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داده‌های تاکسونومیک برای خانواده پاپریده (روزپرک‌ها) (Lepidoptera) در غرب استان مازندران

سمیه اسدیان نارنجی*، استادیار گروه زیست‌شناسی جانوری، دانشکده علوم زیستی، واحد تنکابن، دانشگاه آزاد اسلامی، تنکابن، ایران
(مسئول مکاتبات)

assadiansomayeh@hotmail.com

مرضیه رمضان توبی، کارشناسی ارشد گروه زیست‌شناسی جانوری، دانشکده علوم زیستی، واحد تنکابن، دانشگاه آزاد اسلامی، تنکابن، ایران

ramezantoobi@yahoo.com

مریم رحمتی باوجی، کارشناسی ارشد گروه زیست‌شناسی جانوری، دانشکده علوم زیستی، واحد تنکابن، دانشگاه آزاد اسلامی، تنکابن، ایران

rahmati-m6@yahoo.com

معصومه غلام تمیمی، کارشناسی ارشد گروه زیست‌شناسی جانوری، دانشکده علوم زیستی، واحد تنکابن، دانشگاه آزاد اسلامی، تنکابن، ایران

tamimimasoomah@yahoo.com

چکیده

خانواده پاپریده شامل بیشتر پروانه‌های آشنا است که به پروانه‌های سفید کلم و زرد چمن معروفند. آنها وضعیت ثابت و پایدار گونه‌ای دارند. در پژوهش حاضر، بررسی فونستیک این خانواده در غرب استان مازندران در شمال ایران انجام شد. در مجموع، هفت گونه متعلق به پنج جنس از این خانواده شناسایی شد. این گونه‌ها عبارتند از: *Pieris rapae*، *Pieris (napi) pseudorapae* (VERITY, 1908)، *Pieris brassicae* (LINNAEUS, 1758)، *Pontia (daphidice) edusa* (LINNAEUS, 1758)، *Aporia crataegi* (LINNAEUS, 1758)، *Gonepteryx rhamni* (LINNAEUS, 1758) و *Colias crocea* (FOURCROY, 1758). در این پژوهش، مورفولوژی رو و زیر بال و همچنین ساختار ژنیتالیا در جنس نر بررسی شد.

واژه‌های کلیدی: فونستیک، ژنیتالیا، مازندران، مورفولوژی، پاپریده.



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Taxonomic Data for Family Pieridae (Lepidoptera) in West of Mazandaran Province

Somayeh Assadian Narenji

*Corresponding author: Assistant Professor, Department of Animal Biology, Faculty of Biological Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
assadiansomayeh@hotmail.com

Marzieh Ramezan Toobi

MSc, Department of Animal Biology, Faculty of Biological Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
ramezantooobi@yahoo.com

Maryam Rahmati Baogi

MSc, Department of Animal Biology, Faculty of Biological Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
rahmati-m6@yahoo.com

Massomeh Gholam Tamimi

MSc, Department of Animal Biology, Faculty of Biological Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
tamimimasoomah@yahoo.com

Abstract

The Pieridae family includes the most familiar butterflies also known as Cabbage-White and Grass Yellow. They have a long stable status of the species in this family. A faunistic study on the Pieridae family was carried out in the West of Mazandaran province, Northern Iran. A total of 7 species of the family (5 genera) were collected. These species were: *Pieris brassicae* (Linnaeus, 1758), *Pieris (napi) pseudorapae* (Verity, 1908), *Pieris rapae* (Linnaeus, 1758), *Aporia crataegi* (Linnaeus, 1758), *Pontia (daplidice) edusa* (Linnaeus, 1758; Fabricius, 1777), *Colias crocea* (Fourcroy, 1758), and *Gonepteryx rhamni* (Linnaeus, 1758). For these species, illustrations of adults' upper wing and lower wing morphology and their male genitalia are given.

Keywords: Faunistic, Genitalia, Mazandaran, Morphology, Pieridae.

Introduction

Butterflies are members of the Lepidoptera order (butterflies and moths), which are one of the richest groups among insects (Kaya et al. 2015). They can indicate areas of endemism (Footitt & Alder, 2009; Solis, 1997). Because of their dependence on the climatic, vegetational, and ecological characteristics of the environment, butterflies are employed by many authors as bioindicators (Pollard, 1997; Robbins & Opler, 1997; Perveen & Fazal, 2013).

The Pieridae family is a relatively small family of Lepidoptera comprising about 1100 species placed in four subfamilies and 85 genera (Wahlberg et al., 2014; Naderi, 2012). Pieridae includes the most familiar butterflies also known as Cabbage-White and Grass Yellow. They have a long stable status of the species in this family (Nazari et al., 2011; Abro et al., 2018). These Species have long been the subjects of ecological and evolutionary studies (Wahlberg et al., 2014; Naderi, 2012).

Iran, with an area of 1,648,195 km², with a very high climatic diversity, also has high biodiversity. In terms of animal geography, Iran is located in the Palearctic region and geographically on the desert belt of the northern hemisphere (Naderi & Zehzad, 2019). Despite the rather dry climate of the country, the northern line of the country comprises humid forests. Owing to its various geographic and climatologic diversity, relatively rich fauna and flora are expected in the country (Feizpoor, Shirvani, & Rashki, 2014). Therefore, with at least 400 species of butterflies, it ranks first among neighboring countries (Naderi & Zehzad, 2019).

The study of Iranian butterflies started in 1832 by the Russian scholar Edward Menetries in the Nakhchivan, Talesh, and northern borders of Iran. Jalal Afshar is one of the first Iranian researchers who showed great interest in studying butterflies (1894-1974). Many studies have been continued by many foreign and Iranian researchers such as Bienert (1858-1859), Grant Duff (1905), Davachi (1949), Hashemi Tafreshi (1966), Robert (1970), Freina and Witt (1987, 1990), Eckweiler (1979-2001), Carbonell (1994-2001), Ten Hagen (1998-2001), Naumann, et al. (1999), de Freina (1997, 2001), Mirkarimi (2003), Laszlo et al. (2007), Lehman and Zahiri (2011), and many others (Iraji & Ghorbani-yekta, 2016; Salatin, 2013; Khayrandish, Ahmadzadeh, & Naderi, 2010; Lehmann & Zahiri, 2011). Also, Nazari (2003), Naderi (2012), and Tshikolovets, Naderi, and Eckweiler (2014) have done valuable surveys on the taxonomic status of butterflies in different regions of Iran. So far, about 50 species of the Pieridae family have been identified in the whole of Iran. Vazrik Nazari in his book *Butterflies of Iran* (2003) has reported 20 species of this family throughout Mazandaran province.

