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Zygaena diaphana Staudinger, 1887 bona species (Lepidoptera: Zygaenidae)

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Abstract

Zygaena purpuralis (Brünnich, 1763), *Z. minos* ([Denis & Schiffermüller], 1775) and *Z. diaphana* Staudinger, 1887 with the same label data from Pelister National Park (Mt. Baba) in southwestern North Macedonia were discovered in the Witt collection (Zoologische Staatssammlung Munich). Consequently, on the basis of sympatry and good differences in male genitalia with *Z. minos*, *Z. diaphana* is reinstated to species rank. *Zygaena smirnovi* Christoph, 1884, is also treated as separate species.
KEY WORDS: Lepidoptera, Zygaenidae, *Zygaena purpuralis*, *Z. minos*, *Z. diaphana* stat. rev., *Z. smirnovi* stat. rev., sympatry, Pelister, Balkan Peninsula, North Macedonia.

Zygaena diaphana **Staudinger, 1887 buena especie (Lepidoptera: Zygaenidae)**

Resumen

Fueron descubiertas en la colección Witt (Zoologische Staatssammlung, Múnich), *Zygaena purpuralis* (Brünnich, 1763), *Z. minos* ([Denis & Schiffermüller], 1775) y *Z. diaphana* Staudinger, 1887 con la misma etiqueta y datos del Parque Nacional Pelister (Monte Baba) en el suroeste de Macedonia. Consecuentemente, sobre la base de simpatría y buenas diferencias en la genitalia del macho con *Z. minos*, se restituye al rango de especie a *Z. diaphana*. *Zygaena smirnovi* Christoph, 1884, también se restituye al rango de especie.
PALABRAS CLAVE: Lepidoptera, Zygaenidae, *Zygaena purpuralis*, *Z. minos*, *Z. diaphana* stat. rev., *Z. smirnovi* stat. rev. simpatría, Pelister, Península Balcánica, Macedonia norte.

Introduction

Zygaena Fabricius, 1775 is a Palaearctic genus represented by 108 species according to HOFMANN & TREMEWAN (2017). One of the most problematic groups in *Zygaena* is the *Z. purpuralis* complex. *Z. purpuralis* and *Z. minos* are cryptic species which can be distinguished only by male and female genitalia, larva and larval host-plants. Although initially described as species, they were considered conspecific in the 19th and the beginning of the 20th century until REISS (1940, 1941) examined the genitalia and proved that they are in fact distinct species (*Z. purpuralis* and *Z. pimpinellae* Guhn, [1913] sensu Reiss). ALBERTI (1958-1959) gave priority to the taxon *Z. diaphana* over *Z. pimpinellae*; this was followed by REISS & TREMEWAN (1960, 1967). The correct identity and authorship of both species has been established by TREMEWAN (1981a, 1981b) as *Zygaena purpuralis* (Brünnich, 1763) and *Z. minos* ([Denis & Schiffermüller], 1775). TREMEWAN (1981b) treated *Zygaena diaphana* Staudinger, 1887 as a subspecies of *Z. minos*. According to the last revision of this species group by NAUMANN *et al*. (1983) and NAUMANN & NAUMANN (1985) only two species were recognized, viz. *Z. purpuralis* and *Z. minos*. Another taxon that belongs to this group was also described as a species, *Z. smirnovi* Christoph, 1884. It was downgraded to a subspecies of *Z. minos*

(NAUMANN *et al*., 1983). CESARONI *et al*. (1989) did similar morphometric analysis to that of NAUMANN *et al*. (1983), but they used multivariate statistics and as a result got three clusters *Z. purpuralis*, *Z. minos* and *Z. diaphana* from Yozgat in Turkey. In the same study, results of allozymic analysis were confusing as gene flow seems to occur between sympatric populations of *Z. purpuralis* and *Z. minos* in Abruzzo in Italy. However, allozymes of *Z. diaphana* were not analyzed, nor of any population outside of Italy. The sample was not extensive and did not include many important populations. However, the results showed that a reasonable doubt still exists that more than two cryptic species are involved. HOFMANN & TREMEWAN (1996, 2010, 2017) and NAUMANN *et al*. (1999) followed their initial decisions that there are just two species: *Z. purpuralis* and *Z. minos*.

