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INTRAPOPULATION VARIABILITY OF THE WING PATTERN OF *MELANARGIA PARCE* (STAUDINGER, 1882) (LEPIDOPTERA: NYMPHALIDAE: SATYRINAE) IN TAJIKISTAN

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Abstract - This paper examines the phenotypic variability of *Melanargia parce* in Tajikistan. Studies have shown that this species demonstrates large intrapopulation variability, manifested in the size of butterflies, the wing pattern, and the structure of the genitalia. Two types of wing pattern variants were identified. The first is a butterfly with well-developed and expressive drawings with a high degree of detail and brightness, and the second variant is butterflies with less developed patterns on the wings, which are less contrasting and less pronounced. These deviations were recorded among representatives of various populations collected from different locations in the region, taking into account the elevation factor. The observed variability provides the basis for further studies of intraspecific variability and comparative morphological analysis of this species in various parts of its range, which will allow a deeper understanding of its adaptive features and evolutionary processes.
KEY WORDS: butterflies, morphological features, phenotypic variability, statistical analysis, taxonomy, evolution

Izvleček – INTRAPOPULACIJSKA VARIABILNOST KRILNIH VZORCEV *MELANARGIA PARCE* (STAUDINGER, 1882) (LEPIDOPTERA: NYMPHALIDAE: SATRINAE) V TADŽIKISTANU
Članek opisuje fenotipsko variabilnost *Melanargia parce* v Tadžikistanu. Študije so pokazale, da ta vrsta kaže veliko variabilnost znotraj populacij, tako v velikosti metuljev, kot tudi vzorcu kril in strukturi genitalij. Identificirane so bile dve vrsti variant vzorca krila. Prvi je metulj z dobro razvitimi in izraznimi risbami z visoko stopnjo podrobnosti in svetlosti, druga varianta pa so metulji z manj razvitimi vzorci na krilih, ki so manj kontrastni in manj izraziti. Ta odstopanja so bila zabeležena med predstavniki različnih populacij, zbranih iz različnih lokacij v regiji, ob upoštevanju

faktorja nadmorske višine. Opažena variabilnost je osnova za nadaljnje študije intraspecifične variabilnosti in primerjalno morfološko analizo te vrste v različnih delih njene razširjenosti, kar bo omogočilo globlje razumevanje njenih prilagoditvenih značilnosti in evolucijskih procesov.
KLJUČNE BESEDE: dnevni metulji, morfološke značilnosti, fenotipska variabilnost, statistična analiza, taksonomija, evolucija

Introduction

Butterfly wing color patterns are one of the most colorful and spectacular examples of patterns in nature. The color patterns have evolved as mechanisms for visual communication such as camouflage, sexual signaling, warning coloration, and mimicry (Sekimura 2014). Variability in the wing patterns of lepidoptera as seen in adult butterflies are a result of outer (environmental) as well as genetic factors. They form important material for genetic, taxonomic, morphological, zoogeographical and ecological research (Dabrowski & Dobrański 1996). Variability as a general biological phenomenon has not lost its relevance and deserves the attention of scientists (Batlutskaya 2003; Artemyeva 2005). Variability shows the plasticity of living systems and is associated with the implementation of the adaptive strategy of a population as a minimal evolutionary structure (Yablokov & Yusupov 1998).

The population variability is important also for the taxonomy and evolution of each group of animals. The study of this phenomenon is primarily important to identify the morphological features of certain groupings that may be assigned taxonomic status in the future, or in order to avoid unjustified description of new taxa at intraspecific level. It should be noted that there are not many publications concerning the study of the phenotypic variability of butterflies at the intraspecific level within limited territories. All this could lead to an increase in the number of hasty descriptions of unsubstantiated taxa, which adds to confusion in the systematics of butterflies.

Butterflies of the genus *Melanargia* Meigen 1829 comprise 24 species (Nazari et al. 2010) and are distributed in the Palearctic region (Bozano 2002). There are three species and one subspecies of this genus in Central Asia: *Melanargia parce* (Staudinger, 1882), *Melanargia russiae* (Esper, 1783), *Melanargia repentina* Korb et Stradomsky, 2018 and *Melanargia parce karatavica* (Zhdanko, 2012) (Korb & Stradomsky 2018).