The genital structure has been one of the most important sources of characteristic information in Lepidoptera systematics (Atay, 2012). The morphology of the female genitalia has been important in less specific levels of taxonomic divisions in Lepidoptera, while the male genitalia is commonly used taxonomically in more specific levels as genus and species (Leite, Casagrande & Mielke 2011). Studies have shown that genitalia are more stable and less changeable than other taxonomic traits such as wing color and motifs (Pazuki, 1976). In the article of Singh Kirti and his colleagues

in 2020, the morphological characters, genitalic attributes, and the distribution of six species belong to the genus *Pieris* from India was studied (Kirti, Kaur, Sidhu, & Singh, 2020). Abro et al. studied biodiversity of the Pieridae family from Tandojam Pakistan (2018). The structure of the male genitalia in some genera of this family is also published in the book *Indian Insects, diversity, and Science* (Ramani, Mohanraj, & Yeshwanth 2020). In another study entitled *a review of records for Colias erate (Lep.: Papilionoidea Pieridae) in Cyprus: were they all yellow forms of Colias croceus*, the male genitalia structure was studied (John, Coutsis & Makris, 2006). The external morphological study of *Pieris rapae* (Lepidoptera: Pieridae) in the mid and North of Iraq also aimed to examine the structure of the genitalia (Al-Asady & Aljaf, 2015).

The present study is in the framework of faunistic studies of the dominant fauna of Pieridae and contributes to the knowledge of Lepidoptera species and their distribution in the west of Mazandaran province. This report contains records of 7 frequent species. Illustrations of wing taxonomic characteristics of collected adults and a preliminary study of male genitalia structure are presented for all species.

Since previous studies have not covered all areas in the west of Mazandaran province, more regional research is needed to complete the information. It is hoped that this survey will provide a benchmark for future research.

Material and Methods

Study Area

Mazandaran province is located on the southern coast of the Caspian Sea and along the northern part of the Alborz Mountain range and geographically divided into two parts: the coastal plains and the mountainous areas. It lies between 50°15'E and 56°15'E and between 35°45'N and 38°15'N. The province is characterized by a moderate and humid climate (Ezzatpanah, Latif, Malek, & Salehi, 2010). The province had an average temperature of 25°C in summer and about 9 °C in winter. The mean annual rainfall in the coastline equals to 977 mm, the spatial distribution decrease from West to East.

Given the climatic change and varying rates of rainfall in different parts of Mazandaran province, this region has a variety of climates, including the mild and humid climate of the Caspian shoreline and the moderate and cold climate of the mountainous regions. The western and central plains of the province, up to the northern foothills of the Alborz Mountain Range, experience the mild climate of the Caspian region (Bunalski, Samin & Ghahari, 2016).

Field work was carried out in 6 areas including Ramsar, Abbasabad, Kelardasht, Chalous, Marzanabad, and Kojur (Figures 1 and 2). Sampling stations at different heights (high and low altitudes) were considered. Using GPS, the geographic coordinates of research stations and their height above sea level were measured (Table 1).

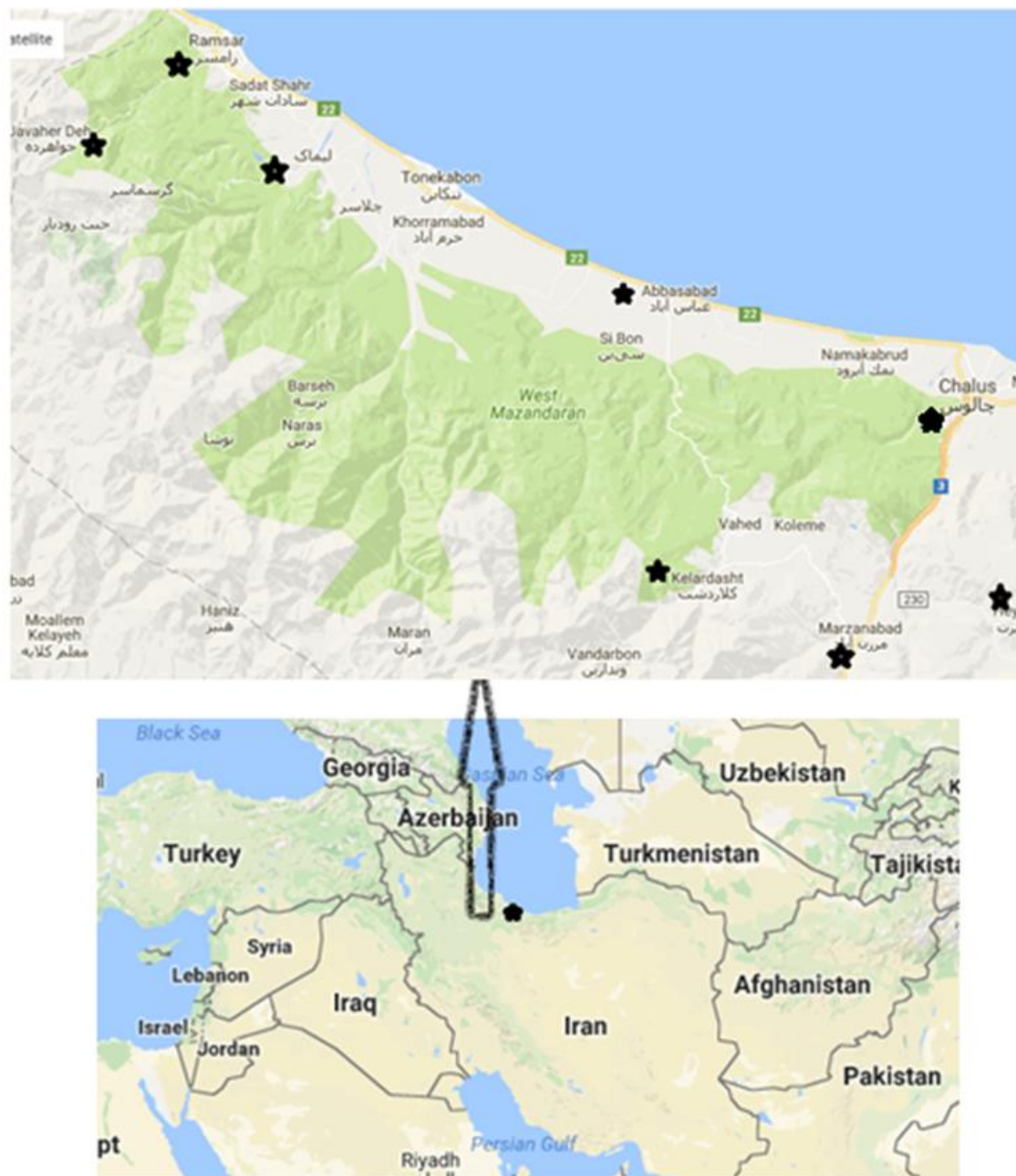


Figure 1. Map of the Study Area Showing the Range of Sampling Localities in Western Mazandaran Province (with Black Stars)