In *Z. minos* ([Denis & Schiffermüller], 1775) two groups are recognized: the *"minos*-group" and the *"diaphana*-group" with differences in male genitalia, larvae and host-plants (NAHIRNIĆ & TARMANN, 2016; HOFMANN & TREMEWAN, 2017). Differences in genitalia are known only in males. In *Z. diaphana* the uncus is slender while the lamina dorsalis has a triangular shape. In *Z. minos*, the uncus is only slender and pointed at the top and the sides of the lamina dorsalis are convex. *Z. minos* final instar larvae are of very light mint blue to light grey. The final instar larvae of *Z. diaphana* from eastern Serbia and western Bulgaria are greenish bright yellow (NAHIRNIĆ & TARMANN, 2016), the final instar larvae from Chelmos are greyish dark olive-green (NAHIRNIĆ & TARMANN, 2016), while those from Turkey are light grey to dark grey, usually with a narrow and sometimes broad, pale yellow mediodorsal line (HOFMANN & TREMEWAN, 2017).

Populations of the *"minos*-group" feed on *Pimpinella* species, while those of the *"diaphana*-group" feed on *Eryngium* species. Both the *"minos*-group" in Crimea (EFETOV, 1990) and the *"diaphana*-group" on Toros dağlari in Turkey (HOFMANN & TREMEWAN, 2017) feed on *Falcaria vulgaris* Bernh.; the latter larvae accept *Eryngium* sp. in captivity (HOFMANN & TREMEWAN, 2017). The distribution of the *"minos*-group" includes Sweden, central and eastern Europe, western Balkan Peninsula to Russia and Transcaucasia, while the *"diaphana*-group" ranges from the southern, central and eastern Balkan Peninsula to Turkey and Transcaucasia. Since both taxa have never been found in sympatry they were considered to belong to the same species by many authors.

New investigations on the *Z. purpuralis* complex with special emphasis on the Balkans including extensive examination of specimens revealed a sympatry of the *"minos*-group" and the *"diaphana*-group" on Mt. Baba in North Macedonia.

Material and methods

Genitalia dissections were done according to ROBINSON (1976). Some of the genitalia were mounted in Euparal on slides, while others are preserved in micro-vials filled with glycerol. All specimens are deposited in the Witt collection in the Zoologische Staatssammlung, Munich, Germany (ZSM). Photographs of genitalia slides were done with an Olympus E-4 camera mounted on an Olympus BH-2 stereo microscope.

Results

New records of *Z. minos* and *Z. diaphana* from the wider area of their sympatry in the Balkans:

Zygaena minos ([Denis & Schiffermüller], 1775)

Republic of North Macedonia, Veles, Topolka Gorge, 150 m, 6 ♂♂, 27-V- 07-VI-1979, leg. (J. de Freina). Pelister [Mt. Baba], 18 km W Bitola, 1900 m, 1 ♂, 11-VII-1980, leg (P. Schaider). Pelister [Mt. Baba], 18 km W Bitola, 1800 m, 1 ♂, 11-VII-1980, leg (P. Schaider). Albania, Devoll river, 2 ♂♂, VI-1934

Zygaena diaphana Staudinger, 1887 **stat. rev.**

Republic of North Macedonia, Pelister [Mt. Baba], 18 km W Bitola, 1900 m, 1 ♂, 11-VII-1980,

leg. (P. Schaider). Pelister [Mt. Baba], 18 km W Bitola, 1800 m, 1 ♂, 11-VII-1980, leg (P. Schaider). Umgebung Bitola, Pelister [Mt. Baba], 1500-1750 m, 1 ♂, 07-08-VII-1979, leg. (J. de Freina)

These are the first reports of *Z. minos* in North Macedonia and Albania. The report of *Z. minos* from Albania in NAHIRNIĆ *et al*. (2013) has to be referred to *Z. diaphana*. The same applies to NAUMANN *et al*. (1983) for North Macedonia since the genitalia of all North Macedonian specimens are illustrated and belong to *Z. diaphana*. *Zygaena purpuralis* is found in the same series as *Z minos* and *Z. diaphana* mentioned above, except at Devoll river.

