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Pontia daplidice (Linnaeus, 1758) and *Pontia edusa* (Fabricius, [1777]) in Tajikistan: one or two species? (Lepidoptera: Pieridae)

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Abstract

DNA barcoding of *Pontia* Fabricius, 1807 species from Tajikistan has shown that only *Pontia edusa* (Fabricius, [1777]), which was previously considered *Pontia daplidice* (Linnaeus, 1758), is found throughout the country. In fact, these are the only finds of this species in Central Asia, and these data will improve our understanding about distribution of *Pontia edusa*, and in the future will help to form an overall picture of the distribution of both taxa. *Pontia edusa* is also reported for the first time for central Iran. Morphological analysis did not reveal significant features in the structure of the genitalia that could distinguish between *P. edusa* and *P. daplidice*.
Keywords: Lepidoptera, Pieridae, DNA barcoding, morphological features, seasonal forms, Tajikistan, Iran.

Pontia daplidice **(Linnaeus, 1758) y** *Pontia edusa* **(Fabricius, [1777]) en Tayikistán: ¿una o dos especies? (Lepidoptera: Pieridae)**

Resumen

El código de barras del ADN de las especies de *Pontia* Fabricius, 1807 de Tayikistán ha demostrado que sólo la *Pontia edusa* (Fabricius, [1777]), que antes se consideraba *Pontia daplidice* (Linnaeus, 1758), se encuentra en todo el país. De hecho, se trata de los únicos hallazgos de esta especie en Asia Central, estos datos mejorarán nuestra comprensión sobre la distribución de la *Pontia edusa* (Fabricius, 1777) y en el futuro ayudarán a formarse una imagen global de la distribución de ambos taxones. También se informa por primera vez de la presencia de *Pontia edusa* en el centro de Irán. El análisis morfológico no reveló características significativas en la estructura de la genitalia que pudieran distinguir entre *P. edusa* y P. *daplidice*.
Palabras clave: Lepidoptera, Pieridae, código de barras del ADN, características morfológicas, formas estacionales, Tayikistán, Irán.

Introduction

Due to the morphological similarity of *Pontia edusa* (Fabricius, [1777]) and *Pontia daplidice* (Linnaeus, 1758), there is still no consensus among entomologists about their status. Some researchers (Geiger & Scholl 1982; Wagener, 1988; Geigeret al. 1988; John et al. 2013) consider both taxa as a separate species, while other authors (Porter et al. 1997; Kurze et al. 2006) consider *P. edusa* to be a subspecies of *P. daplidice*. Tuzov et al. (1997) refer to the study by Geiger & Scholl (1982) on the assignment of the parapatric species *P. edusa* to the complexes of *P. daplidice* species based on data from electrophoretic analysis of enzymes, and emphasize that due to the lack of such a study in Russia and neighboring territories, the distribution of *P. daplidice* is considered by analogy with the distribution of European-African and the Middle Eastern population. Korb & Bolshakov (2016), in their work on Papilionoidea of the former Soviet Union, consider *P. edusa* a subspecies of *P. daplidice*, while noting that both of these taxa are certainly different, but their status is still uncertain. As can be seen from the above data, the situation is not simple, and all this uncertainty has led to the fact that researchers still cannot determine the exact distribution of these two taxa.

According to Bruna et al. (2004) the distribution of *P. daplidice* covers Canary Islands, North Africa, Southwest Europe, Southeast Turkey, Middle East, Western and Southern Iran to Afghanistan and, possibly, Kazakhstan and Tajikistan. It is also a rare migrant to South England and Ireland (Thomas & Lewington, 1991). *Pontia edusa* is common on Central, Eastern and Southeastern Europe, Transcaucasia, Turkey, Northeast Iraq, Northwest Iran and, possibly, Central and Eastern Asia to Japan (Bruna et al. 2004).

The occurrence of *P. daplidice* in northern Africa, the Levant and southwestern Europe has been demonstrated based on molecular data (Geiger et al. 1988, John et al. 2013, Dapporto et al. 2019). The presence of *P. edusa* in the Tien Shan mountains in southern Kazakhstan was shown in the work of Lukhtanov et al. (2009) based on the analysis of mitochondrial DNA barcodes.

