Original article (Orijinal araştırma)

Size and shape variability of the wing in burnet moth, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae)[1]

Burnet güvesi, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae)'nde kanadın boy ve şekil değişkenliği

Tina KLENOVŠEK[2*] ⓘD   **Predrag JAKŠIĆ**[3] ⓘD   **Franc JANŽEKOVİČ**[4] ⓘD

Abstract

The burnet moth, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae) is a distinctly polymorphic species of moth appearing in several color morphotypes. This study examined whether the variability of the *Z. ephialtes* forewing could be explained by geometric morphometric methods. The analysis included 70 male specimens from five localities in Montenegro (Plužine, Tepca, Dobrilovina, Gusinje and Rugovska Gorge), and one locality in North Macedonia (Kožuf Mountain) belonging to the subspecies *Zygaena ephialtes pannonica* Holik, 1937 and *Zygaena ephialtes istoki* Silbernagel, 1944, respectively, collected between 1981 to 2018. The forewing outline and the aposematic color pattern formed by five spots were analyzed separately. Neither forewing size nor forewing spots pattern size exhibited interpopulation heterogeneity. Size variation of the basal spot was independent from the rest. Considering the forewing outline shape, the apical portion was the most variable and different among populations. Two morphological groups were recognized: a group with a narrower pointed forewing (samples: Kožuf Mountain, Rugovska Gorge and Tepca), and a group with a wider forewing with a blunt apex (Plužine, Gusinje). Neither the outline or the spots pattern of the forewing supported the distinction between the subspecies *Z. e. pannonica* and *Z. e. istoki*.

Öz

Burnet güvesi, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae) farklı renk morfotiplerinde görülen belirgin şekilde polimorfik bir güve türüdür. Bu çalışmada, *Z. ephialtes*'in ön kanatlarındaki değişkenliğin geometrik morfometri yöntemleri ile açıklanıp açıklanamayacağını incelenmiştir. Analizde, 1981 ile 2018 yılları arasında toplanan sırasıyla *Zygaena ephialtes pannonica* Holik, 1937 ve *Zygaena ephialtes istoki* Silbernagel, 1944 alt türlerine ait Karadağ'daki beş bölgeden (Plužine, Tepca, Dobrilovina, Gusinje, Rugovska Gorge) ve Kuzey Makedonya'daki bir bölgeden (Kožuf Dağı) 70 erkek örnek kullanılmıştır. Beş noktanın oluşturduğu ön kanat taslağı ve aposematik renk modeli ayrı ayrı analiz edilmiştir. Hem ön kanat boyu hem de nokta desen boyu, popülasyonlar arası heterojenlik göstermemiştir. Bazal noktanın boyut değişimi diğerlerinden bağımsızdır. Ön kanadın anahat şekli göz önüne alındığında apikal kısım, popülasyonlar arasında en değişken ve farklı olanıdır. Daha dar sivri uçlu bir grup (Örneğin: Kožuf Dağı, Rugovska Gorge ve Tepca) ve küt bir tepeye sahip daha geniş bir ön kanatlı bir grup (Plužine, Gusinje) olmak üzere iki morfolojik grup tanımlanmıştır. Ön kanadın ne anahattı ne de benek deseni, *Z. e. pannonica* ve *Z. e. istoki* alt türleri arasındaki ayrımı desteklememiştir.

Introduction

The burnet moth, *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae) is a larger representative of the burnet family with a forewing 15-19 mm long. Adult animals show a pronounced aposematic pattern of coloration (Figure 1). The forewing is black with five or six spots (stigma), in white and yellow or red. The abdomen is black, with yellow or red abdominal belt (cingulum) on only one segment. The species is polymorphic, with two basic forms: peucedanoid and ephialtoid. Both forms come in red and yellow. *Z. ephialtes* is also characterized by mimetic forms, the coloration of the ephialtoid form is similar to the *Amata* genus Fabricius, 1807 (Lepidoptera: Erebidae) (Batesian-Müller mimicry). Only one generation is present in the life cycle, adult specimens having a short life span, and flying from late may to august (Hofmann & Tremewan, 2020). The species inhabits thermophilic dry grasslands on limestone. Its areal entails central and southern Europe, extending to Asia Minor (Türkiye) (Naumann et al., 1999).