M. parce is distributed exclusively in Central Asia from Hissar Mts. in Uzbekistan/Tajikistan to southeast Kazakhstan. In Tajikistan, this species is distributed in Hissar-Alai and Darvaz Mts.. It prefers steppes in midlands with mixed meadows at elevations 1500–2800 m. Depending on the location and habitat, the abundance of this species varies, that is, in some places it is numerous, while in other it can be rare. It is univoltine, flying from May to August.

It should be noted that in representatives of the genus *Melanargia* are variabile in the elements of butterfly wing patterns, and this phenomenon is noted in several publications (Schwanwitsch 1931; Bozano 2002; Zakharova et al. 2017; Korb & Stradomsky 2018). Variability is sometimes manifested to such an extent that all elements of the wing pattern deviate more or less from nominate forms. Such variability

should be carefully studied using large series of material, since in many cases it could be attributed to aberration. In addition, according to Korb & Stradomsky (2018), the use of the variability of the wing pattern of the genus *Melanargia* for the separation of close taxa (treated so far in the rank not higher than subspecies) cannot be justified without statistical support.

For *M. parce*, as well as other representatives of this genus, the variability of the wing pattern is characteristic and these variations are observed in representatives of both sexes. The nature of variability is most evident in the degree of development of dark patterns on both wings, the shape of disc-shaped spots on the forewing, the number and shape of black spots in the postdiscal area of the hindwing, the background colour of the wings, etc. In addition, there are specimens whose wing patterns deviates completely from the rest and they attract most attention. In order to review such variability and make a reasonable conclusion, it is necessary to conduct quantitative analysis of intraspecific forms. This, first of all, will help to establish all types of discontinuous variability of this species, which is important to prevent further hasty and unjustified description of new taxa. As noted by Nazari et al. (2009) the use of poorly defined and 'fluid' diagnostic characters from wing elements, namely morphology or even shape of genitalia in *Melanargia*, has negatively impacted the taxonomy of the group and has obstructed morphological phylogenetic studies of the genus.

It should be noted that the work of Schwanwitsch (1931) examines the structural features, various modifications and evolution of the wing pattern of butterflies of the genus *Melanargia* in the Palearctic region, including of *M. parce*. Naturally, he did not consider the population variability of representatives of the genus *Melanargia* individually and for each specific territory, since this requires a huge amount of material. In addition, his work was carried out in the 30's of the last century, and after almost 100 years, given the ongoing climate change, it is possible that there have been some changes in the morphology of insects, including butterflies, as they respond fast to environmental changes. Taking into account all these trends, the object of our study is *M. parce*, which is a common species in Tajikistan with a peculiar and variable wing pattern. It is important to note that in the design of this study, the author's extensive long-term material was used, collected from various locations in Tajikistan, taking into account the vertical distribution of this species (Fig. 1, Table 1). As a result, a comprehensive analysis was carried out aimed at identifying all types of changes in the wing pattern of *M. parce*.

The purpose of this work is to clarify the patterns of variability of *M. parce* wing patterns at the population level in Tajikistan. The study is aimed at analyzing variations in morphological characteristics, which can help in understanding the ecological and evolutionary processes occurring in this species.

Material and Methods

The material for the study is part of our collections and was collected from different parts of Tajikistan (Central, Northern, South-Western Tajikistan and the Western Pamirs) in the period 2013 to 2024.

All the elements of the drawings were studied on the upper side of both wings. The marginal cell shape, subapical spot, subapical stripe development, discal spot shape, development of line in the discal cell, postdiscal stripe development on the forewings, hind wings - marginal cell shape, development and shape of the ocellus between the veins, development of patterns from the discal to the basal margin, also the background colour of the wings were studied (Fig. 2). A total of 620 males and 330 females were studied and analyzed.

The structure of the genitalia of 20 males was also examined. The diagnostic characteristics of the genitalia used in this work are explained by Korshunov (2002). The photographs of genitalia were taken with a NIKON D7000 digital camera connected to a microscope.

Statistic processing of data, including wingspan, phenotypic diversity and percentages, was performed using a Python program, which ensured high accuracy and efficiency of data analysis. The reliability of the obtained results was determined using t-test, as well as X^2-test at significance levels of 0.05. Due to the fact that the phenotypic variability among the specimens of all samples was manifested in the same way and there was no variability of features characteristic of only one population, a comparative analysis between the samples from different populations was not carried out.