All published works on the Papilionoidea fauna of Tajikistan (Stshetkin, 1960, 1963, 1975; 1981; Tshikolovets, 2003; Davlatov, 2020, 2022) mention *P. daplidice*. For the first time about the findings of *P. edusa* in Tajikistan (Karategin ridge) is reported in the works of Sharafutdinov & Lukhtanov (2019) based on the study of barcodes of mitochondrial DNA and, in fact, this is the only find in Central Asia. After this publication, doubts arose as to whether *P. daplidice* is found in Tajikistan at all or only *P. edusa* is distributed throughout Tajikistan. As noted by Wagener (1988) and John et al. (2013), there is no reliable morphological feature by which these two taxa can be reliably distinguished. At the same time, these two species differ well in mitochondrial and nuclear genes (John et al. 2013). In such difficult situations, especially when the distribution of both taxa has not been fully clarified, morphological features do not give final results in determining, in addition, populations from all Central Asian countries have not been genetically studied, it is impossible to come to a final conclusion about which of these species occurs in Tajikistan. Thus, we set ourselves the task that it is necessary to study populations from all over Tajikistan at the genetic level, since this is the only way to clarify the situation as to which of these two taxa is reliably distributed in Tajikistan, or all such both species are found in this region.

Material and methods

The material for the description of this paper were a collection of authors from 2014 to 2023 from different parts of Tajikistan at altitudes of 300-3800 m above sea level. In total, over 560 specimens of Papilionidae were studied, in particular 310 males and 240 females. First of all, all our efforts were aimed at studying the morphological features of these species, such as the pattern of wings, as well as the structure of the genitals, followed by their comparison with each other. The wing patterns were studied on both sides of the wings. Dissection and preparation of genitalia slides were performed applying standard protocols. The diagnostic signs of the genitals used in this work are given by Korshunov (2002) and Wu (2010). The photographs of Papilionoidea and their genitalia were taken with a NIKON D7000 digital camera connected to a Crystallite ST-7045 Trinocular Microscope.

DNA barcodes of Lepidoptera from Tajikistan and Iran were obtained and analyzed (Table 1). For comparison, samples of *P. daplidice* from France, Italy, and Spain, as well as *P. edusa* from Kazakhstan were used (Table 1). The species identity of the samples from France, Italy, Spain, and Kazakhstan was previously established using DNA barcodes (Lukhtanov et al. 2009, Dapporto et al. 2019).

Standard mitochondrial DNA barcodes (658 bp fragments of the cytochrome c oxidase subunit I gene) were obtained at the Department of Karyosystematics (Zoological Institute RAS, St. Petersburg). DNA was extracted from single legs removed from dried voucher specimens. The target 658-bp fragment of COI was amplifying using the primers LepF1 and LepR1 (Hebert et al 2004). Sequences were obtained using ABI 3730XL sequencers (Applied Biosystems). Sequences were edited to remove ambiguous base calls and primer sequences and aligned using the BioEdit software (Hall, 1999). All new sequences were submitted to GenBank (Table 1).

The TCS haplotype network (Clement et al. 2000) was created and visualized using the PopArt software (Leigh & Bryant, 2015). The within- and intraspecific uncorrected *COI* p-distances (%) were calculated using the MEGA 11 program (Tamura et al. 2021) (Table 2).

Results and discussion

Morphology and variation

The wingspan reaches 35-45 mm. The wing patterns of the studied Lepidoptera are almost identical. The upper side of the wings is white, with black spots on the apex and the marginal area of the forewings up to Cu1 and the hindwings up to Cu2. There is a black discal spot on the forewings with a white stroke in the middle. The underside of the hindwings has a greenish coloration with white spots. When studying the features of the Papilionoidea wing pattern collected by us from different parts of Tajikistan, it became clear that have the same wing pattern features, except for a small change. The change is as follows: the black pattern on the apex of the forewings is sometimes combined with larger white spots, and sometimes these white spots are slightly smaller; the black color is sometimes mixed with developed white scales; some specimens have a strongly developed black discal spot, others to a lesser extent. The patterns on the underside of the hind wings also have different degrees of development, especially light green patterns, and sometimes colorless ones, are often observed; the number of white spots in the middle transverse row varies, decreases in some specimens, and increases in others (see figures 2-13). It should be noted that all these changes are not a sufficient argument for distinguishing into separate species and these changes are primarily undoubtedly related to the season of the year and the number of generations. Stshetkin (1960) notes the variability of the wing patterns of the *Pontia* (*daplidice*) *edusa* and associates these changes with the transition of shape from one population to another.