Figure 2. Images of some Habitats of the Collected Specimens

Table 1. Geographical Characteristics of the Study Area and the Number of Collected Specimens

The study area	Location	Geographical coordinates(Latitude and Longitude)	Altitude	Number of specimens Based on High and Low altitude
1- Ramsar	Ramsar City	36°53'N, 50°41'E	34-70	18
	Javaher Deh road	36°50'N, 50°40'E	100	
	Mar kooh road	36°51'N, 50°43'E	71	
	Telar sar	36°51'N, 50°43'E	103	
	Larsanvar	36°54'N, 50°39'E	300	
	Arbeh kaleh	36°31'N, 51°11'E	600-700	
	Seighal mahalleh of Javaher deh	36°50'N, 50°28'E	1800	6
	Siasarad	36°34'N, 50°30'E	1850	
2- Abbas Abad	Abbas abad city	36°59'N, 50°35'E	34	25
	Baooj kheel	36°41'N, 51°06'E	39	
	Shoja mahalleh	36°40'N, 51°06'E	60	
	Ali abad	36°30'N, 51°17'E	85	
	Kela kordeh	36°54'N, 50°35'E	87	
3- Kellar Dasht	senar	36°30'N, 51°17'E	1037	20
	Lash sar	36°33'N, 51°09'E	1080	
	Kordi chal	36°31'N, 51°12'E	1093	
	Korna sangh	36°30'N, 51°16'E	1147	
	koleno	36°31'N, 51°13'E	1177	
	Makarud	36°33'N, 51°09'E	1227	
4- Chalous	Faraj abad	36°67'N, 51°45'E	10	29
	Shahrak navvab	36°64'N, 51°42'E	45	
	Mazoo poshteh	36°64'N, 51°40'E	100	
5- Marzanabad	Gharmabak	36°45'N, 51°27'E	750	56
	Tovir	36°40'N, 51°26'E	865	
	Senar sofla	36°51'N, 51°29'E	1019	
6- Kojour	Hasan abad	36°51'N, 51°35'E	500	56
	Dasht-e Nazir	36°42'N, 51°42'E	924	
	Mikh saz	36°30'N, 51°57'E	1830	

Butterfly Identification

The sampling was random and conducted from April to September, during the year 2015, throughout the day from 10:00 am to 16:00 pm. On average, two visits per month at each study site for a six-month period were undertaken. Specimens were captured with handheld nets only when necessary to prevent the possibility of handling effect. Species were noted along with the date, location of capture, altitude, and any plant association. Butterflies were identified in the field using field guides by Naderi (2012). Images of some habitats of the collected specimens are shown in Figure 2. Habitats are very diverse and include pastures, mountain heights, wooded areas, flower gardens, and areas with suitable vegetation. A small number of the collected specimens were killed in killing jars with ethyl acetate, then put in an especial envelope and all materials were brought to the laboratory for preparation taxonomic study. After softening for 2-3 days, they were pinned with standard insect pins of the appropriate number, stretched on stretching boards (stall) and dried. A stall with a groove close to the size of the abdomen of the butterfly was prepared. Detailed taxonomic and color patterns identification was done using keys available in the book *Butterflies of Iran* (Braby, 2000; Nazari, 2003, Naderi, 2012, and Ramani et al., 2020 (Figure 3).