Discussion

Specimens of *Z. purpuralis, Z. minos* and *Z. diaphana* have the same label data from Pelister National Park (Mt. Baba). All three were collected at the same place at elevations of 1800 and 1900 m. There are not many roads "18 km west from Bitola" which lead to these elevations. In fact, there are two possibilities: the slope next to the road to Široka or the meadows above the Hotel "Molika" which is 12 km west from Bitola where the road ends and then a few kilometers uphill on the foot above the "Kopanki" mountain hut. Paul Schaider used frequently to collect around the hotel "Molika" in the 1980s (Predrag Jakšić, pers. comm.). As *Z. minos* and *Z. diaphana* were found to be sympatric and synchronic on one mountain range, and most probably syntopic, *Z. diaphana* Staudinger, 1887 must be reinstated to species rank. Moreover, there are clear differences in male genitalia (Fig. 1) and habitus between the males of *Z. minos* and *Z. diaphana* from that series. However, on Mt. Baba determination based on habitus is not possible because *Z. minos* and *Z. diaphana* can't be easily distinguished from *Z. purpuralis* due to its high variability. The male genitalia of *Z. purpuralis* are also illustrated on figure 1. De Freina collected only *Z. diaphana* on Mt. Baba at altitudes of 1500-1750 m. This locality could be above hotel "Molika" which is at 1450 m, and around mountain hut "Kopanki" which is at 1630 m. In the 1980s, Schaider and de Freina were both frequently collecting only above hotel "Molika" accompanied by Predrag Jakšić (Predrag Jakšić, pers. comm.). Although this was after the years when they collected *Z. minos* and *Z. diaphana*, the most accessible locality for collecting at that time was the area of hotel "Molika" and its surroundings.

Other discoveries of *Z. minos* in the Balkan Peninsula show that *Z. minos* is geographically closely distributed to *Z. diaphana* (Figure 2). COUTSIS (2017) found *Z. diaphana* on one more locality at Mt. Baba on its southern side which belongs to Greece (in Greece known as Varnús or Peristeri). COUTSIS (2017) reported it as *Z. minos* but according to his description of male genitalia it is clear that all specimens belong to *Z. diaphana*. In the West on the nearby Mt. Galičica, separated by Prespa Lake from Mt. Baba, *Z. diaphana* is a common species (NAUMANN *et al*., 1983, NAHIRNIĆ & TARMANN, unpublished). The southernmost occurrence of *Z. minos* in the Balkans is at Devoll River in Albania in the South-West from Mt. Galičica. Another locality in the southern distributional limit of *Z. minos* is the Topolka River Gorge in central North Macedonia. This population is quite isolated from others of *Z. minos* and *Z. diaphana*. In recent years, knowledge of the distribution of *Z. minos* and *Z. diaphana* has considerably improved. New localities and new country records in the Balkans have been published in NAHIRNIĆ *et al*. (2013), COUTSIS (2017) and NAHIRNIĆ *et al*. (2019). Nonetheless *Z. minos* has been reported as new for Belgium based on old museum material (RENNESON, 2018). The intensive ongoing research will probably reveal more closely distributed and sympatric populations.

Genitalia slides of *Z. minos* from Mt. Baba were already prepared by Karl-Heinz Wiegel, though the date of these genitalia preparations remains unknown to the author of this paper. It is strange that Wiegel didn't notice that NAUMANN *et al*. (1983) published *Z. diaphana* (then as *Z. minos*) from Mt. Galičica and illustrated the genitalia different that those he had done from nearby Mt. Baba. If he (or anyone else who could have seen these genitalia slides) had been triggered by the presence of two different genitalia on two nearby mountains and consequently examined more specimens from Mt. Baba they would likely have found *Z. diaphana* on Mt. Baba sympatric with *Z. minos* and resolved this problem a long time ago.