There are no major changes in the structure of the genitals, except for the shape of the valva, which is sometimes elongated with a pointed tops, and in large cases it has an oval shape. Bruna et al. (2004) use genital structures to distinguish between both taxa, in particular, it was shown that the discal margin of the valva in *P. edusa* is more angular and pointed than in daplidice, but at the same time it is noted that this feature is insufficiently pronounced and unstable. Wagener (1988) also gives drawings of the valvae of two taxa (see figures 17-22), but as can be seen from the drawings, there is no greatest difference between the structures of these valvae, and such features can be observed in the genitals studied by us (see figures 14-16).

Ecology

P. edusa is one of the most common in Tajikistan. It is found everywhere except in sandy deserts at an altitude of 300-3500 m but is most often found on agricultural lands such as alfalfa fields, orchards, and other crops. Depending on the altitude, locality and weather conditions, this specie's flight begins in late February or early March and lasts until November, while during the year from four to six generations develop, respectively. According to Stshetkin (1960), in the conditions of the Vakhsh valley (Southern Tajikistan), *P. edusa* develops to the 6th generation within a year, and probably the 6th generation is incomplete. In other parts of Tajikistan, it produces up to 4 generations per year.

Molecular work

The TCS network clearly demonstrated that all studied specimens from Tajikistan and Iran form a single cluster together with previously identified specimens of *P. edusa* from Kazakhstan. All *P. daplidice* specimens from France, Italy and Spain form another distant cluster (Fig. 1). Interspecific uncorrected COI p-distance between *P. edusa* and *P. daplidice* was within 7.8 - 8.3%. Intraspecific uncorrected COI p-distance between samples of *P. edusa* was in the range of 0 - 0.6%. Intraspecific uncorrected COI p-distance between samples of *P. daplidice* was within 0.3 - 1.0% (Table 2). Thus, the interspecific distances significantly exceeded the intraspecific distances.

We also checked the species identity of the studied samples using algorithms and the BOLD system database (https://www.boldsystems.org/index.php/IDS_OpenIdEngine).This method also showed unequivocally that all newly studied specimens belonged to *P. edusa*.

Thus, not a single specimen of *P. daplidice* was identified in the sample collected in different parts of Tajikistan. In contrast, all studied specimens from Tajikistan turned out to be *P. edusa*. The only specimen studied from central Iran was also found to be *P. edusa*.

Table 1. List of the analyzed *COI* barcodes of *Pontia daplidice* and *P. edusa.*