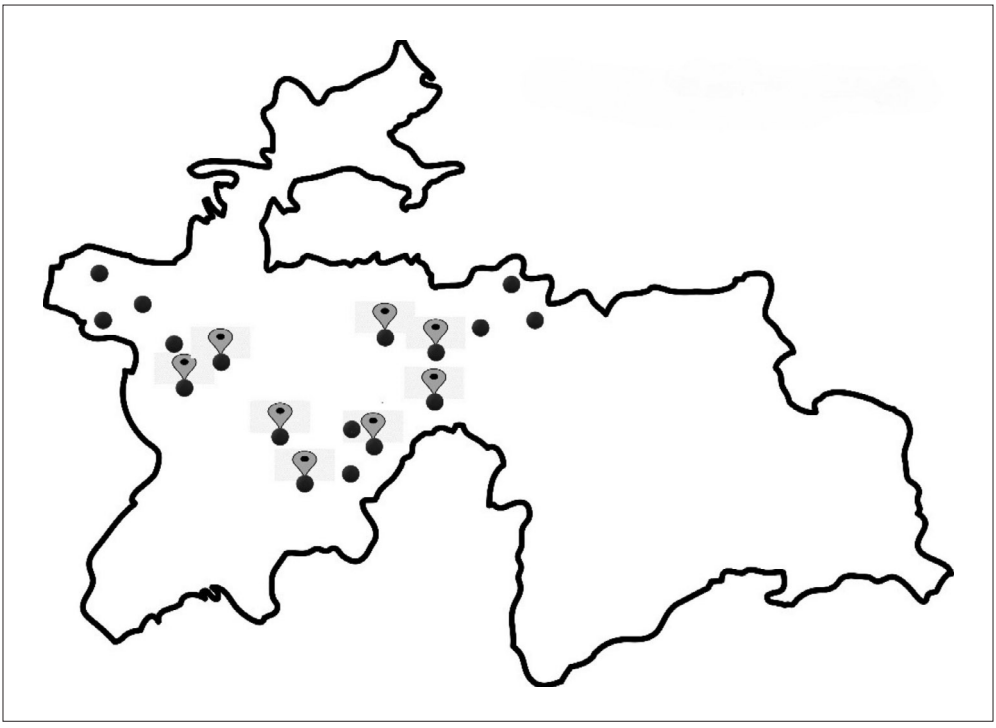

Fig. 1. The distribution of *Melanargia parce* in Tajikistan. The dots indicate the distribution, and the pin indicates the collection points.

Fig. 2. The studied elements of the wing patterns in *M. parce*. a- development of patterns from the discal to the basal margin; b- marginal cell shape; c- shape, development and shape of the ocellus between the veins; d- development of the postdiscal stripe; e- line development in the discal cell; f- discal spot shape; g- subapical stripe development; h- subapical spot; i- marginal cell shape.

Tab. 1. The number of the collected specimens (leg. et coll. Abdulaziz Davlatov) with indication of their geographical location from the territory of Tajikistan.

Number	Collection point	Year	Month	Altitude	Sample size		Coordinates	
					Males	Females	N	E
1	Hazrati Shoh ridge	2014-2023	June, July	1500-2200	130	51	37°55’16.70”	070°02’45.88”
2	Peter the Great ridge	2013, 2014,	June, July, August	1850-2850	75	49	39°00′12.98	070°25′04.95
3	Karategin ridge	2022-2024	June, July	1500-1800	90	46	39°13′41.27	070°51′08.83
4	Vakhsh ridge	2020	May, June	1530	86	38	38°29′26.53	070°04′45.63
5	Rangontau ridge	2023	June	1500	59	34	38°21′19.70	068°44′27.61
6	Sanglok ridge	2023	June	1560	69	43	38°19′27.08	069°14′58.57
7	Hissar ridge	2023	June	1600	65	40	38°43′30.54	069°18′06.31
8	Darvaz ridge	2023	July	1800	46	29	38°32′21.43	070°48′14.28

Results

Butterfly wingspan

The measurements show that the wingspan of *M. parce* males among the studied samples ranges from 30.8 to 40.9 mm, in females from 40 to 50.7 mm. On average,

the wingspan of males is 35.8 mm, and that of females is 45.3 mm. Thus, males are visibly smaller than females. Among the collected samples of male and female *M. parce* taken in different years and at different elevations, there was no discernable differentiation among populations. This indicates that the wingspan of *M. parce* butterflies does not undergo significant changes either among males or females, and, as a result, maintains a stable size, which may indicate the genetic or ecological stability of this character.