Preliminary field studies on Mt. Baba could not confirm the presence of *Z. minos* nor *Z. diaphana*.

The visited localities were Kopanki at 1500-1900 m and the road to Široka. Overgrowing of vegetation was noticed in both visited areas. The reason for their possible absence may be that habitats favorable for *Eryngium amethystinum* L., which is the host-plant of *Z. diaphana* on nearby Mt. Galičica (Nahirnić, pers. obs.), or potential host-plant *Eryngium campestre* L. which was observed in lower altitudes on Mt. Baba, are both subject to succession. *Eryngium campestre* could have occupied early successional habitats made by construction of the ski center "Kopanki" which was officially put into operation on 04-XI-1975.

Differences in habitats occupied by *Z. minos* and *Z. diaphana* on the Balkan Peninsula are evident. If all Balkan populations of *Z. diaphana* were considered to belong to *Z. minos*, it would be very difficult to explain why would a xero-mesophilous species like *Z. minos* have made a sudden shift to xero-thermophilous habitats in the central Balkan Peninsula when proceeding to the South. In the Dinaric mountains *Z. minos* inhabits *Mesobromion* grasslands close to the coniferous forest at an altitude of 1200-1300 m. On Mt. Baba it probably inhabits clearings in *Pinus peuce* Griseb. forest. In the southern Balkans, *Z. diaphana* is found in dry rocky grasslands mainly between 1200-1800 m. In the eastern part of the Balkans it is found also in dry rocky grasslands from 350 to 1000 m (NAHIRNIĆ *et al*., 2019). Localities where *Z. minos* were found are sheltered and always in vicinity of the forest while those of *Z. diaphana* are open and windy, very often on exposed mountain slopes. Moreover, why should this species make a host-shift? At several localities where *Z. minos* occurs, *E. amethystinum* or *E. campestre* occur as well. The same applies for localities of *Z. diaphana*, where *Pimpinella* sp. were found.

Another question which could challenge reinstatement of *Z. diaphana* is what could be the refugium of each species during the Pleistocene glaciations? *Z. diaphana* is much more widely distributed in the Balkans than *Z. minos*. One of the reasons for this maybe the availability of the larval host-plants as *E. campestre* is common throughout all of the Balkans and *E. amethystinum* is common in the mountains in southern Balkans. *Z. diaphana* has a continuous range in the Balkans while *Z. minos* is very local with geographically distant populations. The majority of the localities of *Z. minos* on the Balkans are near or at refugial localities such as deep gorges or small gorges of east-west direction. Populations from North Macedonia and Albania are well-differentiated from other Balkan populations which means that they had time to differentiate and that they were present in the Balkans before the postglacial period. Dispersed distribution of *Z. minos* in the Balkans could well be explained as relict populations from Pleistocene which migrated over the Dinaric Alps and Scardo-Pindhic mountain system and found refugia there. As *Z. minos* occurs in Crimea, Georgia, Armenia and Dagestan, it is possible that one way of expansion during the glaciations to the south was along the Black Sea to Transcaucasia and further to the South. Another way could be along the Apennine Peninsula. A possible refugium of *Z. diaphana* could be southern Balkans and Turkey. *Zygaena diaphana* shows an expansion which is most probably postglacial. Apparently different origins of *Z. minos* and *Z. diaphana* in the Balkans indicate the possibility that they were already different species during the last Glacial Age. They must have been in contact during the glacial and interglacial stages, but in the whole Balkan Peninsula specimens with intermediate male genitalia have not been found so far.

A check list of the subspecies of *Z. minos* and *Z. diaphana,* primarily based on male genitalia morphology, is provided below. All these subspecies were treated as *Z. minos* by HOFMANN & TREMEWAN (1996) and EFETOV (2004).