Field/ BOLD ID	GenBank ID	Haplo-type	Species	Country	Locality	Source
RVcoll.11-E86	MN145195	d1Fr	*P. daplidice*	France	Corsica, North Corsica, L'Inzeca	Dapporto et al. 2019
RVcoll.12-O547	MN144058	d2It	*P. daplidice*	Italy	Sardinia, Ogliastra, Tortoli	Dapporto et al.2019
RVcoll.11-H637	MN143626	d3It	*P. daplidice*	Italy	Sicily, Agrigento, Isola di Lampedusa	Dapporto et al.2019
RVcoll.12-O278	MN143616	d4It	*P. daplidice*	Italy	Sardinia, Olbia-Tempio, La Maddalena	Dapporto et al.2019
RVcoll.08-L132	MN143573	d3It	*P. daplidice*	Spain	Granada, Andalucía, Laguna Seca	Dapporto et al.2019
RVcoll.08-H595	MN143491	d6Sp	*P. daplidice*	Spain	Guadalajara, Castilla-La Mancha, Durón	Dapporto et al.2019
RVcoll.14-E068	MN143442	d4It	*P. daplidice*	Italy	Piedmont, Piano della Casa	Dapporto et al.2019
LOWA029-06	FJ664011	e1Ka	*P. edusa*	Kazakhstan	Tienschan, Dzhambul Region, Kurdai Pass, 43.33N 74.95E	Lukhtanov et al. 2009
LOWA029-06	FJ664010	e2Ka	*P. edusa*	Kazakhstan	Tienschan, Dzhambul Region, Kurdai Pass, 43.33N 74.95E	Lukhtanov et al. 2009
LOWA561-06	FJ664009	e3Ka	*P. edusa*	Kazakhstan	Dzhungarsky Alatau, Lepsy River, 45.72N 80.28E	Lukhtanov et al. 2009
LOWA561-06	FJ664008	e3Ka	*P. edusa*	Kazakhstan	Dzhungarsky Alatau, Lepsy River, 45.72N 80.28E	Lukhtanov et al. 2009
VL728	PV793518	e5Ta	*P. edusa*	Tajikistan	Khrebet Khozratisho, A. Davlatov leg.	This study
VL729	PV793519	e6Ta	*P. edusa*	Tajikistan	Hissarsky Khrebet, A.Davlatov	This study
VL730	PV793520	e7Ta	*P. edusa*	Tajikistan	Darvazsky Khrebet, A.Davlatov	This study
VL731	PV793521	e5Ta	*P. edusa*	Tajikistan	Tabakchi, A.Davlatov	This study
VL732	PV793522	e5Ta	*P. edusa*	Tajikistan	Khrebet Petra Pervogo, A. Davlatov	This study
VL733	PV793523	e10Ta	*P. edusa*	Tajikistan	S. Tajikistan, Tabakchi, A.Davlatov	This study
BPAL2653-14	PV793524	e11Ta	*P. edusa*	Tajikistan	Alai Mts, Komarob, 39.14N 70.22E, 1400m, V. Lukhtanov	Sharafutdinov, Lukhtanov, 2019
BPAL2654-14	PV793525	e11Ta	*P. edusa*	Tajikistan	Alai Mts, Komarob, 39.14N 70.22E, 1400m, V. Lukhtanov	Sharafutdinov, Lukhtanov, 2019
BPAL2655-14	PV793526	e11Ta	*P. edusa*	Tajikistan	S. Tajikistan, Sarband, 37.88N 68.94E, 504m, V. Lukhtanov leg.	This study
BPAL2796-15	PV793527	e3Ka	*P. edusa*	Iran	Qamsar, 33.72N 51.5E, 2000m, 16July2009, V. Lukhtanov leg.	This study

Table 2. Inter- and intraspecific uncorrected COI p-distances between specimens of *P. daplidice* (specimens 1-7) and *P. edusa* (specimens 8-21).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	0,0000																				
2	0,0030																				
3	0,0106	0,0076																			
4	0,0091	0,0061	0,0015																		
5	0,0106	0,0076	0,0000	0,0015																	
6	0,0122	0,0091	0,0046	0,0030	0,0046																
7	0,0079	0,0048	0,0016	0,0000	0,0016	0,0032															
8	0,0780	0,0780	0,0810	0,0826	0,0810	0,0856	0,0811														
9	0,0780	0,0780	0,0810	0,0826	0,0810	0,0856	0,0811	0,0031													
10	0,0776	0,0776	0,0809	0,0825	0,0809	0,0858	0,0801	0,0017	0,0017												
11	0,0765	0,0765	0,0795	0,0810	0,0795	0,0841	0,0795	0,0015	0,0015	0,0000											
12	0,0778	0,0778	0,0809	0,0824	0,0809	0,0855	0,0797	0,0047	0,0047	0,0033	0,0031										
13	0,0778	0,0778	0,0809	0,0824	0,0809	0,0855	0,0797	0,0016	0,0016	0,0000	0,0000	0,0031									
14	0,0762	0,0762	0,0793	0,0809	0,0793	0,0840	0,0780	0,0063	0,0063	0,0050	0,0047	0,0016	0,0047								
15	0,0778	0,0778	0,0809	0,0824	0,0809	0,0855	0,0797	0,0047	0,0047	0,0033	0,0031	0,0000	0,0031	0,0016							
16	0,0778	0,0778	0,0809	0,0824	0,0809	0,0855	0,0797	0,0047	0,0047	0,0033	0,0031	0,0000	0,0031	0,0016	0,0000						
17	0,0762	0,0762	0,0793	0,0808	0,0793	0,0838	0,0780	0,0061	0,0061	0,0050	0,0046	0,0016	0,0047	0,0031	0,0016	0,0016					
18	0,0765	0,0765	0,0795	0,0810	0,0795	0,0841	0,0795	0,0046	0,0046	0,0033	0,0031	0,0000	0,0031	0,0016	0,0000	0,0000	0,0015				
19	0,0765	0,0765	0,0795	0,0810	0,0795	0,0841	0,0795	0,0046	0,0046	0,0033	0,0031	0,0000	0,0031	0,0016	0,0000	0,0000	0,0015	0,0000			
20	0,0765	0,0765	0,0795	0,0810	0,0795	0,0841	0,0795	0,0046	0,0046	0,0033	0,0031	0,0000	0,0031	0,0016	0,0000	0,0000	0,0015	0,0000	0,0000		
21	0,0765	0,0765	0,0795	0,0810	0,0795	0,0841	0,0795	0,0015	0,0015	0,0000	0,0000	0,0031	0,0000	0,0047	0,0031	0,0031	0,0046	0,0031	0,0031	0,0031	0,0000

