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Cupido carswelli (Stempffer, 1927), endemism from SE. Spain, a different species of *Cupido minimus* (Fuessly, 1775): updated distribution, identification of its larval host-plant and notes on taxonomy, ecology and morphology

(Lepidoptera, Lycaenidae)

by

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Abstract: The taxonomic status of *Cupido carswelli* (Stempffer, 1927), an endemic taxon, one of the smallest European butterflies, very local and scarce in southeastern Spain, is still open to debate. In spite of being considered, as a valid species (which is my opinion), different from *Cupido minimus* (Fuessly, 1775), in a good number of references and guides of butterflies, based on differences in genitalia, morphology, ecology and biology, but yet, despite their evident differences that will be discussed in the current work, a minority of authors still considers it as a synonym of *C. minimus* (Fuessly).

A recent molecular study (DNA barcoding, 2015), where the DNA sequences of these and others taxa from the Iberian Peninsula (Spain), proved that *C. carswelli* (Stempffer) is a different species to *C. minimus* (Fuessly). Being the latter absent in the range of distribution of *C. carswelli* (Stempffer). *Cupido carswelli* (Stempffer) is a taxon closely related to *Cupido lorquinii* (Herrich-Schäffer, 1847), but has substantial differences in adult morphology, presenting allopatric distribution as well as important different ecological preferences.

In the current work, the known distribution of *C. carswelli* (Stempffer) is updated, with new localities, which increase the area occupied by their colonies by a 26.6%. Furthermore, the larval host plant of this butterfly is identified for the first time to subspecific level, being *Anthyllis vulneraria reuteri* Cullen, an exclusive plant from SE. Iberian Peninsula (SE. Spain) and N. Africa (Rif). Differences in morphology (adults and pupae) and distribution (allopatric) and ecology of both *C. carswelli* (Stempffer) and *C. lorquinii* (Herrich-Schäffer, 1847), are discussed and illustrated. Notes on taxonomy, ecology and biology are also added.

I consider that *C. carswelli* (Stempffer) and *C. lorquinii* (Herrich-Schäffer, 1847) have recently diverged from each other. Moreover it has been widely accepted that recently diverged species might not be distinguishable on basis of their DNA sequencing (COI), since these taxa have acquired only few genetic differences.

Resumen: El estatus taxonómico de *Cupido carswelli* (Stempffer, 1927), un taxón endémico, muy local y escaso del sureste de España, todavía está abierto a debate. Pese a ser considerado, mayoritariamente, como una especie válida (que es mi opinión), diferente de *Cupido minimus* (Fuessly, 1775), en un buen número de referencias y guías de mariposas, basándose en diferencias en su genitalia, morfología, ecología y biología, todavía existen algunos autores, en minoría, que lo consideran como un sinónimo de *C. minimus* (Fuessly), pese a sus evidentes diferencias que serán comentadas en el trabajo actual.

Un estudio molecular reciente (codificación de barras del ADN, 2015), donde se estudiaron las secuencias de ADN de estos y otros taxones de la Península Ibérica (España), ha determinado que *C. carswelli* (Stempffer) es un taxón diferente de *C. minimus* (Fuessly). Este último no existente en el rango de distribución de *C. carswelli* (Stempffer). Siendo *C. carswelli* (Stempffer) un taxón estrechamente relacionado con *C. lorquinii* (Herrich-Schäffer, 1847), del cual es muy diferente en la morfología de los adultos, presentando una distribución alopátrica y con diferentes preferencias ecológicas.

En el trabajo actual, se actualiza la distribución conocida de *C. carswelli* (Stempffer), con nuevas localidades, que aumentan en un 26,6 % la superficie ocupada por sus colonias. También, la planta nutricia larval de este lepidóptero es identificada, por primera vez, a nivel subespecífico, resultando ser *Anthyllis vulneraria reuteri* Cullen, una planta exclusiva del SE. de la Península Ibérica (SE. Spain) y N. África (Rif). Se exponen e ilustran las diferencias en morfología (adultos y crisálida), distribución (alopátrica) y ecología entre *C. carswelli* (Stempffer) y *C. lorquinii* (Herrich-Schäffer, 1847). Se añaden notas sobre taxonomía, ecología y biología.