Understanding the pronounced polymorphism of the *Z. ephialtes* is a challenge for researchers (Hofmann, 2003a, b, c). The entire Zygaenoidea group is the subject of in-depth phylogenetic analyses (Yen et al., 2005 and the literature collected there). Attempts have been made to explain interpopulation variability by morphometric and genetic approaches (Hofmann, 2003a, b, c). Researchers have been looking for links to evolutionary changes during the last glacial period, where populations survived in various refuges in which they had previously evolved. Zoogeographic interpretation of morphological variability seeks answers in recolonizations from multiple refuges with partially specialized populations, which has led to contact between different haplotypes, thereby also greatly increasing morphological variability (Hofmann, 2003a, b, c). Genomic analysis does not yield unambiguous results on subspecific taxonomy. Taxonomic affiliation at the subspecies level is based primarily on geographical distribution. According to Hofmann (2003c), populations of *Z. ephialtes* used in this study belong to the subspecies *Zygaena ephialtes pannonica* Holik, 1937 and *Zygaena ephialtes istoki* Silbernagel, 1944. The discriminating characteristic in taxonomy for the subspecies *Z. e. pannonica* and *Z. e. istoki* is that specimens of subspecies *Z. e. pannonica* are always five-spotted with spots 1 and 2 and cingulum on abdomen yellow, whereas specimens of *Z. e. istoki* are five-spotted with spots 1 and 2 and abdominal ring (cingulum) either red or yellow. The percentage of red and yellow forms differs according to the region (more yellow in the north, more red towards the south of the range of this subspecies). However, research on morphological characteristics and anatomical features conducted so far does not allow a reliable distinction between subspecies (Hofmann, 2003a, b, c). Another source of phenotypic variability may be due to adaptations to local ecological conditions, leading to ecotypic diversity (Bai et al., 2015; Martin et al., 2016).

The primary purpose of this study was to contribute to the understanding of the phenotypic variability of the burnet moth, *Z. ephialtes* in the western Balkan Peninsula. We analyze the forewing outline size and shape variability as well as the spots pattern using methods of geometric morphometrics (Adams et al., 2013). More specifically, we intend to find answers about (1) whether the morphometric variability of the forewing coincides with the geographical distribution of the populations, and (2) whether the interpopulation variability corresponds with the subspecies affiliation.

Figure 1. The burnet moth, *Zygaena ephialtes* is a characteristic species of dry grasslands. Suva Planina Mountain, photo by P. Jakšić.

Materials and Methods

The sample comprised 70 specimens of *Z. ephialtes* collected at six localities in Montenegro and North Macedonia (Table 1 and Figure 2) in grassland habitats at altitudes between approx. 690 and 925 m. To exclude variability associated with sexual dimorphism, only males were used in the analysis. The specimens were collected and identified by coauthor Predrag Jakšić (PJ) in the period from 1981 to 2018 as part of various biodiversity research projects, and are deposited in a dry collection of PJ in Belgrade.

Table 1. Localities of populations (MN, Montenegro and MK, North Macedonia), subspecies, altitude, geographic coordinates and number of specimens of *Zygaena ephialtes*