1 – Pontia_daplidice_MN145195_RVcoll.11-E863_France_North_Corsica
2 – Pontia_daplidice_MN144058_RVcoll.12-O547_Italy_Sardinia_Ogliastra_Tortoli
3 – Pontia_daplidice_MN143626_RVcoll.11-H637_Italy_Sicily_Agrigento_Isola_di_Lampedusa
4 – Pontia_daplidice_MN143616_RVcoll.12-O278_Italy_Sardinia_Olbia-Tempio_La_Maddalena
5 – Pontia_daplidice_MN143573_RVcoll.08-L132_Spain_Granada_Andalucia_Laguna_Seca
6 – Pontia_daplidice_MN143491_RVcoll.08-H595_Spain_Guadalajara_Castilla-La_Mancha_Duron
7 – Pontia_daplidice_MN143442_RVcoll.14-E068_Italy_Piedmont_Piano_della_Casa
8 – Pontia_edusa_FJ664011_LOWA029-06_Kazakhstan_Tienschan|Dzhambul_Region|Kurdai_Pass_43.33N_74.95E
9 – Pontia_edusa_FJ664010_LOWA030-06_Kazakhstan_Tienschan|Dzhambul_Region|Kurdai_Pass|43.33N_74.95E
10 – Pontia_edusa_FJ664009_LOWA561-06_Kazakhstan_Dzhungarsky_Alatau_Lepsy_River_45.72N_80.28E
11 – Pontia_edusa_FJ664008_LOWA562-06_Kazakhstan_Dzhungarsky_Alatau_Lepsy_River_45.72N_80.28E
12 – Pontia_edusa_VL728_Tajikistan_Khrebet_Khozratisho_A.Davlatov
13 – Pontia_edusa_VL729_Tajikistan_Hissarsky_Khrebet_A.Davlatov
14 – Pontia_edusa_VL730_Tajikistan_Darvazsky_Khrebet_A.Davlatov
15 – Pontia_edusa_VL731_Tajikistan_Tabakchi_A. Davlatov
16 – Pontia_edusa_VL732_Tajikistan_Khrebet_Petra_Pervogo_A. Davlatov
17 – Pontia_edusa_VL733_Tajikistan_Tabakchi_female_A. Davlatov
18 – Pontia_edusa_BPAL2653-14|CCDB-17967_H04|Tajikistan_Komarob_39.14N_70.22E_1400m_VLukhtanov
19 – Pontia_edusa_BPAL2654-14|CCDB-17967_H05|Tajikistan|Komarob_VLukhtanov
20 – Pontia_edusa_BPAL2655-14|CCDB-17967_H06|Tajikistan|Sarband|37.88N_68.94E_504m_VLukhtanov
21 – Pontia_edusa_BPAL2796-15|CCDB-17969_D05|Iran_Qamsar_33.72N_51.5E_2000m_16July2009_VLukhtanov

Conclusion

As can be seen from the above data, there are no significant morphological differences between these two taxa. The only way to distinguish these two taxa is by the genetic method, which is currently widely used in insect taxonomy. Thanks to the obtained genetic data, it has now become clear that only *P. edusa* reliably inhabitant in Tajikistan, and the taxon *P. daplidice*, which have so far been cited for our fauna, does not occur in Tajikistan at all. It is likely that only *P. edusa* inhabitant in all Central Asian countries, since this is a migratory species, but to clarify this issue, it is necessary to involve material from all these countries for genetic analysis.

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Conflict of Interest

The authors declare that they have no known financial interest or personal relationship that could have influence the work presented in this article.