Considero que *C. carswelli* (Stempffer) y *C. lorquinii* (Herrich-Schäffer, 1847) son taxones que han divergido recientemente entre sí. Es ampliamente aceptado que las especies recientemente divergidas pueden no ser distinguibles sobre la base de sus secuencias de ADN (COI), ya que estos taxones han adquirido pocas diferencias genéticas.

Introduction: *Cupido carswelli* (Stempffer, 1927) is one of the smallest European butterflies. It is an endemic from SE. Iberian Peninsula (Spain), where it exists in local and small colonies, each with scarce number of individuals.

In 1926, the lepidopterist M. Carswell, caught a butterfly in the Sierra Espuña (W. Murcia Province, SE. Spain), which was new to him, it was sent to Stempffer, who identified it as a new subspecies, which he named in Carswell's honour as „*Cupido minimus carswelli*“ described in Stempffer (1927). The same year, Riley (1927) points out the

specific status of this taxon, with specimens from the same locality caught by another collector, who named it as a new species: „*Cupido arcilasis*“, not knowing Stempffer's previous description. The following year, Stempffer (1928), after a detailed morphological examination (genitalia and imago's morphology) of the butterfly and its congeners, concluded it was a new species, not a mere subspecies of *Cupido minimus* (Fuessly, 1775). Thereafter in Riley (1928), the synonymy of *C. carswelli* (Stempffer) = *C. arcilasis* Riley was carried out.
Higgins (1975) performed further genital studies, these showed that, in spite of the fact that specimens were scarce and that examinations were somewhat incomplete, he had no doubt in considering it to be a valid species.
In Gil-T (2003), for the first time, the preimaginal stages of *C. carswelli* (Stempffer) are described, pointing out some differences in the painting of the pupae between *C. carswelli* (Stempffer) and *C. minimus* (Fuessly). According to the results obtained in this work, I stated that *C. carswelli* (Stempffer) was taxonomically closer to *C. lorquinii* (H.-S.) rather than *C. minimus* (Fuessly), an affirmation that turned out to be correct, as we will see later. In Gil-T (2006) the genital differences of *C. carswelli* (Stempffer), *C. lorquinii* (H.-S.) and *C. minimus* (Fuessly) pointed out by Stempffer (1928) are confirmed. In addition, new data are provided regarding other morphological differences found in pupae of the three previous taxa, mainly in the color of their pilosity, these shall be dealt with later.
Cupido carswelli (Stempffer) is a protected butterfly (Gil-T, 2008) included in the Red Book of the Invertebrates of Andalusia Region (S. Spain), the species is also protected in Murcia Region (SE. Spain).
Finally, in Dinca et al. (2015), where the DNA sequences of the known butterflies from the Iberian peninsula (Spain) are studied, in addition to other interesting taxonomic results obtained, it is stated that *C. carswelli* (Stempffer) is a different taxon from *C. minimus* (Fuessly). Being *C. carswelli* (Stempffer) a taxon closely related to *C. lorquinii* (H.-S.) in accordance to their mitochondrial DNA. Both taxa are very different in the external morphology of their adults, in addition to other differences (ecology, biology, morphology of the chrysalis) which I will mention later.
In the current work, the known distribution of *C. carswelli* (Stempffer) is enlarged. Furthermore, and for the first time, the host plant is identified at subspecific level.

