is exemplified below.

Molecular and morphological diversification

Callisto coffeella-group (Figures 8 & 9): *Callisto coffeella* (Zetterstedt, 1839) (Gracillariidae) is a species with Arctic/Alpine disjunction, which was originally described from Scandinavia. In the eastern Alps the species occurs in two genetic lineages, one in the northern and central Alps, which is close to Scandinavian populations (Mutanen in litt.), and another lineage in the south-eastern Alps. Only three haplotypes were found in Alpine populations. Genetic distances from the COI barcode fragment are significant but moderate, ranging from 1.70% to 1.89%. The adult moths are easily separable by the basal silvery line of the forewings, which is transverse in south-eastern Alpine populations but vertical in all other populations. However, genitalia structures, which are of high diagnostic value in the genus, are virtually indistinguishable. Summing up: the taxonomy remains uncertain, but cryptic diversity is obviously involved, at least on a subspecies level.

Eulamprotes libertinella-group (Figures 10–13): *E. libertinella* (Zeller, 1872) was described from several specimens collected in Styria (Austria) and in Engadin (Switzerland) (Zeller 1872) and later recorded from a major part of the Alps, whereas records from Bohemia and Spain are dubious (Elsner et al. 1999). Biology and host plant are unknown, although *E. libertinella* may be common and is found in several habitats ranging from the lower montane zone up to altitudes of about 3000 m. The species shows a conspicuous brachyptery in the female. The genetic diversity of this species is remarkable and runs to five units. Intraspecific sequence variation is high, with a mean distance of 2.77% and a maximum distance of 5.25% in four clusters, and a mean distance of 9.7% to the fifth cluster. Four genetic lineages are correlated with geogra-

phy and confined to the south-western, central, southern and south-eastern Alps with an allopatric pattern of distribution. The fifth lineage is restricted to the south-western Alps and specimens from this area occur syntopically with the lineage first mentioned (Figures 10 & 11). In this lineage we face a situation where speciation has gone far and gene flow is already interrupted. Examination of voucher material resulted in distinct morphological characters of the fifth lineage compared with other samples (Figures 12 & 13). However, the taxonomic status of four genetic lineages is insufficiently resolved due to a lack of female specimens which offer the most reliable morphological characters.

Intra- or interspecific molecular divergence

Caryocolum peregrinella-group: *Caryocolum peregrinella* (Herrich-Schäffer, 1854) as currently defined (Huemer & Karsholt 2010a) splits into three genetic lineages correlated with the south-western and south-eastern Alps and the Balkans (Dinaric Mountains to Olymp) (Figure 14). Sequence variation at COI between clusters is high, with deep splits of 4.46% to 5.34%, whereas it is low within the lineages (Figure 15). However, preliminary analysis of morphological characters is at odds with barcode differentiation and does not support specific status. Despite this shortcoming, *Caryocolum peregrinella* could well represent an overlooked case of cryptic diversity. Even though this may be scientifically proven in future, one problem has to be considered: the type locality of the species is unknown, the abdomen of the lectotype is missing and the age of more than 150 years (Huemer 1988) will be a major hindrance for recovering DNA.

Discussion

Species diversity is increasingly threatened as documented for instance in the IUCN Red List of Threatened Species and multiple national or regional Red Data Books. Intensified agriculture and forestry, pollution of soil, water and air, excessive infrastructure etc. lead to a dramatic loss of diversity in terms of species and habitats. Protection measures will be of particular importance for endemics with limited distribution, which define the uniqueness of many Alpine areas and should be regarded as a specific treasure (Komposch 2010; Rabitsch & Essl 2009). But what do we really know about endemics in the Alps? Do we rely on biased spatial data? And is alpha-taxonomy of species sufficiently explored? Lepidoptera may be a case in point. The detection of a considerable number of allegedly Alpine endemic species outside the Alps, in the Abruzzi, shows up the huge gaps in knowledge about actual distribution. The discovery of such species in central Italy is not *per se* unexpected, but the quality and quantity of taxa is surprising. Extensive and specific search in related European mountain systems will be necessary to prove the endemic status of every single