| Locality	Subspecies	Altitude (m)	Coordinates	Specimens
Plužine (MN)	*Z. e. pannonica*	690	43°09'41" N, 18°47'41" E	15
Tepca, Tara River Canyon (MN)	*Z. e. pannonica*	900	43°12'08" N, 19°04'26" E	7
Dobrilovina, Tara River Canyon (MN)	*Z. e. pannonica*	800	43°01'37" N, 19°23'50" E	5
Gusinje, Prokletije Mountain (MN)	*Z. e. pannonica*	925	42°32'50" N, 19°49'31" E	12
Rugovska Gorge, Prokletije Mountain (MN)	*Z. e. pannonica*	820	42°41'09" N, 20°10 40" E	14
Kožuf Mountain (MK)	*Z. e. istoki*	810	41°11'29" N, 22°18'37" E	17
Total				70
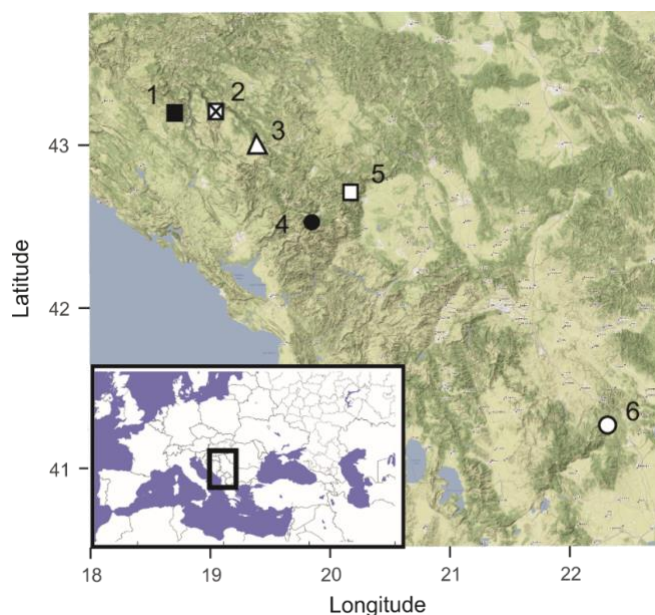
Figure 2. Balkan Peninsula depicting localities of *Zygaena ephialtes* (1, Plužine; 2, Tepca; 3, Dobrilovina; 4, Gusinje; 5, Rugovska Gorge; and 6, Kožuf Mountain).

Images of the dorsal side of left forewings were taken under constant conditions with a Nikon D 3200 camera with AF-S Micro Nikon lens. Landmarks were used to describe the forewing morphology from two aspects, the forewing outline and the pattern of color spots (Figure 3). In total, 14 landmarks were used to describe the outline of the wing, and 22 landmarks to describe the shape and arrangement of the five color spots. Landmarks were digitized using the TpsDig2 software (Rohlf, 2015). Generalized Procrustes analysis (GPA) was performed to standardize size and remove the differences in landmark configurations due to position and orientation. Size information was preserved as centroid size (Bookstein, 1991) and shape information as Procrustes coordinates (Zelditch et al., 2012). The GPA and all subsequent analyses were performed for the forewing outline and the spots separately. Therefore, we analyzed the centroid size and shape of the forewing outline, as well as the centroid size and shape of the forewing spots pattern. Additionally, to get centroid sizes of individual spots, we performed a GPA for each spot.
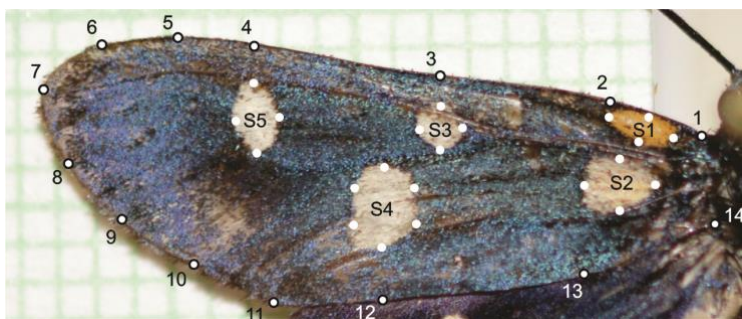
Figure 3. Left forewing of the *Zygaena ephialtes* with 36 landmarks (LM). Landmarks on the outline of the wing (LM 1-14), and spots S1-5 (LM 15-36). Forewing outline: 1, base of the wing; 2, at distal edge of S1; 3, at midst of S3; 4, at midst of S5; 5 and 6, at 1/3 and 2/3 outline distance between LM 4 and 7; 7, apex; 8, 9 and 10, at 1/4, 2/4 and 3/4 outline distance between LM 7 and LM 11; 11, juncture of the outer and the rear wing edge; 12, at midst of S4; 13, at distal edge of S2; and 14, base of the wing. Spots: S1-5: landmarks at proximal, distal, upper and lower edges of spots (in S4 proximal and distal edges described with two equally distanced landmarks).