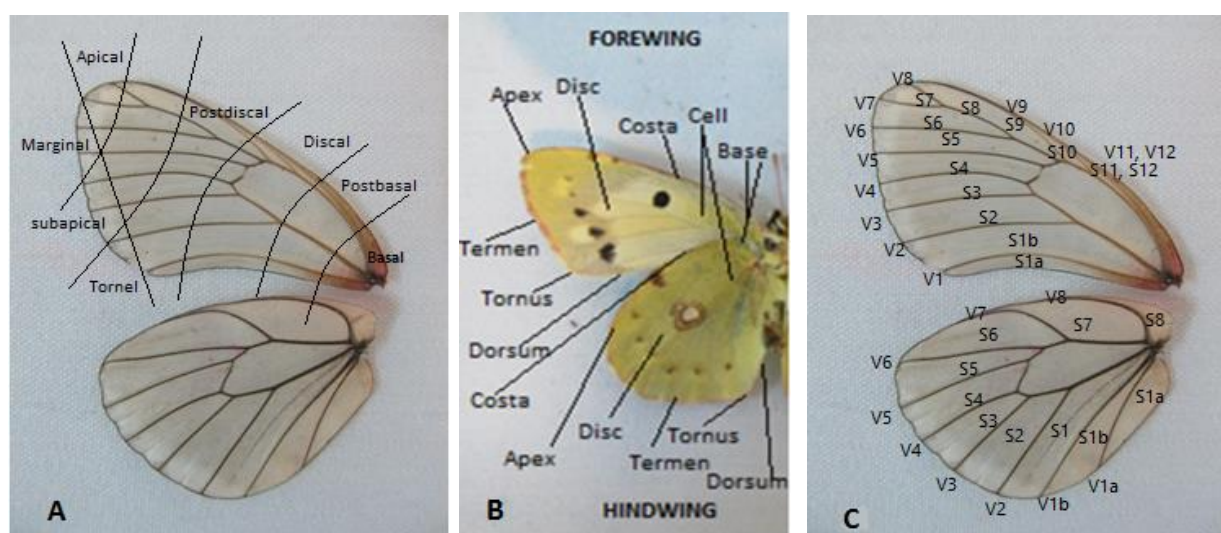


Figure 3. **A, B:** Specialized terminology related to the forewing and hindwing structure of Adults; **C:** The numerical system simply numbers veins from dorsum to costa. **Terminology:** **Apex:** The tip of a wing between the leading edge and the trailing edge; **Basal:** At the base of the wing; **Base:** Where the wing is attached to the thorax; **Cell:** An area of a wing enclosed by veins; **Costa:** The leading edge of the wing when the butterfly is in flight; **Cross bands or fascia:** a band of colour across the wing from leading edge to trailing edge; **Termen:** the outer edge of the wing - between the tip and the trailing corner; **Dorsum:** The trailing edge of a wing when the butterfly is in flight; **Tornus:** the trailing corner of the wing - the corner between the outer and trailing edges of the wing; **Ocellus:** A rounded spot or marking on the wings, effectively a "false-eye" that may temporarily startle a predator, or divert attack away from the body of the butterfly; **Lunule:** A crescent shaped mark, typically found in a series around the wing margins in some families; **Apical:** relating to or denoting an apex; **Discal:** Located in a cell near the middle of a wing that is relatively free of veins; **Marginal:** An edge of a wing, usually the farthest from the body, more accurately called the outer margin; **S: Sub marginal:** Somewhat inward from the edge of the margin; **V: Vein:** A tubular blood vessel, particularly in reference to the tubes supporting the membrane of butterfly wings (Braby, 2000; Nazari, 2003, Naderi, 2012; Ramani et al., 2020).

Genitalia Preparation

After the morphological identification of species, male genitalia dissection was performed. In this study, two pairs of pointed forceps, a very fine camel-hair brush and several very fine needles were the most requirements. For examination of male genitalia, the complete abdomen of the specimen was removed from the body. The abdomen was placed into 5 ml of 10% potassium hydroxide (KOH 10%) solution for at least 24 hours (depending on the size of the abdomen) to soften the tissues. Then, the abdomen was washed in water to remove KOH. In the next step, the abdomen was transferred to 20%, 70%, and 90% alcohol, for 10 min in each. After dehydration, samples were immersed in xylene for 5-7 min, then stained with yellow eosin. Eventually, the abdomen was dissected with the help of fine-pointed forceps from the lateral side. Genitalia was separated from the abdomen (of 7th abdominal segment), washed with distilled water, and then examined under the dissecting monitoring stereo microscope. Genitalia material was preserved into a micro vial with a drop of glycerin and pinned with the specimen. Genitalia terminology is used by many scholars (Klots, 1970; Winter, 2000; Neild, Nakahara, Fratello, & Harvey, 2014; Wahlberg et al., 2014; Leite et al., 2011; Mal, Memon, Shah, Memon, & Shah, 2015; Bonfantti, Casagrande & Mielke, 2013, Kirti et al., 2020, Leite, Casagrande, & Mielke, 2013) (Figure 4). All measurements were taken in millimeters.

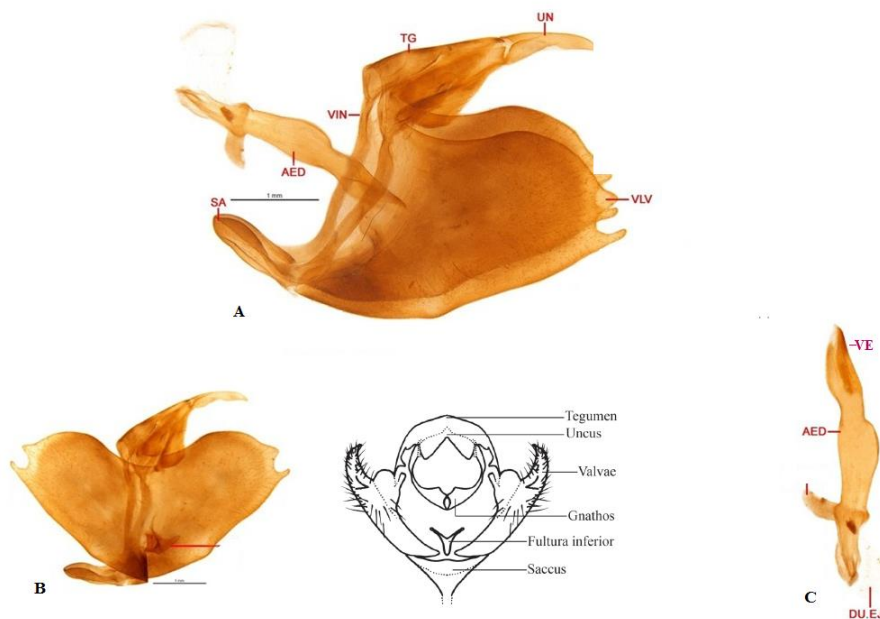


Figure 4. **A:** Specialized terminology related to the male genitalia structure in butterfly family Pieridae. **A:** Lateral View; **B:** Caudal View; **C:** Aedeagus. **Terminology:** **VLV:** Valva; **UN:** Uncus; **TG:** Tegumen; **VIN:** Vinculum; **SA:** Saccus; **AED:** Aedeagus; **VE:** Vesica; **DU.EJ:** Ejaculatory Duct (Kirti et al., 2020; Leite et al., 2013)

Statistical Analysis

Statistical evaluation was carried out by the Kolmogorov-Smirnov test to examine the normal distribution. Data were analyzed using the t-test to compare the means of the number of samples at two heights and one-way analysis of variance (ANOVA) for comparison of the means of the number of samples in different locations, with software SPSS version 22. In these results, $p < 0.05$ was considered as a significant level.

Results

In total, 7 species of the Pieridae family, Pierinae subfamily, and Tribus Pierini are known from the study area. Three species belong to the genus *Pieris* and the other four species are in the genus *Aporia*, *Pontia*, *Colias*, and *Gonepteryx*. The results are listed below:

1. Species: *Pieris Brassicae* (LINNAEUS, 1758)

Subspecies: *Pieris Brassicae Brassicae*

Morphological Characteristics

Males: forewing length in male is between 28-33 mm; **upper side of wings:** background is white; the anterior margin of the forewings are covered with a dust of black scales; apex of the forewing is black that extends beyond V_3 ; on the hindwing, a black spot is located on the S_7 of the anterior margin (Figure 5-1A).

The underside of wings: background is white; the apex of the forewing and the whole of hindwing are somewhat greenish and covered with specific gray dust; the forewing has two spots on S_{1b} and S_3 ; hindwing has a dark shadow on the anterior margin (Figure 5-1B).

Females: forewing length in female is between 30-37 mm; **upper side of wings:** background is white; the forewing has two specific and large black spots on S_{1b} and S_3 and a long dark triangular spot on the inner margin; on the hindwing, black spot in S_7 in female is larger than male (Figure 5-1C); **the underside of wings:** it is similar to males in females (Figure 5-1D).