Background colour of the wings

The background colour of the wings is mainly white, white-yellow and yellow. This phenomenon is observed in both males and females in all samples, and there is no population with a single wing background. Butterflies with a white-yellow tint are most common among specimens (62.11%), while specimens with a pure white or exclusively yellow background are found in lower numbers (white 22%, yellow 15%).

The wing patterns

The basis of the drawings of the forewing of *M. parce* butterflies are the marginal lunules, a subapical spot between the veins M1-M2, a subapical stripe, a line in the central cell, a spot in the discal cell and a postdiscal stripe. On the hindwing, it is characterized by marginal lunules, ocelli between the veins Sc-R, R-M1, M2-M3, M3-Cu1, Cu1-Cu2, drawings in the basal and the discal area. The variability of the wing pattern of *M. parce* is shown in Fig. 3.

The marginal pale lunules are clearly visible and in a few cases partially covered with dark scales. Despite the coverage the marginal lunules retain their shape. This phenomenon is detected only when studying large amount of material. The subapical black spot is only poorly developed only in few cases but otherwise uniform among specimens of all locations. A postdiscal stripe at subapical spot is always present, but sometimes poorly developed. A large oval spot in the discal cell has the same shape in individuals of both sexes, but there is a difference in the degree of coverage with black scales, which is observed in both males and females. In most specimens from all locations, this discal spot is covered with dense scales, but in the middle of spot the scale coverage is partially reduced. There are also individuals whose discal spot is completely covered by dark scales, and few cases without any coverage. Such individuals are present in each sample, mostly in 2 or 3 specimens.

The postdiscal stripe is observed on a constant basis, but manifests itself in two forms: more developed and less pronounced. The line passing through the central cell and connecting both sides of it remains constant, although the degree of its development varies. Very few individuals are completely devoid of this trait.

The marginal lunules on the hindwing remain stable in all samples, clearly defined and not covered with dark scales. The ocelli are present in two forms – discrete and forming a single line. The second option occurs when the space between the ocelli is covered with thick black scales, but despite the degree of covered scales, the shape of individual ocelli is visible. The number of ocelli on the hindwing is 5,

with the ocellus between the veins of Cu2-A2 noticeably smaller in size compared to others. The location of the ocelli in the cells between the veins remains constant in all species. The black markings between the veins M2 and M3 are variable. Sometimes it is expressed as a connecting line between the ocellus M2 and Cu1, forming a stripe, and in other instances it is completely absent.

The basal and the discal area are uniform in its pattern, but the degree of development of black scales is variable. There are specimens with well-developed black scales, and in this case the drawings look clear, but in others they are poorly developed apart from the veins which are always covered by dark scales. In this case, the patterns from the underside of the wing are visible, since the drawings of both sides of the wings are similar.

Based on general shape of the drawings of the both wings, two variations can be distinguished: with well-developed and poorly developed drawings. In the case of well-developed drawings, they are characterized by pronounced elements, which makes them more visible and noticeable. The marginal lunules of such wings are partially covered with black scales, in particularly on the forewings, the apical spot, subapical lines, postdiscal lines and the line in the discal cell is wider, while the large discal spot is completely or almost completely covered with black scales, which gives the wings an expressive and contrasting appearance. In contrast, poorly developed patterns make such wings less contrasting. In these cases, the marginal lunules are usually not covered with scales, and the subapical and postdiscal lines are represented by thin lines, sometimes barely distinguishable. The line in the discal cell also turns out to be thin or almost imperceptible, and the large discal spot is either barely covered with scales or remains white. This lack of pronounced pattern elements gives these wings less expressive appearance.

Summarizing the above phenotypic differentiation, two main variations of patterns on the wings of the Tajik *M. parce* population can be distinguished. The first variant includes butterflies with well-defined and well-developed patterns, which indicates a high level of pigmentation and a variety of black scales. The second variant, on the contrary, includes individuals with less pronounced and poorly developed patterns. These marked differences in phenotype highlight the importance to study the genetic background on the development of black scales and their effects on the visual perception of wings.