Notes on taxonomy: In general, *C. carswelli* (Stempffer) is mentioned in both, past and new European references, as a valid species, eg.: Manley & Allcard (1970), Higgins & Hargreaves (1983), Tolman & Lewington (1997), Lafranchis (2004), Leraut (2016) as well as others. With some exception, as in FAUNA EUROPAEA (2013), where, surprisingly, it is yet mentioned as a subspecies of *C. minimus* (Fuessly): none of the co-authors responsible for this project had knowledge of some of the previous references mentioned?
It also is really surprising (and wrong, as we will see later), the results obtained in the „morphometric analysis of genitalia and wing pattern“ of Prieto et al. (2009), where these authors seem be in possession of the absolute truth, contrary to the results obtained by Riley (1927), Stempffer (1928), Higgins (1975) and Gil-T (2006) [genitalia, morphology and consideration of its specific validity], it is stated that *C. carswelli* (Stempffer) and *C. minimus* (Fuessly) are the same species, show no differences in their genitalia, and there are no morphological differences between adults (wing pattern), differences obtained and mentioned in Riley (1928), Stempffer (1928), Gil-T (2003) as well as by other authors. The previous statements are not strange to me, because I have observed a certain degree of obstinacy in some authors of this reference (Prieto et al., 2009), mainly by its second co-author, in considering to *C. carswelli* (Stempffer) as a synonym of *C. minimus* (Fuessly), and whom I have corrected several times, in certain number of articles, for his erroneous affirmations on ecology, biology, distribution and taxonomy of other Spanish butterfly species.
In Dinca et al. (2015) is demonstrated that the affirmations in Prieto et al. (2009) regarding *C. carswelli* (Stempffer) are not true. On the other hand, I consider that, with the improvement and current use of techniques and molecular phylogenetic studies, to differentiate species, the use of „morphometric analysis of genitalia and wing pattern“ is an inaccurate, unreliable and non-definitive tool to separate related species, since there is a wide range of variation for some traits (wings patters, size and characters of the genitalia), among other factors, I would stand out, the size variation of the adults used in the sample. Another example can be found in Kolev (2005), where a morphometric analysis was used, similar to the procedure taken up by Prieto et al. (2009), within the aim to demonstrate the specific validity of *Polyommatus andronicus* Coutsis & Ghavalas, 1995: a taxon which subsequent molecular studies demonstrated as being a synonym of *Polyommatus icarus* (Rottemburg, 1775).
In Dinca et al. (2015), DNA sequencing of Iberian butterfly fauna was undertaken, and amongst the valuable taxonomic data obtained, it is concluded that *C. carswelli* (Stempffer) is a species different to *C. minimus* (Fuessly): in the phylogenetic tree got in that work, *C. minimus* (Fuessly) forms a separate clade of the clade composed of *C. lorquinii* (H.-S.) and *C. carswelli* (Stempffer). As a result, in this reference is stated: „the current DNA barcode data suggest that *carswelli* is distinct from *minimus* and more closely related to *lorquinii* rather than to *minimus*, despite obvious differences in ♂♂ (blue upperside in *lorquinii* and dark brown in *carswelli*)“.
In fig. 1 it is clearly noticeable the morphological differences between *C. carswelli* (Stempffer) and *C. lorquinii* (H.-S.): a striking fact considering that they are „closely related“ taxa. The close taxonomical relation between *C. carswelli* (Stempffer) and *C. lorquinii* (H.-S.) was mentioned for the first time in Gil-T (2003), by analizing the pre-imaginal stages of *C. carswelli* (Stempffer). Summarizing: Riley (1927), Stempffer (1928), Higgins (1975), and Gil-T (2006) were right to separate to *C. carswelli* (Stempffer) from *C. minimus* (Fuessly) when they studied their genitalia.
The case of „*carswelli-lorquini*“ is alike to that between other taxa very taxonomically related, considered species, for example between *Polyommatus golgus* (Hübner, 1813) and *Polyommatus dorylas* (Denis & Schiffermüller, 1775),

considered in Dinca et al. (2015) in the category of "lumped" (specimens of two or more species that were recovered as a single genetic entity), two species closely related [Additional note: I believe and I am convinced that *Polyommatus sagratrox* (Aistleitner, 1986) should be in the same „lumped" group („*golgus-dorylas*"), but this taxon is not mentioned, inexplicably, in the work of Dinca et al. (2015). Further information on this can be found in Gil-T (2013) and Gil-T & Ibáñez (2009)].
The application of DNA barcoding has been hindered by the difficulty of distinguishing closely related species, especially in cases of recently diverged taxa. It is accepted that recently diverged species might not be distinguishable on the basis of their COI (mitochondrial cytochrome oxidase I gene, DNA) sequences. This is on account of the fact that the COI has not yet accumulated sufficient sequence differences, therefore these species have acquired very few genetic differences, meaning that there is a scarce number of characters to discriminate them. I am convinced that this can be the case of *C. carswelli* (Stempffer) with respect to *C. lorquinii* (H.-S.).
The characteristics between different species (morphology, reproductive incompatibily, different ecology, etc) are acquired at different times during the process of lineage divergence. On account of this, recently diverged species may not yet have acquired the entire set of these characters (and as a consequence are often difficult to separate). *Cupido carswelli* (Stempffer) is easy to separate from *C. lorquinii* (H.-S.), since these taxa are very different in their morphology, present allopatric distribution, and show ecological and biological differences, which will be mentioned in the following sections.