Male Genitalia Structures

The valva is more or less symmetrical, broad, and thick. The upper margin (dorsal) of the valva is arcuate but in lateral edge is slightly straight and in the distal part of ventral margin armed with strong thorn-like appendage; ventral margin of the valve is smooth, without bending. Uncus in dorsal view is V-shaped and in lateral view is narrow and slightly bent down at the tip. Tegumen is sclerotized and oriented upwards. The vinculum is wavy. Saccus is large, sclerotized, rounded at the base, and bent upwards. Aedeagus is thick, and relatively short, protruding in the middle part. Thecal appendage is well visible. Ejaculatory duct is almost thick and has a sharp point. Vesica is blunt and sharp (Figure 6-1).

2. Species: *Pieris (Napi) Pseudorapae* (Verity, 1908)

Subspecies: *Pieris Pseudorapae Mazandarana* (Eitschberger, 1987)

Morphological Characteristics

Males: forewing length in males is between 26-28 mm; **upper side of wings:** background is white; apex of the forewing has a black spot that extends to V_3 . The third cell is spot because the prominent black scales are different. The first cell's spot is big and wide, round or drop-like with dust of white scales. The second cell's spot is similar to the first spot and by black scales attached to it. Basal dust is clear; hindwing has a big and specific spot on the anterior margin (Figure 5-2A).

The Underside of Wings: the apex of forewing and the whole of hindwing is bright sulfur yellow; in forewing, the cell's spots are bigger and stronger than the spots of upper side of wings. The third spot is joined to black area of the apex and is less clear. The basal area is covered by black dust. On the hindwing, the spot of the anterior margin is smaller than upper side of the wing but is clearer. Black dust on veins is very weak and in the cell area is absent (Figure 5-2B).

Females: forewing length in females is between 25-27 mm; **upper side of wings:** is similar to males. Black spot on the apex of the forewing is bigger; the spot of the end of the cell is clearer. The basal area has more dense gray scales; the background of the hindwing is light yellow; the spot of anterior margin is large but is brighter than the forewing designs. Wing veins to the outer margins become black; spaces between veins have dark scales (Figure 5-2C).

The underside of wings: is similar to male. In the forewing, the cell area has much weaker dark scales. Also, the darkness of wings is unclear without density even to the near of cell (Figure 5-2D).

Male Genitalia Structures

The valva is more or less symmetrical, broader, and thicker than *Pieris brassicae*. The upper margin of the valva is relatively curves and the apex is round. The lateral and ventral edge of the valva is without thorn-like appendage unlike the *Pieris brassicae*. Small thorns are seen on the dorsal margin of the valve. The ventral margin of the valva is slightly concave near the base. The uncus is V-shaped in dorsal view and narrow and long with a very sharp tip in the lateral view that is larger than *Pieris brassicae*. Tegumen is sclerotized and broad. The vinculum is wavy. The saccus is almost similar to the saccus of *Pieris brassicae*, which is large, sclerotized, rounded at the base, and bent upwards. Aedeagus with medium size and is narrower and more curved than the aedeagus of *Pieris brassicae*. The thecal appendage is well visible; ejaculatory duct is angular; vesica is almost blunt and sharp (Figure 6-2).

3. Species: *Pieris rapae* (Linnaeus, 1758)

Subspecies: *Pieris Rapae Rapae*

Morphological Characteristics

Male: upper side of wings: forewing length in males is between 18-25 mm; the apex of the forewing is sharp; the background is white; dark dust on the basal area is distinctive which extends on the anterior margin; a black spot of the apex of the wing is inconspicuous and rarely exceeds V_6 , even sometimes absent. Black spots are usually absent but in some instances, S_3 spot of the forewing and a black spot on the anterior margin of hindwing have a weak presence (Figure 5-3A).

The underside of wings: S_3 spot of the forewing and sometimes S_{1b} spot are present; the apex of the forewing and the whole of hindwing is light yellow and covered with dust mass of gray scales (Figure 5-3B).

Females: in first-generation, **upper side of wings:** forewing length in females is between 23-26 mm; the background is grayish-white; basal dust is bold and extensive especially in the forewing; the spot of S_{1b} and S_3 is black and distinctive. On the hindwing, the black spot on the anterior margin is usually obvious (Figure 5-3C). **The underside of wings:** is similar to males (Figure 5-3D).

Male Genitalia Structures

The valve is like two previous species with the exception of a few small difference; the upper and lateral margin without thorn-like appendages; the valva is triangular and the apex has been sharper than in the *Pieris brassicae* and *P. pseudorapae*. The ventral margin of the valva is slightly concave near the base. The uncus is straight with a sharp tip but has a shorter and smaller size than the two previous species. Tegumen is sclerotized and straight. The vinculum is sclerotized, broad, straight, and without bending. The saccus is almost similar to the saccus of *Pieris brassicae*, which is large, sclerotized, and rounded at the base. The aedeagus is shorter than the two previous species and gently arcuate. The thecal appendage is well visible; the ejaculatory duct is short; vesica is rounded at the tip (Figure 6-3).

4. Species: *Aporia Crataegi* (Linnaeus, 1758)

Morphological Characteristics

Males: forewing length in males is between 25-36 mm; **upper side of wings:** the background is white; all wing veins are completely black near the apex of the forewing, and outer margins are slightly wider and are seen as small transparent triangles; the spot of the end of the cell is large and long; upper side of hindwing is unsigned; inner margin is convex (Figure 5-4A). **The underside of wings:** the wing veins are darker; the background especially in hindwing is more yellowish and accompanied with dispersing black scales (Figure 5-4B).

Females: forewing length in females is between 33-37 mm. It is similar to males but is bigger; **upper side of wings:** the forewing is bright, and only a small area of white scales is seen near the margins; the spot of the end of the cell is rarely seen; wing veins towards the basal area are yellow-brown and black and darker towards the margin; marginal small transparent triangles are weak. Like the forewing, the hindwing is usually bright but sometimes has a relatively stronger white background (Figure 5-4C). **Underside of wings:** the apex of the forewing and whole of hindwing have a yellow color darker than males, with more scattered black scales (Figure 5-4D).