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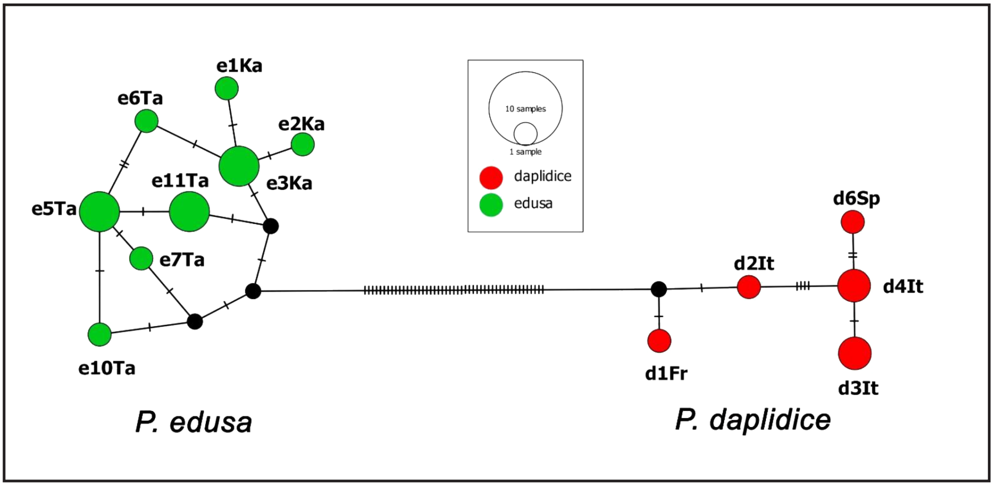
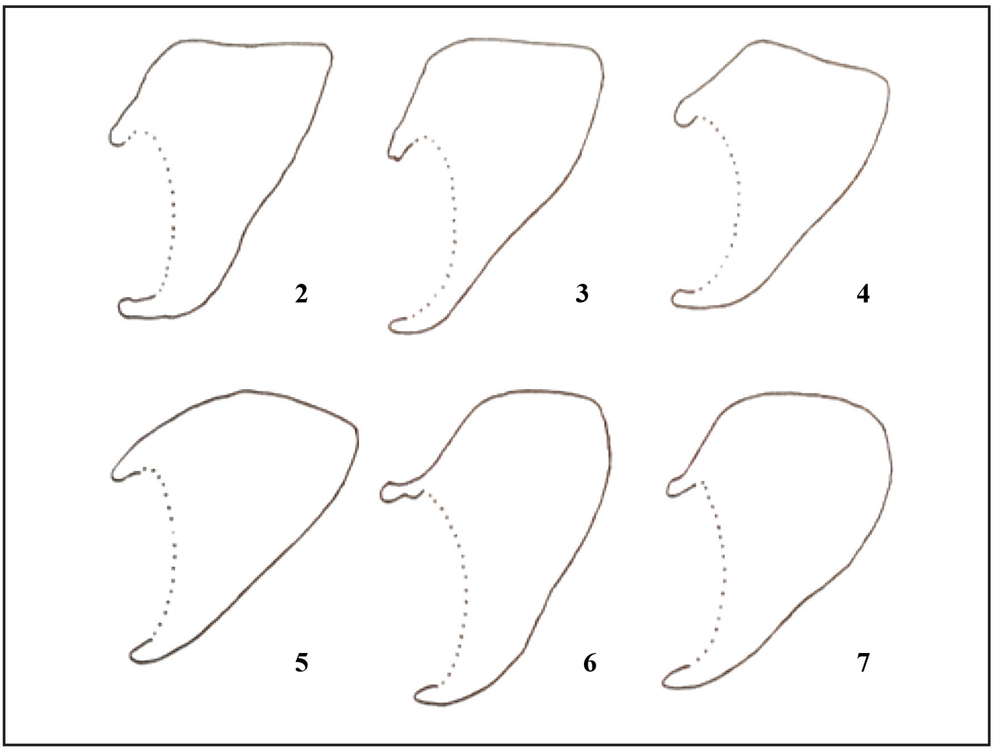
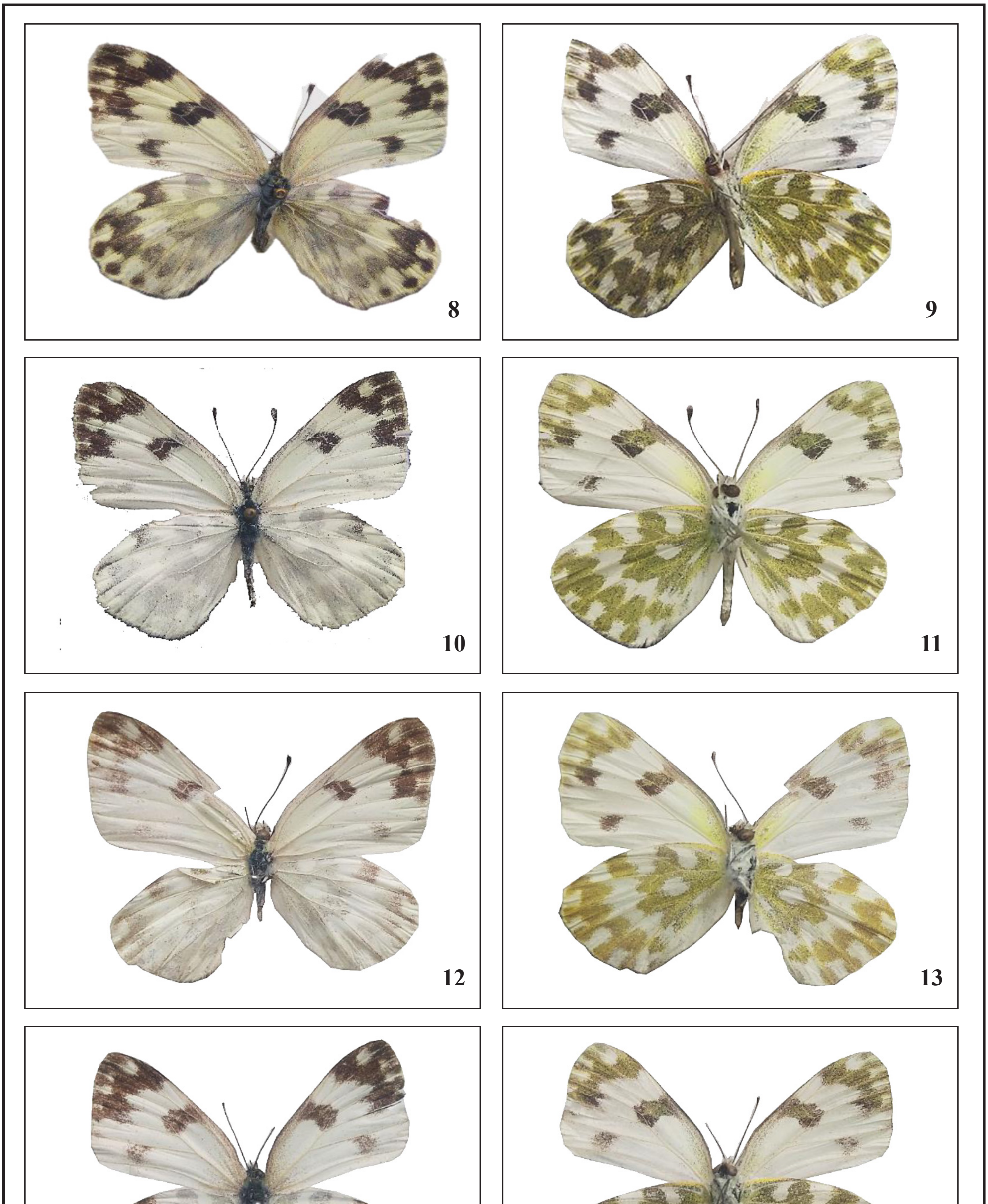
Figure 1. The TCS haplotype network of the analyzed samples of *P. edusa* and *P. daplidice*. Circles are proportional to sample size for each haplotype. Smallest black dots represent unsampled but predicted haplotypes, and mutations are shown as dashes. The species clusters are highlighted in different colors. **Fr** is France, **It** is Italy, **Ka** is Kazakhstan, **Sp** is Spain, and **Ta** is Tajikistan.



Figures 2-7. Types of valves of *Pontia daplidice* and *Pontia edusa* by Wagener, 1988. **2-4.** *Pontia edusa*. **5-7.** *Pontia daplidice*.



Figures 8-15. *Pontia edusa* in Tajikistan. **8-9**. Tabakchi Mountain. **10-11**. Hazratisho Ridge. **12-13**. Darvaz Ridge. **14-15**. Hissar Ridge.



Figures 16-22. 16-17. Peter the Great Ridge. **18-19.** Turkestan Ridge. **20-22.** Genitalia of *Pontia edusa* in Tajikistan.