Zygaena minos ([Denis & Schiffermüller], 1775)
- *Z. minos minos* ([Denis & Schiffermüller], 1775)
- *Z. minos sareptensis* Rebel, 1901
- *Z. minos normanna* Verity, 1922
- *Z. minos viridescens* Burgeff, 1926
- *Z. minos ingens* Burgeff, 1926
- *Z. minos dagestana* Sheljuzhko, 1936

Zygaena diaphana Staudinger 1887, **bona sp., stat. rev.**

Z. diaphana diaphana Staudinger, 1887, **stat. rev.**

Z. diaphana clavigera Burgeff, 1914, **comb. n.**

Z. diaphana peloponnesica Holik, 1937 **comb. n.**

Z. diaphana alagezi Holik & Sheljuzhko, 1953, **comb. n.**

Z. diaphana alanyca Reiss & Reiss, 1972, **comb. n.**

Z. diaphana tatvanica Reiss & Reiss, 1973, **comb. n.**

Further revision is needed as several taxa are provisionally treated here as *Z. diaphana*. Preliminary results of sequencing of the 658-bp region the COI mitochondrial gene *Z. minos persica* Burgeff, 1926, initially described as *"Smirnowi* [sic] Christoph var. *persica* n. v." showed a distance of 5% from all other samples of *Z. purpuralis*, *Z. minos* and *Z. diaphana* which originated from Italy, Austria, Balkans, Ukraine and Turkey (NAHIRNIĆ & TARMANN, 2016; NAHIRNIĆ & TARMANN, unpublished). Based on the male and female genitalia and the external appearance *persica* and *smirnovi* are most probably conspecific, and this would lead to the reinstatement of *Z. smirnovi* because it has priority over *persica*. If *Z. smirnovi* would be accepted here as conspecific with *Z. diaphana* to which it is morphologically closer than to *Z. minos* this would cause synonymy of *Z. diaphana* as *Z. smirnovi* was described earlier. *Z. smirnovi* Christoph, 1884, stat. rev., is therefore also treated here as separate species and *Z. smirnovi persica* Burgeff, 1926 as subspecies of the same species. There is a constant difference in male and female genitalia between *Z. smirnovi* and other taxa from *Z. purpuralis* complex. *Zygaena smirnovi* has a slender uncus but it is not so slender as in *Z. diaphana*. The lamina dorsalis is close to that of *Z. purpuralis* but it is slightly triangular. Lastly, female genitalia are similar to those of *Z. minos* and *Z. diaphana*. The known larval host-plant of *Z. smirnovi persica* is *Eryngium creticum* Lam. (Keil, 2014). Even *tatvanica* could be closely related to *Z. smirnovi*. There is an ongoing research on *Z. smirnovi* which will reveal more detailed information. The genitalia morphology of *alagezi* is more similar to that of *Z. diaphana* than to that of *Z. minos* while its biology is unknown.

Based on this discovery the distribution of *Z. minos* is as follows: France, Belgium, Italy, Germany, Switzerland, Austria, the Czech Republic, Denmark, Sweden, Poland, Lithuania, Estonia, Belarus, Slovakia, Hungary, Bosnia and Herzegovina, Montenegro, Albania, North Macedonia, Romania, Ukraine, Russia, Georgia and Armenia. *Zygaena diaphana* is distributed in Serbia, Albania, North Macedonia, Bulgaria, Greece, Turkey, Armenia and Iran.