Male Genitalia Structures

The valva is more or less symmetrical; the total structure is very sclerotized; the valve is similar to previous species but with less width and thickness. The valve in the dorsal margin is slightly convex; the length of upper margin is almost half of the ventral margin of the valve. The apex has a triangular shape and slightly rounded. The uncus is long, arched from middle to the distal end, distal tip pointed, downcurved and sharp. Tegumen is sclerotized and curved. The vinculum is almost wavy. Succus is broad, large, sclerotized, and rounded at the base; external surface of cylindrical

succus is like a series of the loop, which has a ring-like appearance. The aedeagus is relatively long, thin, and bent. The thecal appendage is well visible; vesica is straight and short (Figure 6-4).

5. Species: *Pontia (Daplidice) Edusa* (Linnaeus, 1758; Fabricius, 1777)

Morphological Characteristics

Males: forewing length in males is between 20-25 mm; **upper side of wings:** the background is white; dark dust on the basal area is distinctive. In the forewing, the spot of the end of the cell is large and black, with a white line in the middle. The margin design is black and specific but rarely extending from the V_3 . On the hindwing, marginal designs are reduced or absent; sometimes a black spot is seen in S_7 ; designs of the ventral surface of wings are seen from upper side (Figure 5-5A).

The underside of the wings: In the forewing, the spot of the end of the cell is distinctive and extends to the anterior margin. Marginal designs are green-dark yellow; the spot of S_3 is slightly darker; hindwing has a basal, cellular, and marginal large spot, all of which are green (Figure 5-5B).

Females: forewing length in females is between 20-24 mm; **upper side of wings:** is similar to males, with a broader and deeper dark designs; forewing has a large and specific black spot in S_{1b} . upper side of hindwing is darker than males. The surface of the wing has specific marginal designs with a dust of scattered black scales (Figure 5-5C).

The underside of the wings: is similar to males; the forewing has a specific and black spot in the S_{1b} area (Figure 5-5D).

Male Genitalia Structures

The valva is more or less symmetrical, broad, and thick; the dorsal margin of the valva has a convex bulge, which is different from other species. The apex of the valva is relatively sharp and triangular. The uncus is similar to *Pieris brassicae* but its point is bent slightly inward; tegumen is sclerotized and oriented upwards. The vinculum is wavy. Saccus is large and has a crescent-like shape. Aedeagus has a medium size, relatively thick, and completely arcuate shape; thecal appendage is short. The ejaculatory duct is narrow with a sharp tip; vesica is blunt with an oblique edge (Figure 6-5).

6. Species: *Colias Crocea* (Fourcroy, 1758)

Morphological Characteristics

Males: forewing length in males is between 23-30 mm; **upper side of wings:** the background is dark yellow-orange. The basal of the wing is broader in hindwing with gray dust; in the forewing, the spot of the end of the cell is specific and black; the marginal black strip is complete and is wider at the apex. Veins in the apex of the wing are usually seen as weak yellow transverse rays; on the hindwing, the spot of the end of the cell is different with background, sometimes lighter and sometimes more bold; sexual spot on anterior margin has a light cream color; the marginal dark strip is continuous and complete and will not be broken (Figure 5-6A). **The underside of wings:** the background is light green to yellow; the cell area in the forewing is lighter with orangish color. Beneath of cell is yellow, the spot of the end of the cell is similar to upper side of the wing, usually with white centers. Series of sub-marginal spots are present, sometimes atrophied, but usually the

spots of areas S1b, S2, and S3 are well visible. In hindwing, the background is combined with dark dust; the spot of the end of the cell is two-part and 8-like; series of sub-marginal spots are light and fading, usually with the exception of the first spot on the anterior margin, which is always large and specific, the rest are barely recognizable (Figure 5-6B).

Females: forewing length in female is between 22-32 mm; **upper side of wings:** the background is similar to males, often a little brighter. Hindwing is covered with dark dust scales; in the forewing, the basal area have dark dust, sometimes broad, and extends to the middle cell; dark marginal bars in both wings have bright yellow spots, usually large, complete, and distinctive (Figure 5-6C). **The underside of wings:** is similar to males (Figure 5-6D).

Male Genitalia structures

The size of genitalia is very small and appearance is different with other identified species in this study. The valva has a unique shape; in the dorsal margin is narrow with a very small thorn-like frill, then downwards and becomes broad and wide in the ventral margin. Uncus is small, narrow, with a sharp tip. Tegumen is sclerotized, relatively small, and almost oriented upwards. The vinculum is not straight. Saccus is round and short. The aedeagus is relatively short (Figure 6-6).

7. Species: *Gonepteryx Rhamni* (Linnaeus, 1758)

Morphological Characteristics

Males: forewing length in male is between 28-33 mm; **upper side of wings:** in the forewing, the anterior margin is concave; the background is bright lemon yellow across both wings. In the forewing, the spot of the end of the cell is very small and dot-like with brown-red color, and in hindwing is unclear; the end of veins in the margin of the wing usually has a small spot that is barely recognizable; in hindwing, wing vein of V2 is sharp and tail-like (Figure 5-7A). **The underside of wings:** the background is much brighter; the spot of the end of the cell is like the upper side of the wing and is very small and dot-like; in hindwing, the background is yellow-white and very bright; wing vein in anterior margin is white; the spot of the end of the cell is like the upper side surface, and has a small silver-colored center (Figure 5-7B).

Females: forewing length in females is between 28-31 mm; **upper side of wings:** the background is pure white, designs are similar to male; in the forewing, the spot of the end of the cell is very small and dot-like; in hindwing, this spot is clearer, with yellow to orange color (Figure 5-7C). **The underside of wings:** background of anterior margin of forewing and whole of the hindwing are bright apple green; the areas of the cell, sub-cell, and outer margin is white; the spot of the end of the cell in both wings has brown-red color; other features are similar to males (Figure 5-7D).

Male Genitalia Structures

The valva is long with fine hairs; the dorsal margin of the valva has an irregular crescent shape; the apex of the valva is very sharp; futura inferior can be seen in the form of two large cones in the middle area of the two valvae. The uncus is narrow and small. Tegumen is sclerotized and arc-shaped. The vinculum is almost straight. Saccus is sclerotized, very long, and drop-shape. The aedeagus is very narrow and long, compared to other identified species, without bending; thecal

appendage is difficult to see; the ejaculatory duct is very short; vesica is narrow and sharp (Figures 6-7).