Analysis of variance was used to examine wing and spots pattern size variability between geographic localities (populations). The strength of connection in size among individual spots was analyzed by the Pearson correlation coefficient. Principal component analysis (PCA) was used to explore the pattern of variation among specimens in the forewing and spots shape as well as to reduce the number of shape variables. Scatter plots of the first two principal components (PCs) were used for visualization. Shape changes along the first PC were presented by wire-frame graphs based on the thin plate spline algorithm (Bookstein, 1991). PCA was used also to search for outliers. One outlier from Kožuf Mountain and one from Rugovska Gorge were excluded from forewing shape and spots shape analyses, respectively. Differences among localities with larger sample sizes (n > 10) were tested using multivariate analysis of variance (MANOVA) and nonparametric permutation test of Procrustes distances (Bookstein, 1991) among populations mean shapes. To control results for increased likelihood of type I error from multiple comparisons, we used a Bonferroni correction. Given the relatively small sample sizes, MANOVA was performed with a reduced set of shape variables (first 10 PCs explaining 94.2% and 82.3% of total variance in the forewing outline and spots pattern, respectively). We estimated the effect of wing and spots size on overall variation in wing and spots shape by multivariate regression of shape onto size and estimated the statistical significance of the regression by a permutation test with 10,000 iterations against the null hypothesis of complete independence between shape and size. All analyses were performed using the MorphoJ software (Klingenberg, 2011) and SPSS Version 28.0.

Results

Forewing size and shape

There was no statistically significant variation in forewing size ($F_{5,64}$ = 0.63, p = 0.674) among the six geographic samples of *Z. ephialtes*. However, highly significant differences were observed in the outline shape (Wilks' λ < 0.001, $F_{30,129}$ = 109, p < 0.001). A PC 1 vs 2 scatter plot, explaining 52.58% of the total shape variation, revealed grouping of specimens from the Kožuf Mountain, Rugovska Gorge and Tepca. Specimens from these locations had mostly positive PC 1 scores (Figure 4) and were characterized by a narrower distal (costal and apical) part and wider basal part of the forewing (Figure 4). Specimens from Plužine and Gusinje had the widest distribution interval and mostly negative PC 1 scores, which was characterized by a broader apical part of the wing. Specimens from Dobrilovina had average values on both PCs and therefore the mean forewing shape. No distinction among the six samples was visible along PC 2. Wing shape differences between the two groups of samples according to the PC 1 vs 2 graph (Kožuf Mountain, Rugovska Gorge, Tepca vs Plužine and Gusinje) were statistically significant (Wilks' λ = 0.266, $F_{10,53}$ = 14.6, p < 0.001).
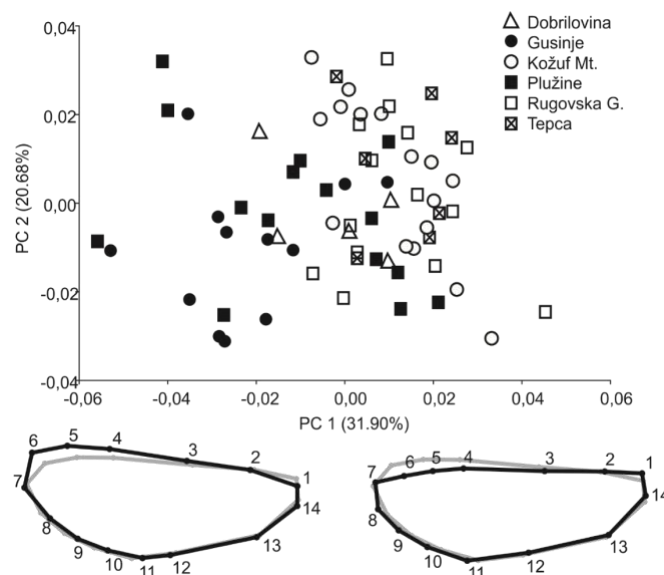
Figure 4. Scatter plot of the first two PCs for the forewing outline of the six populations of *Zygaena ephialtes* with wire-frame graphs showing wing shape changes along the first PC axis for the unit of 0.1 (black) in the negative (left) or positive direction (right) compared to the mean of PC 1 (gray).