Notes on morphology (adult, chrysalid, genitalia): Really, the ♂♂ of *C. carswelli* (Stempffer) and *C. lorquinii* (H.-S.) are very different morphologically (fig. 1): ♂♂ of *C. lorquinii* (H.-S.) presents almost the entire upperside of violet-blue color, with broad dark marginal borders extending basically along the veins. However the upperside of the wings of *C. carswelli* (Stempffer) is very dark gray-brown (fig. 1), with only some blue-violet scales at the base of the upperside wings, sometimes scarcely visible [note: blue-silvery scales in *C. minimus* (Fuessly)]. The wings underside of *C. carswelli* (Stempffer) and *C. lorquinii* (H.-S.) are similar, also the arrangement of the postdiscal black spots of the hind wings underside: black spots on the spaces s2-s5 (see fig. 5) are aligned or slightly arched (the postdiscal black spot in s2 is inwardly displaced in *C. minimus* (Fuessly). All of these morphological features have been described and accepted by several authors.
The preimaginal stages of *C. carswelli* (Stempffer) were described for the first time in Gil-T (2003) and the chrysalis of *C. lorquinii* (H.-S.), also for the first time in Gil-T (2002). In Gil-T (2003) certain differences between the chrysalises of *C. carswelli* (Stempffer) [very small; length: 5,5 - 6,7 mm; wide abdomen: 2 - 2,3 mm] and of *C. minimus* (Fuessly) are pointed out: the lateral zone of the abdomen of *C. minimus* (Fuessly) is marked with large black spots (fig. 2: M), being this a constant feature when compared to those existing in *C. carswelli* (Stempffer) (fig. 2: C) chrysalis.
The pupae pattern in both, *C. carswelli* (Stempffer) and *C. lorquinii* (H.-S.), were of a similar appearance, but according to new observations in Gil-T (2006), the color of the pilosity (thorax and abdomen covered with long hairs) is different: in *C. carswelli* (Stempffer) these are clearly of yellow (Fig. 2: C), while in both, *C. lorquinii* (H.-S.) and *C. minimus* (Fuessly), the pilosity is white (fig. 2: L and M).
The genitalia differences were dealt with in detail in Gil-T (2006).

Distribution of *Cupido carswelli* (Stempffer, 1927), with new localities: During years it was believed that *C. carswelli* (Stempffer) is only present in the Sierra Espuña, W. Murcia Province (the UTM grid shown most to the east in map of the fig. 3) subsequently new finding were recorded in localities outside of Sierra Espuña, some references to these are: Bretherton (1966) and Tarrier (1993) in the NE. Granada Province; Andújar & Gómez (1985) in the Sierra de Alcaraz, S. Albacete Province, mentioned as „*C. minimus*" (sic); in Gil-T (1998), for the first time registered in Almería Province (Sierra de María); Gil-T (2003) in the Sierra de Orce, NE. Granada Province; Tolman & Lewington (1997) and Obregón (2011) in NE. Jaén Province, and others.
In fig. 3 we can appreciate the presently known distribution of *C. carswelli* (Stempffer), which is limited to S. Albacete, W. Murcia, NE. Jaén, NE. Granada and N. Almería Provinces. Showing the four new UTM 10 x10 km (located in Sierra de Segura, NE. Jaén Province, NE. Andalusia Region) where have been located new colonies of this scarce and local butterfly. I have also located this taxon in the E. Sierra de Cazorla (NE. Jaén Province, Borosa river), new locality, but its respective UTM grid is not new, the site is located in the westermost area in the map in fig. 3. These new colonies, as well as the others previously known, occupy an insignificant area in each UTM grid. These four new UTM grids, plus the other three mentioned in Gil-T (1998, 2003), represent 46.6% of its total known distribution.
It is important to notice that *C. minimus* (Fuessly) is absent in Andalusia Region, S. Spain. *C. lorquinii* (H.-S.) exists in Morocco, Algeria, S. Spain (S. Andalusia Region and S. Extremadura Region) and southern Portugal. In fig. 4 we can see a map of Andalusia showing two polygons obtained after connecting the end of the distribution areas of both, *C. lorquinii* (H.-S.) and *C. carswelli* (Stempffer). We are able to see the allopatric distribution of these two closely related taxa, according to the molecular study mentioned before, but as we have already seen, with separate distribution and different morphology. In the following section we expose their ecological and biological differences.