Three specimens stood out in their morphological features and were noticeably different from all the others. Two of these specimens were collected from the Hazrati Shoh ridge, and one from the Peter the Great ridge. These unique specimens are of particular interest for further research and may shed light on the diversity of morphological characteristics of *M. parce* in this region. Despite repeated visits to these locations, we have not been able to find additional butterflies with similar morphological features in subsequent visits. The butterflies collected on the Hazrati Shoh ridge are notable for the fact that one of them has the outer half of the forewing covered with black scales, and the line in the discal cell and postdiscal lines are barely noticeable (Fig. 4). In other individuals, the marginal lunules on the fore and hind wings are completely covered with black scales. In addition, the discal spot is com-

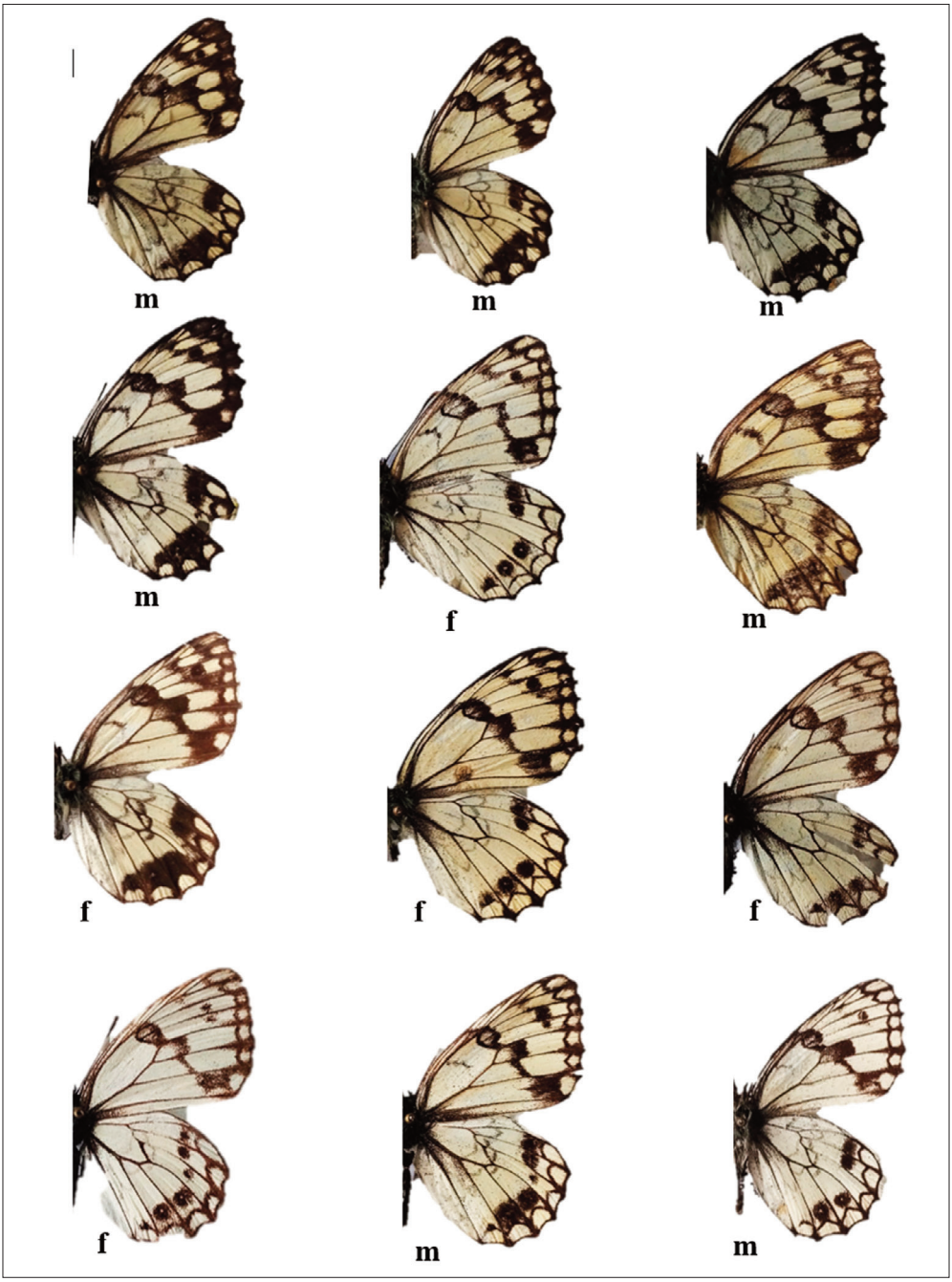

Fig. 3. Wing patterns of *Melanargia parce* and their variability. m) male, f) female.