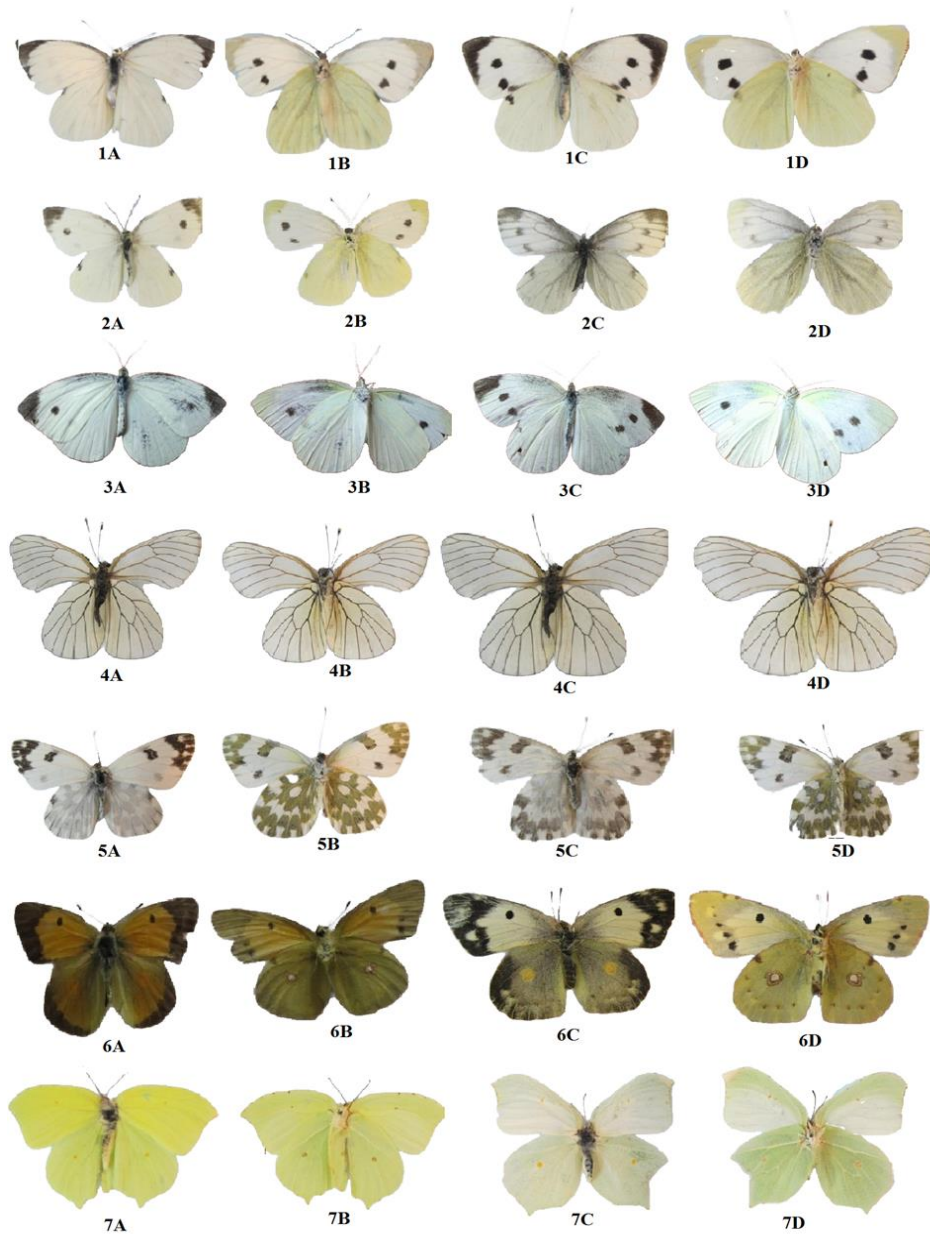


Figure 5. The plate of identified species of family Pieridae based on the Morphologic characteristics of forewing and hindwing. **1:** *Pieris brassicae brassicae*; **2:** *Pieris pseudorapae mazandarana*; **3:** *Pieris rapae rapae*; **4:** *Aporia crataegi*; **5:** *Pontia (daplidice) edusa*; **6:** *Colias crocea*; and **7:** *Gonepteryx rhamni*. In all species, **A:** upperside of male wings, **B:** underside of male wings, **C:** upperside of female wings, **D:** underside of female wings