Pair wise tests for differences in the mean wing outline shape (permutation test for Procrustes distances between mean shapes) showed specimens from Gusinje had in average the most unique wing shape, especially compared to specimens from Kožuf Mountain and Rugovska (Procrustes distances >0.035 and p<0.001; Table 2). Specimens from Rugovska and Kožuf Mountain were the most similar. The influence of allometry on the wing outline shape was statistically significant (p = 0.001) but low 6.05%.

Table 2. Procrustes distances and p-values from permutation tests for Procrustes distances among populations (n > 10) of *Zygaena ephialtes*. Significance after Bonferroni correction for multiple comparisons is p<0.0125

	Gusinje	Kožuf Mountain	Plužine
Kožuf Mountain	0.041; <.001		
Plužine	0.025; 0.007	0.025; 0.003	
Rugovska G.	0.041; <.001	0.010; 0.654	0.024; 0.007

Forewing spots pattern

There was no statistically significant variation in the total spots size ($F_{5,64}$ = 1.72, p = 0.143) among the six populations. We also investigated whether the variability in the sizes of individual wing spots is related. The correlation analysis showed significant but weak (r < 0.5) positive correlations between spots S2-5 (Table 3). The variability in the size of spot S1 was completely independent and did not correlate with any other spot.

Table 3. Correlation coefficients and p-values among centroid size values for individual spots (CS_{S1-5})

	CS_{S1}	CS_{S2}	CS_{S3}	CS_{S4}
CS_{S2}	0.17; 0.159			
CS_{S3}	0.14; 0.261	0.44; <0.001		
CS_{S4}	-0.12; 0.311	0.32; 0.007	0.27; 0.023	
CS_{S5}	-0.17; 0.168	0.19; 0.107	0.38; 0.001	0.34; 0.004

No differences were observed between geographic locations in the wing spots shape pattern (Wilks' λ = 0.456, $F_{30, 132}$ = 1.36, p = 0.124). Also, PC 1 vs 2 scatter plot, explaining only 33.0% of the total shape variation, showed no grouping of the specimens (Figure 5). The scatter of specimens along PC 1 showed the main variation in relative position and shape of spots S3 and S4 compared to the whole pattern (Figure 5).

In specimens with positive values on PC 1, S3 had a more proximal position, closer to S1 and S2, and S4 was, compared to specimens with negative values on PC 1, narrower. PCA was repeated separately for S3 and S4 landmark subsets (results not shown). Similarly, to the whole set of spots, no grouping of specimens according to populations was visible either for S3 or S4 along PC 1 or 2.

Pairwise tests for differences in the mean shape of spots pattern showed significant differences (p < 0.0125 after Bonferroni correction for multiple comparisons) only between specimens from Gusinje and Kožuf Mountain (p = 0.006). The influence of allometry on the wing spots pattern was not significant (p = 0.145).
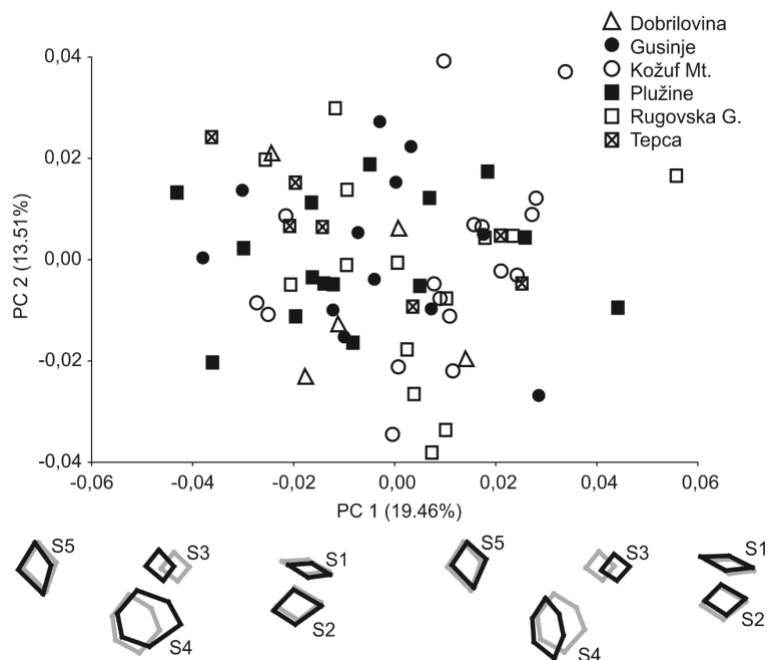