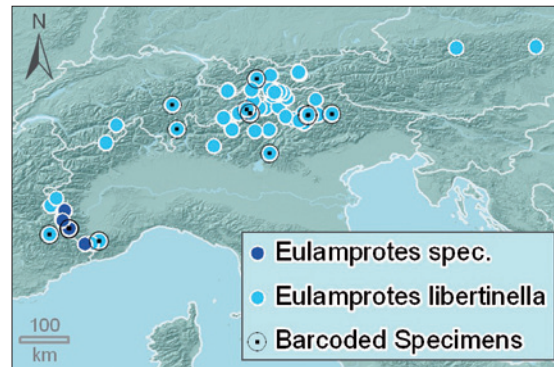


Figure 10 – Distribution pattern of *Eulamprotes libertinella*-complex. © TLM

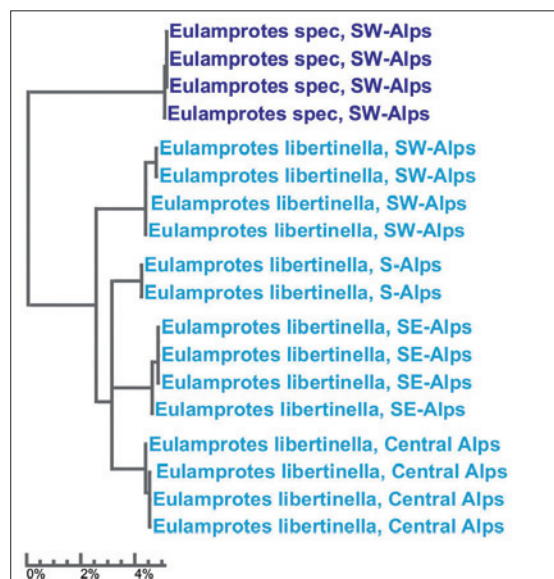


Figure 11 – Genetic distance from the COI barcode fragment of the *Eulamprotes libertinella*-complex. © TLM

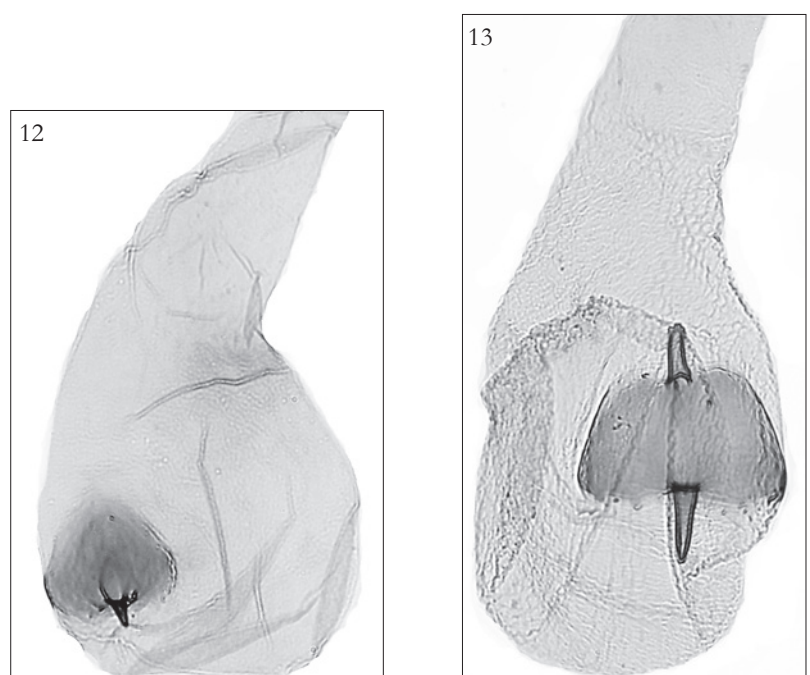


Figure 12 & 13 – Diagnostic and morphological characters of female genitalia in the *Eulamprotes libertinella*-complex. © TLM