Identification of its larval host plant at subspecific level. **Notes on ecology and biology**: Tennent (1993) states an altitude range of 1400-2600 m in north Africa for *C. lorquinii* (H.-S.). In S. Spain (Andalusia) however, other references indicate an altitude from 0 to 1800 m, although in reality it can reach up to 2050 m in the Sierra Nevada (unplublished and new data, pers. obs.)

In NE. Andalusia *C. carswelli* (Stempffer) lives between 1350 and 1800 m (only pers. obs.), while in the Sierra Espuña (W. Murcia prov.) it probably flies lower as these mountains are of minor altitude.
Within the altitude range in Spain I have personally observed two larval host plants for *C. lorquinii* (H.-S.): *Anthyllis vulneraria gandogeri* (Sagorski), which exists between 0-1500 m and is the subspecies of *Anthyllis vulneraria* most widespread in Spain; and *Anthyllis vulneraria reuteri* Cullen. In Novoa et al. (1995) is also mentioned as host plant to *Anthyllis vulneraria maura* (Beck), which exists between 100-1300 m. *Cupido lorquinii* (H.-S.) probably uses other subspecies of *Anthyllis vulneraria* throughout its wide range of altitudes.
The only larval host plant I have observed and can confirm for *C. carswelli* (Stempffer) has been *Anthyllis vulneraria reuteri* Cullen (fig. 5). This plant can be found in an altitude range between 500-2000 m, its distribution is limited only to SE of the Iberian Peninsula and N. Africa (Rif). A characteristic of this subspecies of *Anthyllis vulneraria*, different to others, is the presence of various auxiliar glomerului (1-4) underneath the terminal glomerulus (fig. 5). In Cuvelier & Tarrier (2002: 392) is mentioned erroneously as „*Hippocrepis* sp." (sic) as a host plant of *C. carswelli* (Stempffer).
In Gil-T (2002) is demonstrated that *C. lorquinii* (H.-S.) can have two annual generations, the second one partial, based on the results obtained in connection with its ecology, phenology and experimental rearing in captivity. *Cupido carswelli* (Stempffer) however has only one. *C. lorquinii* (H.-S.) flies from the end of March (at low altitudes) up to mid July (at higher altitude). On the other hand *C. carswelli* (Stempffer) is normally in flight from the end of April until the end of May.
We appreciate that the two taxa, of allopatric distribution, show different ecological preferences.