Figure 5. Scatter plot of the first two PCs for the pattern of forewing spots of the six geographical localities of *Zygaena ephialtes* with wire frame graphs showing spots pattern shape changes along the first PC axis for the unit of 0.1 (black) in the negative (left) or positive direction (right) compared to the mean of PC1 (gray).

Discussion

The morphometric study of the forewing of the burnet moth, *Z. ephialtes* in the western Balkan Peninsula showed no difference in the size of either the wing or the spots pattern among the populations. However, the wing outline shape distinguished two groups, one with narrow pointed forewings and the other with broad forewings with a blunt apex. Morphometric variation did not coincide with the geographical distribution of the populations. In the spots pattern, the relative position of the middle two spots was the most variable, without relation to population structuring. The assumption of a morphological distinction between subspecies *Z. e. pannonica* and *Z. e. istoki* was not apparent in any aspect of forewing morphology.

Forewing size and shape

Phenotypic variability in body size and shape is well expressed in insects (Blanckenhorn, 2009; Whitman & Agrawal, 2009). Larger specimens exhibit greater fitness, and usually come from environment with rich and quality food supply. Body size of insects is directly correlated with the quality and quantity of nutrients (Blanckenhorn, 2009; Whitman & Agrawal, 2009). Inadequate nutrition has a strong inhibitory effect on physical development of all organ systems in insects, as well as a strong effect on reduced fertility, especially evident from smaller number and size of eggs. Environmental variability (e.g., climatic conditions)

indirectly affects insect body size, but according to Whitman & Agrawal (2009), short-lived insects with an r-strategy do not have a large need for plasticity. They build their evolutionary success on a large number of offspring, the life cycle of short-lived adults being too short for selection pressures for phenotypic adaptations to particular climate environment to be asserted. In our study, we did not confirm significant differences in forewing size of the *Z. ephialtes* from different locations (populations), which could also be explained by a pronounced r-strategy of *Z. ephialtes*, as adult *Z. ephialtes* are short-lived and live only a few days, maximum two to three weeks (Naumann et al., 1999). In accordance with Whitman & Agrawal (2009), the absence of interpopulation variability in body size can also be explained by similar environmental and dietary conditions, such as food quantity and quality. The climatic conditions, in which the studied populations live, are probably different, especially in the southern Kožuf Mountain, but analysis of climate has not been carried out due to the small number of populations.

Interpopulation variability of the forewing of *Z. ephialtes* was well expressed in the wing shape. The apical portion of the forewing was the most variable feature and also discriminated between two groups of populations. An example of the phenotypic response of butterfly populations living in different environments is given by Bai et al. (2015) for the *Pieris rapae* (L., 1758) (Lepidoptera: Pieridae) species. It mentions that the shape of the fore and hindwings differ between populations, and that interpopulation variability is consistent with zoogeographical distribution, while boundaries between morphotypes run along zoogeographical boundaries. In our study, variability in the shape of the forewing of the *Z. ephialtes* was most evident in the distal area of the wing, the costal-apical region widening and narrowing. The pattern of forewing shape variability did not coincide with the geographical distribution of the populations. The geographically distant population from the Kožuf Mountain belonged to the group with a narrower wing alongside populations from the Tara River Canyon (Tepca) and the Rugovska Gorge. Except from geographic variation, Shkurikhin & Oslina (2016) discovered seasonal variation of the forewing in the polyvoltine *Pieris napi* (L., 1758) and *P). rapae.* Spring generations had smaller, elongated and pointed wings, and summer (and autumn) generations had larger, broad and rounded wings, which was an adaptation to dispersal. *Z. ephialtes* is not polyvoltine, therefore seasonal variation is less probable. Also, the effect of forewing size on the overall shape variation was minimal, which means the pattern of shape variability could not be explained by allometry. Wing shape affects flight type, which is anteromotoric in lepidopterans, i.e., the forewings have the leading locomotor role (Dudley, 2002). Longer and more slender forewings produce greater acceleration (Berwaerts et al., 2002) and large broader wings enable longer-distance flights. The flight of *Z. ephialtes* has been described as slow and undulating, nevertheless the species has also been found during quick flying events over longer distances (Horák, 2013). Relationships between forewing morphometry and possible sources of variability (e.g., geographical distance, bioclimatic conditions, diet and dispersion) were not tested in our study. This challenge remains for future research, especially with a larger number of specimens and more populations.