pletely covered with dark scales, and the line in the discal cell and the postdiscal lines are well developed. The ocellus on the hindwings are indistinguishable due to the density of the scales, and the patterns from the basal to the discal area are clearly pronounced (Fig. 5). So, this appears to be the extreme dark form of the well-marked individuals.

The specimen collected from the Peter the Great ridge has a number of specific morphological features, but resembles the specimen in Fig. 4 in general appearance. The marginal lunules on the forewing are 95% covered with black scales, as are the apical and subapical areas. There is no line discernable in the discal cell, and also the postdiscal lines are barely visible. On the hindwings, the marginal lunules, with the exception of the first two cells, are completely filled with black scales – this is the only case where the marginal lunules are not visible. The ocelli are also not visible due to the thick coating of scales, which merge with the marginal lunules, forming almost complete dark border. This is in striking contrast to the basal and discal area, which is almost white with visible background pattern from the underside of the wings (Fig. 6).

The structure of the genitalia and their variability

The genital characteristics are also variable (Habel et al. 2017). Our study revealed some variability in the structure of the genitals of *M. parce*, which includes various aspects such as the shape of the tegumen, the shape and size of the uncus, the shape of the valve and the number of teeth at its vertices, as well as the shape and size of the gnathos branch. To a lesser extent, variability was observed in the form of aedeagus (Fig. 7, Fig. 8).

Statistical analysis

The difference in wingspan between the samples of males and females is significant: on average, males have a wingspan of 35.8 mm, a standard deviation of 2.91 mm, while females have 45.3 mm, a standard deviation of 3.09 mm. Thus, there is a noticeable difference that may be significant for the study of sex differences in a given population. According to statistical analysis, there is indeed a statistically sig-

Figs. 4–6. Butterflies requiring further investigation. 4–5) material from the Hazrati Shoh ridge, 6) material from the Peter the Great ridge.

nificant difference in size between males and females, averaging 9.5 mm ($t \approx 46{,}02$, $p < 0.05$). This difference could be related to their different physiology or behavior. X^2 test (≈ 359.5) reveals that significant differences between the observed and expected frequencies of the forms is significant level of $a = 0.05$. Consequently, the distribution of butterfly background colours is not uniform, and the white-yellow background prevails in the Tajik population of *M. parce*.

Tab. 2. The number and percentage of types of variation of *Melanargia parce* wings patterns. The table clearly shows that butterflies with poorly developed wing patterns predominate among the studied specimens.

Number	Samples	Number of specimens with well-developed wing patterns	%	Number of specimens with poorly developed wing patterns	%
1	Hazrati Shoh ridge	70	38.7	111	61.3
2	Peter the Great ridge	48	38.7	76	61.3
3	Karategin ridge	53	39.0	83	61.0
4	Vakhsh ridge	48	38.7	76	61.3
5	Rangontau ridge	36	38.7	57	61.3
6	Sanglok ridge	43	38.4	69	61.6
7	Hissar ridge	41	39.0	64	61.0
8	Darvaz ridge	29	38.7	46	61.3

The partition in two wing pattern forms is strikingly uniform among different locations. Variants with well-developed wing patterns are less common, which indicates the predominance of individuals with weakly pronounced patterns in the *M. parce* population inhabiting in Tajikistan. The observed pattern indicates possible adaptive mechanisms underlying the formation of this morphological feature.