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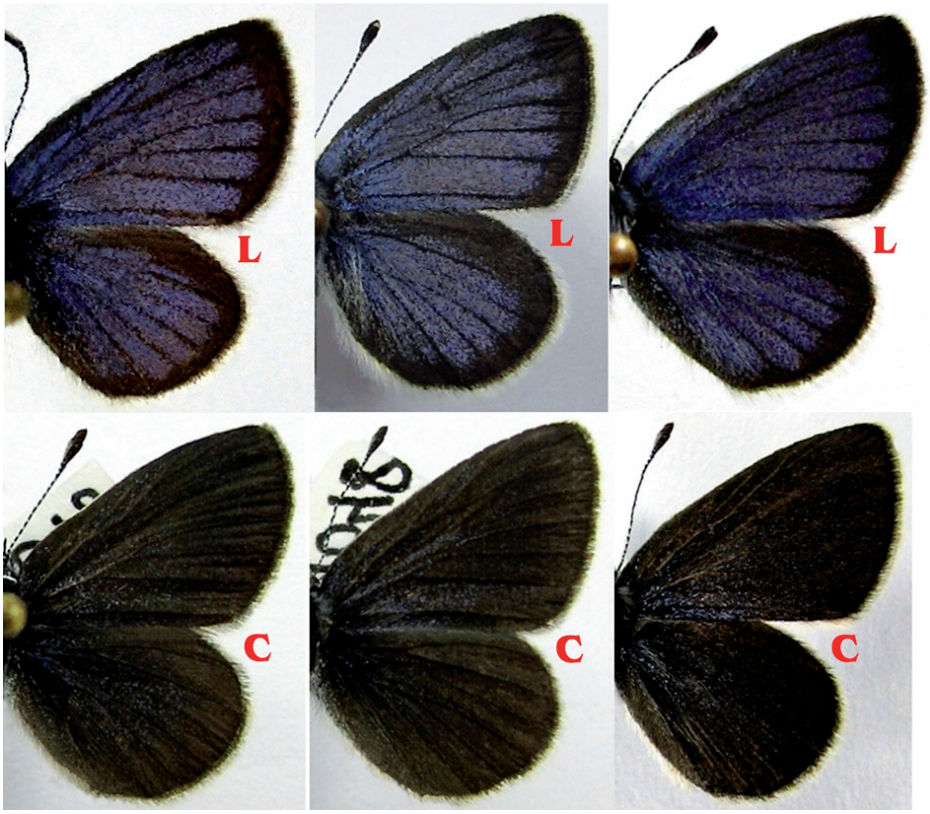

Fig. 1: ♂♂ - Morphology markedly different of *Cupido carswelli* (Stempffer, 1927) (C), and *Cupido lorquinii* (Herrich-Schäffer, 1847) (L).

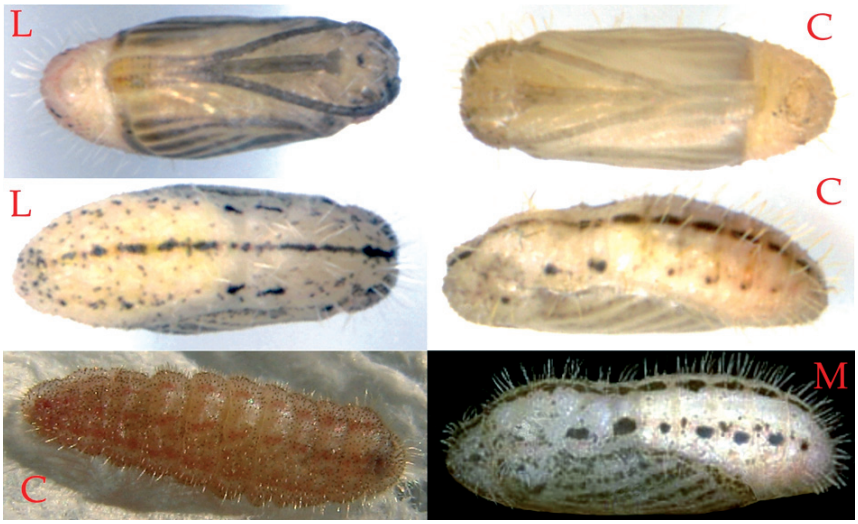

Fig. 2: C = Pupa (yellow pilosity) and larva of *Cupido carswelli* (Stempffer, 1927). L = pupa (white pilosity) of *Cupido lorquinii* (Herrich-Schäffer, 1847). M = pupa (abdominal lateral black spots), very different from the previous ones, of *Cupido minimus* (Fuessly, 1775).