Forewing spots pattern

The aposematic coloration of the whole body, and especially the color pattern formed by the size and shape of the wing spots, has an important defensive function against predators of the *Z. ephialtes*, especially against insectivorous birds and lizards. Moths larvae feed on plants that contain cyanogenic glucosides from which they synthesize the toxic linamarin and lotaustralin (Zagrobelny & Møller, 2011; Nishida, 2017). Cyanogenic toxins remain in the body even after the transformation of larvae into adult animals, the highest concentration of toxins in adult butterflies remaining in the wings (Muhtasib & Evans, 1987). The defense mechanism in the wings is reasonable, as the wings first come into contact with the predator. The color pattern might be under strong selection pressure exerted by butterfly predators, yet evidence also show no association between spots size and toxicity across species of aposematic burnet moths (Briolat et al., 2019). We studied the five spots pattern (S1-5) on the forewing of *Z. ephialtes*. The first two, S1 and S2 were yellow in subspecies *Z. e. pannonica*, and red or yellow in 60 or 40% ratio in

subspecies *Z. e. istoki*, while the remaining three, S3-5, were white in both subspecies. We expected a close correlation between the sizes of individual spots, but the relationships among them were more complex. In other words, the size of each spot was more or less independent of the size of the others. Of particular note was spot S1, which varied completely independently of the rest. Among the remaining spots, the association was weak to moderately strong. The size and the shape of the forewing color pattern showed no population or subspecies structuring. Due to the lack of comparable data with other populations or species in the genus *Zygaena*s, these findings remain a reference for further comparisons.

None of the four datasets, of the forewing size and shape or the size and shape of the spots pattern, showed a recognizable morphometric distinction between subspecies *Z. e. istoki* and *Z. e. pannonica*. Nevertheless, research on a higher number of populations, especially of subspecies *Z. e. istoki*, is needed for meaningful conclusions.

References

Adams, D. C., F. J. Rohlf & D. E. Slice, 2013. A field comes of age: geometric morphometrics in the 21st century. Hystrix, 24 (1): 1-8.

Bai, Y., L. Bin Ma, S.-Q. Xu & G.-H. Wang, 2015. A geometric morphometric study of the wing shapes of *Pieris rapae* (Lepidoptera: Pieridae) from the Qinling Mountains and adjacent regions: An environmental and distance-based consideration. Florida Entomologist, 98 (1): 162-169.

Berwaerts, K., Van H. Dyck & P. Aerts, 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology, 16 (4): 484-491.

Blanckenhorn, W. U., 2009. "Causes and Consequences of Phenotypic Plasticity in Body Size: The Case of the Yellow Dung Fly *Scathophaga stercoraria* (Diptera: Scathophagidae), 369-422". In: Phenotypic Plasticity of Insects: Mechanism and Consequences. (Eds. D. W. Whitman & T. N. Ananthakrishnan). Science Publishers, Enfield, NH, USA, 904 pp.

Bookstein, F. L., 1991. Morphometric Tools for Landmark Data. Cambridge University Press, Cambridge, UK, 456 pp.

Briolat, E. S., M. Zagrobelny, C. E. Olsen, J. D. Blount & M. Stevens, 2019. No evidence of quantitative signal honesty across species of aposematic burnet moths (Lepidoptera: Zygaenidae). Journal of Evolutionary Biology, 32 (1): 31-48.

Dudley, R., 2002. The Biomechanics of Insect Flight: Form, Function, Evolution, Princeton, NJ: Princeton University Press, USA, 496 pp.