Discussion

The studies show that in the *M. parce* populations inhabiting Tajikistan, females are generally larger than males, which indicates a pronounced sexual dimorphism of this species. It should be noted that the samples collected from various regions of Tajikistan are almost identical in the degree of development of the wing pattern elements. Initially, the study allowed us to identify two types of variants in the wing pattern. One is characterized by well-developed and expressive drawings with a high degree of detail and brightness. The second variant is represented by butterflies with less developed patterns on the wings, which are less contrasting and less pronounced. Since the ratio between both forms is strikingly similar in all studied populations it is likely that these are not under selective pressure from the environment indicating possible presence of common environmental and genetic factors affecting the development of the organisms under study. This will require further research to better

understand their relationships and adaptations. In addition, it is worth considering the possibility of the existence of genetic predispositions, which can determine not only the development of specific wing patterns depending on heredity, but also the degree of their adaptation to various environmental conditions. Genetic diversity within a population can contribute to its flexibility and adaptability in a rapidly changing environment.

The genitals also show variability in their structure, but this turns out to be less significant compared to the variability of wing patterns (Fig. 7, Fig. 8). The genitalia usually have a more conservative and stable characteristics, which indicate their important role in the process of reproduction and evolutionary stability of species. Given the intrapopulation variability of the wing pattern of *M. parce*, the variability of the wing pattern and the structure of the genitals described by us, is nothing more than variations within the species. Additional studies, in particular molecular, are needed to obtain confirmation of this observation.

Further analysis of the morphological characteristics of this species in the context of ecology and genetics may lead to new discoveries, respectively deepening our understanding of biological diversity and its evolutionary foundations. Thus, the study of these differences is important not only for deepening our understanding of biological variability, but also for analyzing the mechanisms of evolution and adaptation of these species to changing environment.

Fig 7. The structure of the genitals of *Melanargia parce*, demonstrating morphological variations in different specimens.

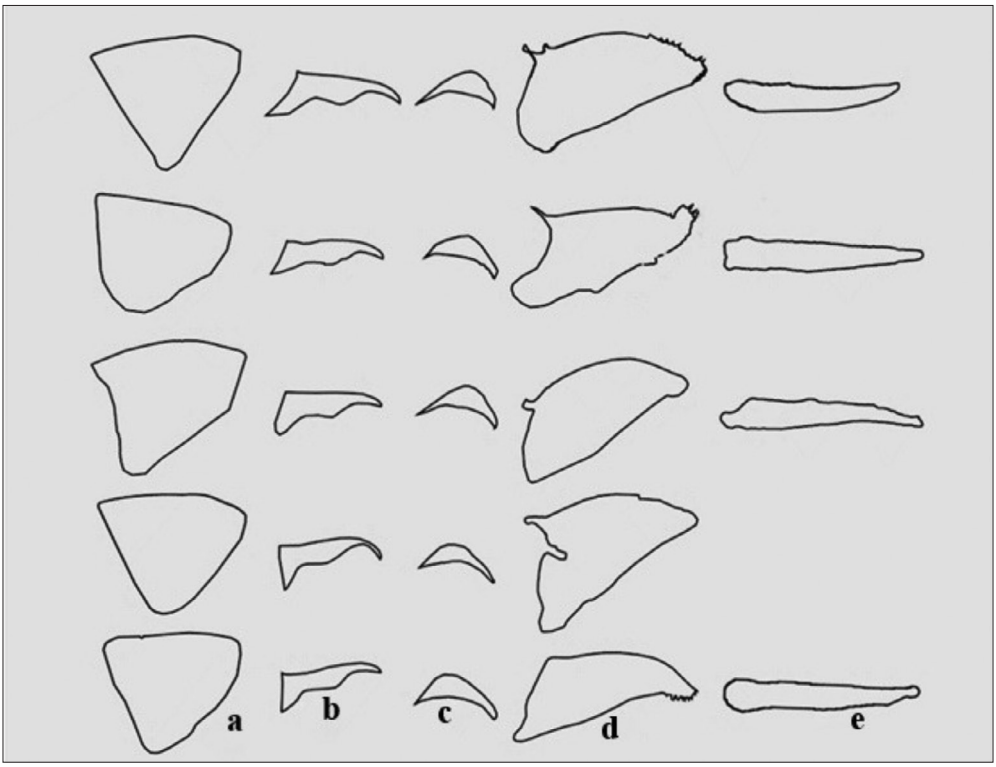

Fig. 8. Variability of individual parts of the genitalia of *Melanargia parce*. a) tegumen, b) uncus, c) gnathos branch, 11), valva, 12) aedeagus.

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