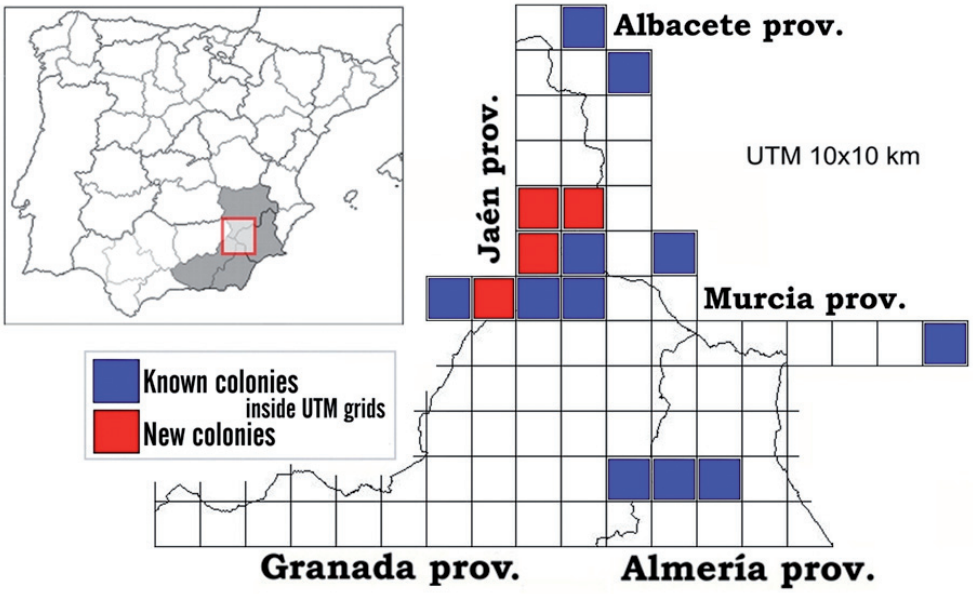

Fig. 3: Update distribution of *Cupido carswelli* (Stempffer, 1927) in SE. Iberian Peninsula (Spain).

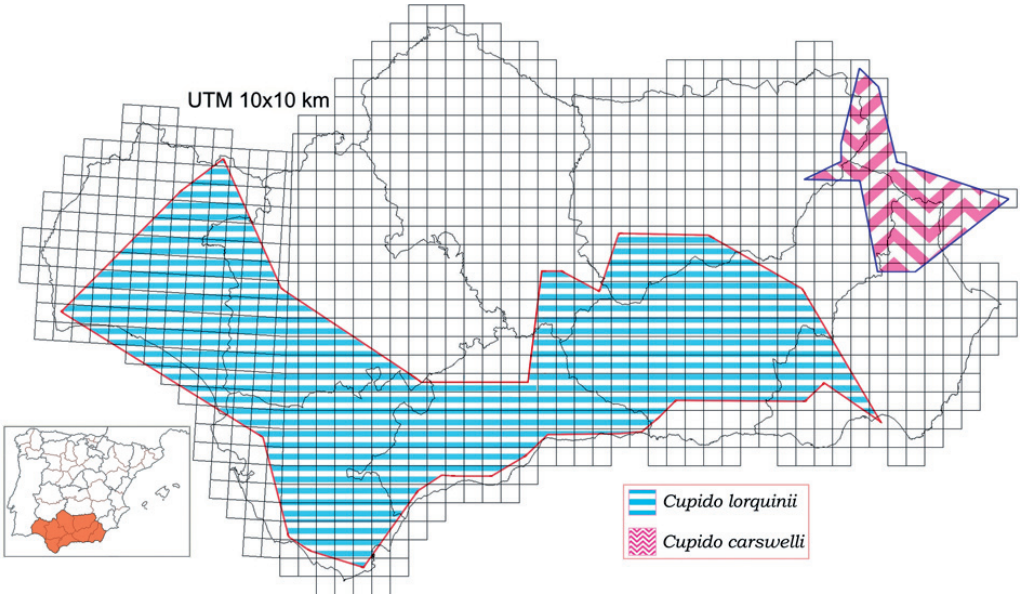

Fig. 4: Allopatric distribution of both, *Cupido carswelli* (Stempffer, 1927) and *Cupido lorquinii* (Herrich-Schäffer, 1847), in Andalusia Region, S. Spain.