Hofmann, A., 2003a. *Zygaena* (*Zygaena*) *ephialtes* (L., 1767) in südlichen Balkan Peninsula nebst Anmerkungen zur Entstehung von Polymorphismus sowie melanistischer Zygaena-Formen im Mittelmeerraum (Lepidoptera: Zygaenidae). Entomologische Zeitschrift mit Insekten-Börse, 113 (2): 50-54 (in German).

Hofmann, A., 2003b. *Zygaena* (*Zygaena*) *ephialtes* (L., 1767) in südlichen Balkan Peninsula nebst Anmerkungen zur Entstehung von Polymorphismus sowie melanistischer Zygaena-Formen im Mittelmeerraum (Lepidoptera: Zygaenidae). Entomologische Zeitschrift mit Insekten-Börse, 113 (3): 75-86 (in German).

Hofmann, A., 2003c. *Zygaena* (*Zygaena*) *ephialtes* (L., 1767) in südlichen Balkan Peninsula nebst Anmerkungen zur Entstehung von Polymorphismus sowie melanistischer Zygaena-Formen im Mittelmeerraum (Lepidoptera: Zygaenidae). Entomologische Zeitschrift mit Insekten-Börse, 113 (4): 108-120 (in German).

Hofmann, A. F. & W. G. Tremewan, 2020. The Natural history of Burnet moths (*Zygaena* Fabricius, 1775) (Lepidoptera: Zygaenidae), Part 3. Proceedings of the Museum Witt Munich, 6 (3.1): 1-508; 6 (3.2): 509-1097.

Horák, J., 2013. Habitat requirements of conspicuous burnet moth *Zygaena ephialtes* (L., 1767) (Lepidoptera: Zygaenidae). North-Western Journal of Zoology, 9 (1): 1-5.

Klingenberg, C. P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources, 11 (2): 353-357.

Martin, M. A., C. Craioveanu, C. Sitar & L. Rákosy, 2016. Why morphometrics: a short review and a case study on *Zygaena carniolica* (Scopoli, 1763). Entomologica Romanica, 20 (1): 57-62.

Muhtasib, H. & D. L. Evans, 1987. Linamarin and histamine in the defense of adult *Zygaena filipendulae*. Journal of Chemical Ecology, 13: 133-142.

Naumann, C. M., G. M. Tarmann & W. G. Tremewan, 1999. The Western Palaearctic Zygaenidae. Apollo Books, Stenstrup, Denmark, 304 pp.

Nishida, R., 2017. "Chemical Ecology of Poisonous Butterflies: Model or Mimic? A Paradox of Sexual Dimorphisms in Mullerian Mimicry, 205-220". In: Diversity and Evolution of Butterfly Wing Patterns (Eds. T. Sekimura & H. F. Nijhout). Springer, Singapore, 333 pp.

Rohlf, F. J., 2015. The tps series of software. Hystrix, 26 (1): 9-12.

Shkurikhin, A. O. & T. S. Oslina, 2016. Seasonal variation of the forewing in polyvoltine whites *Pieris rapae* L. and *P. napi* L. (Lepidoptera: Pieridae) in the forest-steppe zone of the Southern Urals. Russian Journal of Ecology, 47 (3): 296-301.

Whitman, D. W. & A. A. Agrawal, 2009. "What is Phenotypic Plasticity and Why is it Important?, 1-63". In: Phenotypic Plasticity of Insects: Mechanism and Consequences (Eds. D. W. Whitman & T. N. Ananthakrishnan). Science Publishers, Enfield, NH, USA, 904 pp.

Yen, S.-H., G. S. Robinson & D. L. J. Quicke, 2005. The phylogenetic relationships of Chalcosiinae (Lepidoptera, Zygaenoidea, Zygaenidae). Zoological Journal of the Linnean Society, 143 (2): 161-341.

Zagrobelny, M. & B. L. Møller, 2011. Cyanogenic glucosides in the biological warfare between plants and insects: The Burnet moth-Birdsfoot trefoil model system. Phytochemistry, 72 (13): 1585-1592.

Zelditch, M. L., D. L. Swiderski & H. D. Sheets, 2012. Geometric Morphometrics for Biologists: A Primer. Elsevier, Academic Press, London, UK, 488 pp.