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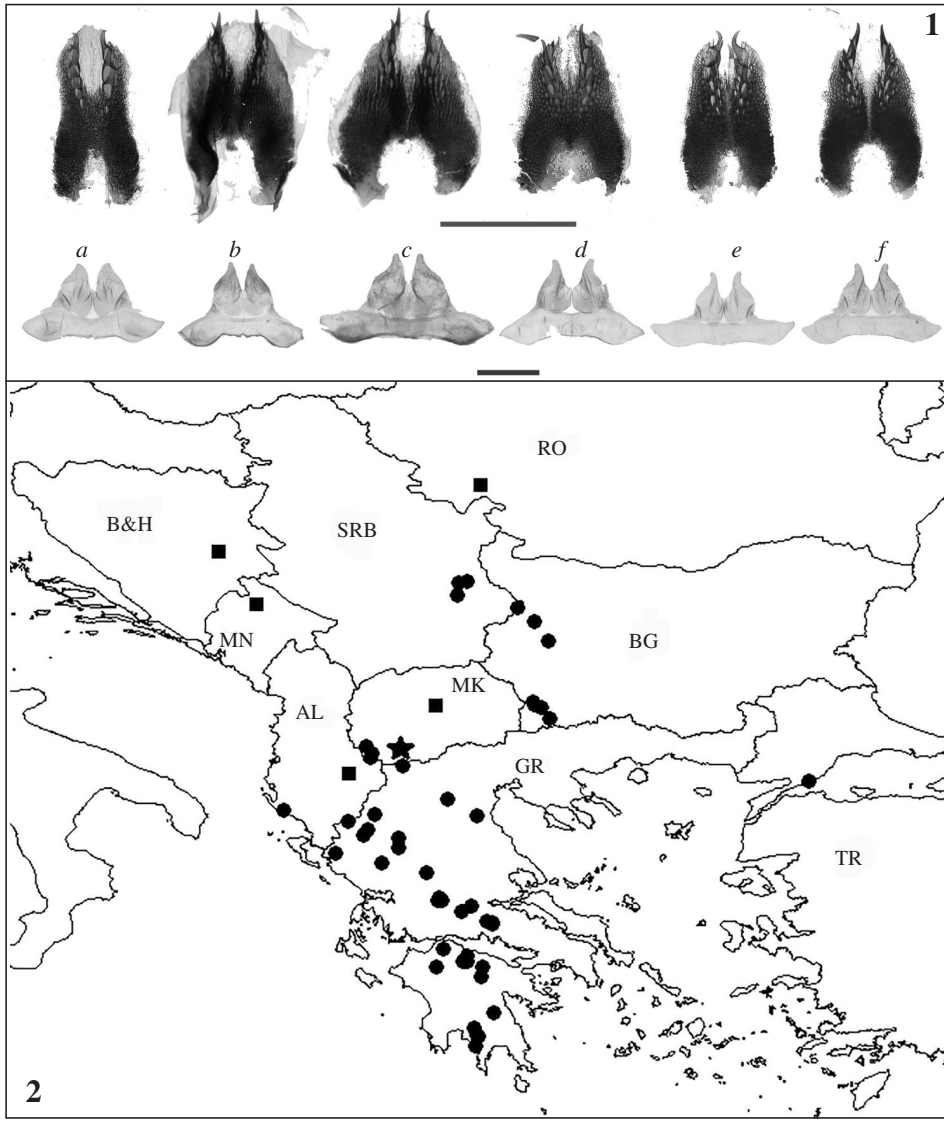

Figures 1-2.– 1. Laminae dorsales (upper row) and uncus-tegumen complexes (lower row) of *Zygaena purpuralis* (Brünnich, 1763), *Z. minos* ([Denis & Schiffermüller], 1775) and *Z. diaphana* Staudinger, 1887 from Mt. Baba. **a.–** *Z. purpuralis*, Mt. Baba, 18 km W Bitola, 1900 m; **b.–** *Z. minos*, Mt. Baba, 18 km W Bitola, 1900 m; **c.–** *Z. minos*, Mt. Baba, 18 km W Bitola, 1800 m; **d.–** *Z. diaphana*, Mt. Baba, 18 km W Bitola, 1800 m; **e.–** *Z. diaphana*, Mt. Baba, 18 km W Bitola, 1900 m; **f.–** *Z. diaphana*, Mt. Baba, Umgebung Bitola, 1500-1750 m. Scales for laminae dorsales and uncus-tegumen complexes are 1 mm. **2.** Distribution of *Zygaena minos* ([Denis & Schiffermüller], 1775) and *Z. diaphana* Staudinger, 1887 on the Balkan Peninsula. Squares - *Z. minos*, circles - *Z. diaphana*, asterisk - *Z. minos* and *Z. diaphana* sympatric. B&H - Bosnia and Herzegovina, MN - Montenegro, SRB - Serbia, RO - Romania, AL - Albania, MK - Republic of North Macedonia, BG - Bulgaria, GR - Greece, TR - Turkey. Locality in Romania is not on the Balkan Peninsula but it is very close to its border.