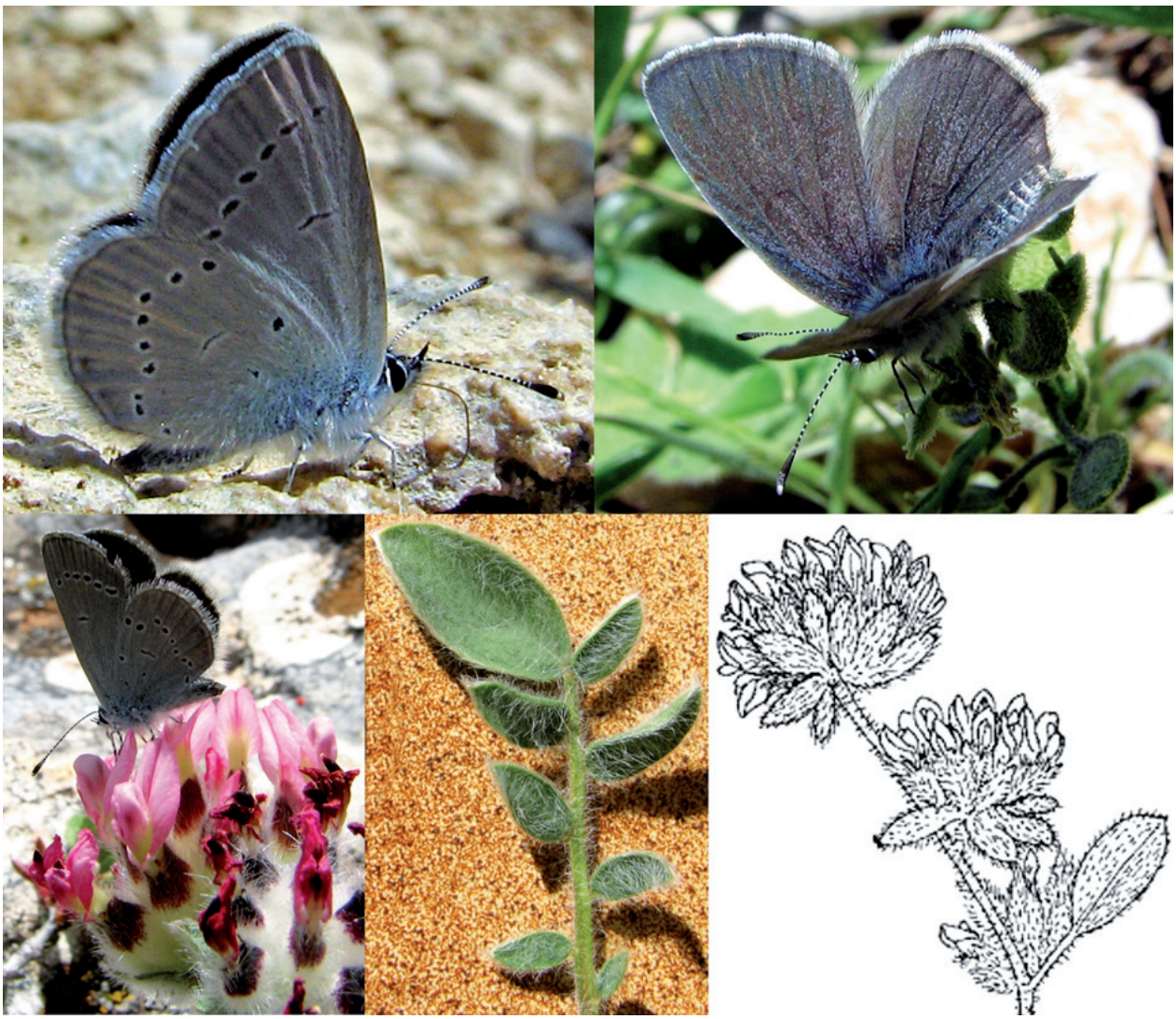

Fig. 5: *Cupido carswelli* (Stempffer, 1927) in nature. Leaves and flowers (glomerulus) of its larval host plant *Anthyllis vulneraria reuteri* C.

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