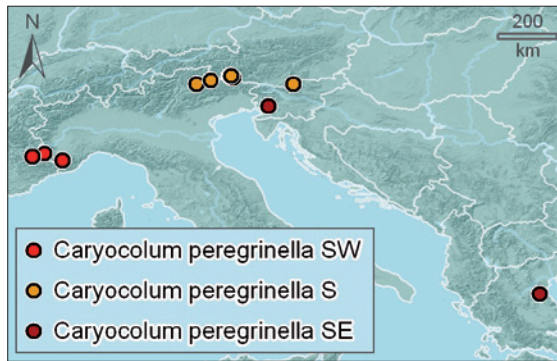


Figure 14 – Distribution pattern of *Caryocolum peregrinella* in the Alps. © TLM

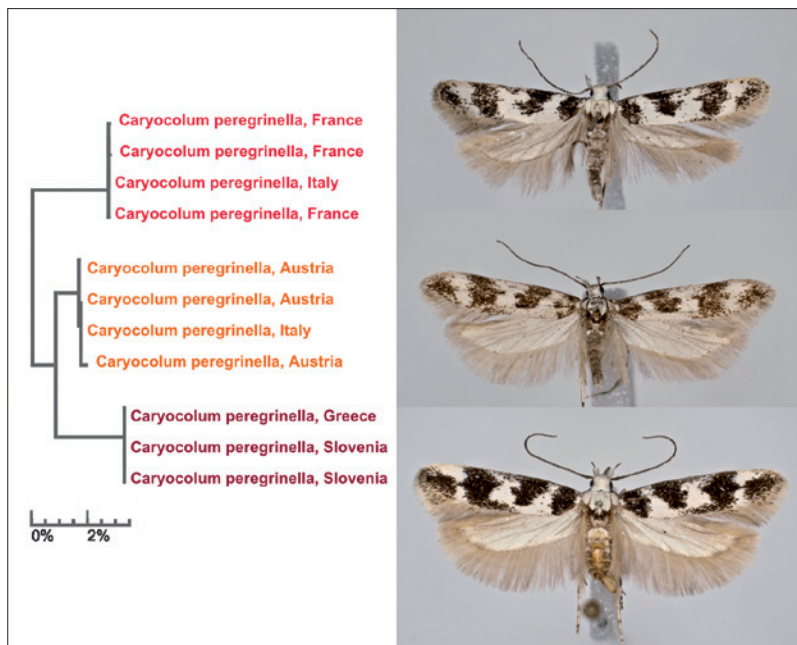


Figure 15 – Genetic distance from the COI barcode fragment and habitus characters of *Caryocolum peregrinella*. © TLM

taxon. Particularly the Balkans, the mountains of central Italy and the Pyrenees and Cantabrian mountains are insufficiently explored, despite their close affinities to the Alps. It is very likely that further endemics will be lost with increasing field research outside the Alps. Conversely, molecular datasets may throw up a considerable number of taxa in the Alps unrecognized to date. Different genetic lineages, frequently reflected in morphology, are widespread in Alpine organisms. The most common feature in plants is a differentiation into four genetic units confined to the south-western, western central, eastern central and eastern Alps (Schönswetter et al. 2005), whereas in animals, genetic units may be similar or often reduced to a western and an eastern lineage (Schmitt 2009). Genetic and morphological differences may be pronounced, as observed for instance in the Alpine endemic butterfly *Erebia melampus* (Fuessly, 1775), see Figure 16, where they are likely to be of interspecific value (Haubrich & Schmitt 2007). However, the number of Lepidoptera species analysed so far is limited and molecular research is usually restricted to butterflies. Our bar-

coding project is a comprehensive attempt to test the usefulness of molecular data for taxonomy of Alpine Lepidoptera. First results are promising but the coverage of species with successfully sequenced barcodes is still limited. Despite of the long tradition of Lepidopterology in Europe and large collections in museums, the lack of suitable material is a major hindrance for a rapid progress in barcoding. In many cases, age and conservation status of specimens have turned out to be inadequate for successful sequencing. Furthermore, even major collections are seriously incomplete and particularly lack rare species such as many endemic micromoths. Despite these shortcomings, initial amounts of molecular data point to a high amount of hidden species richness in the Alps. Such cryptic diversity seems to be higher than average in flightless species and hitherto unknown examples are found in several genera, e.g. *Kessleria*, *Symmoca*, *Eulamprotes* and *Sattleria*. Partial reduction of wings (brachypterous, stenopterous and micropterous species) or complete reduction (apterous species) is a rare exception in Alpine Lepidoptera. It is only observed in less than 1% of the world fauna, though present in 25 families and adopted as a successful family strategy in the Psychidae (Sattler 1991). However, flightlessness is remarkably widespread in Alpine endemics, with about one fifth of the species inventory (Huemer 1998). In the Alpine environment it is interpreted as adaptation to climatic risks such as strong wind and low temperatures. Important consequences of flightlessness are reduced dispersal capacities, which probably have led to increased isolation and disturbance or interruption of gene flow. Further examples of cryptic diversity are to be expected in such comparatively immobile species. Moreover, barcodes – even though still fragmentary – give some evidence to several genetic lineages in fully winged species. Particularly noticeable diversification can be observed in the southern Alps (e.g. Dolomites), south-western Alps and north-eastern Alps. Several of these genetic lineages may be attributed to species complexes, but morphological data are still lacking for many of them. Integrative taxonomy with involving multiple disciplines (Schlick-Steiner et al. 2010) may be of crucial value for delimiting such cryptic diversity in future.

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Figure 16 – *Erebia melampus*, a well studied example of allegedly cryptic diversity in butterflies. © TLM & S. Erlebach

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