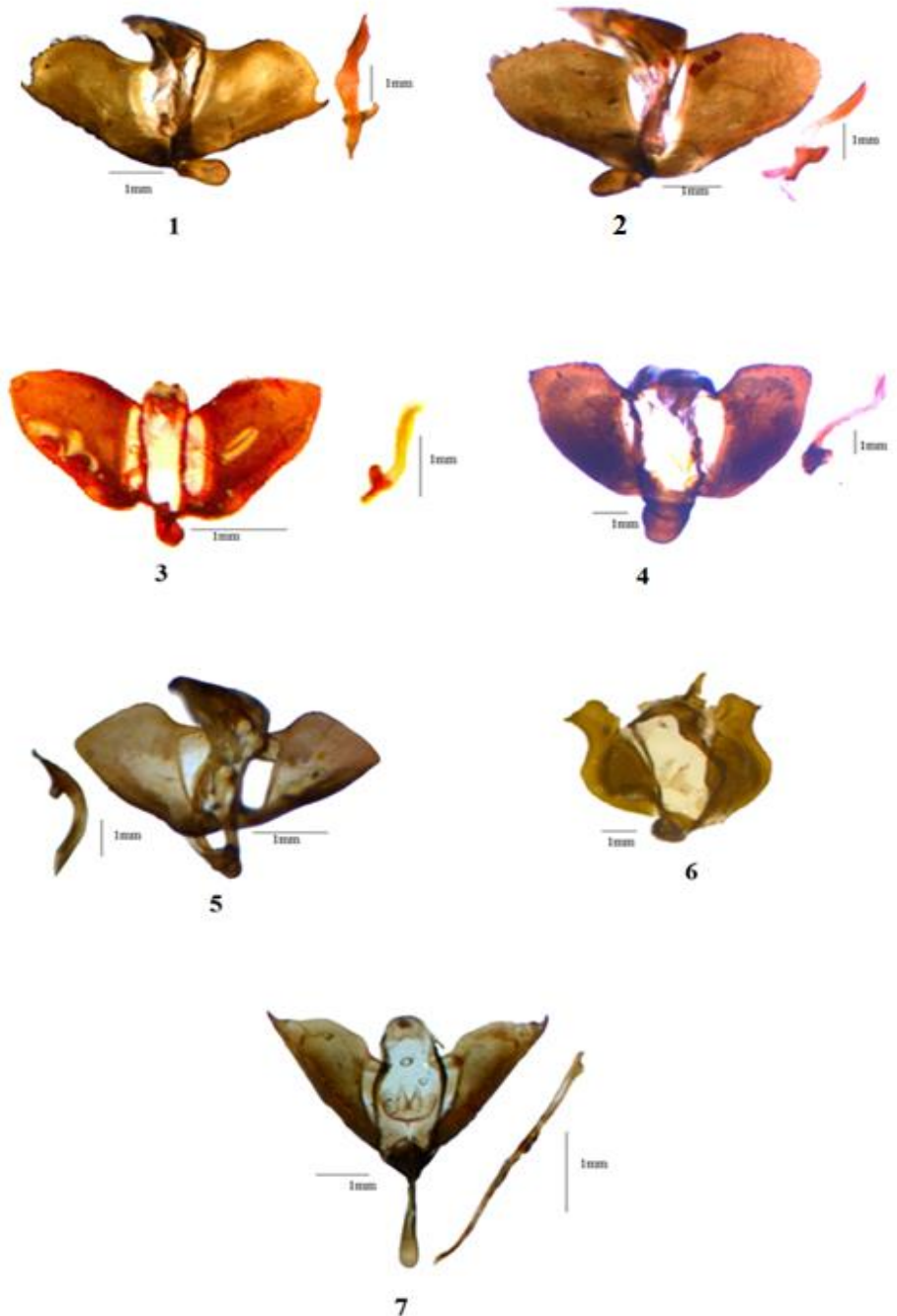


Figure 6. The plate of identified species of family Pieridae based on the male genitalia structure. Male genitalia in marked with a number **1**: in *Pieris brassicae brassicae*; **2**: in *Pieris pseudorapae mazandarana*; **3**: in *Pieris rapae rapae*; **4**: in *Aporia crataegi*; **5**: in *Pontia (daplidice) edusa*; **6**: in *Colias crocea*; and **7**: in *Gonepteryx rhamni*

Statistical Results

After the t-test, no significant difference was observed between the means of the number of specimens at high and low altitudes ($p=0.76$) (Figure 7 A). The means of the number of specimens at different stations were compared using the one-way analysis of variance. The results were not significant ($p=0.69$) (Figure 7 B).

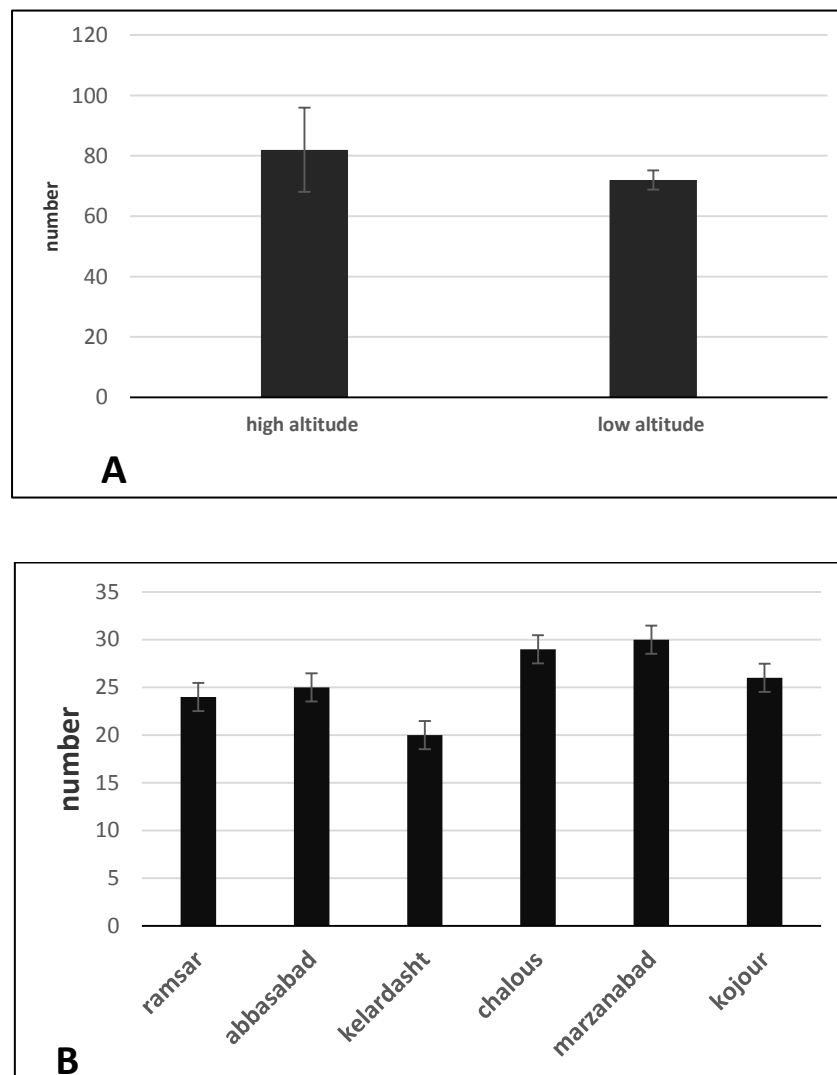


Figure 7. Statistical analysis results to compare the means of the number of specimens **A**: at high and low altitudes and **B**: at different stations

Discussion and Conclusion

According to the results, *Pieris brassicae* (Linnaeus, 1758), *Pieris (napi) pseudorapae* (Verity, 1908), *Pieris rapae* (Linnaeus, 1758), *Aporia crataegi* (Linnaeus, 1758), *Pontia (daplidice) edusa* (Linnaeus, 1758; Fabricius, 1777), *Colias crocea* (Fourcroy, 1758) & *Gonepteryx rhamni* (Linnaeus, 1758) have been studied taxonomically from west of Mazandaran province. The male

genitalia of all the species has been studied and good results were obtained. Using statistical analysis, no significant difference was observed between the mean number of samples at low and high altitudes and at different stations.

The findings of the present study showed that three species of the genus *Pieris* are very similar but there are also some differences that separated them at the level of species. The differences can be seen in terms of wing morphology and structure of genitalia. The other four known species of *Aporia crataegi*, *Pontia edusa*, *Colias crocea*, and *Gonepteryx rhamni* belong to four separated genera and there are significant differences between them. In *Colias crocea* and *Gonepteryx rhamni* these differences are very obvious, especially in the form of valve, uncus, saccus, and aedeagus of genitalia and wing morphology.

The morphology of the wings was consistent with previous studies by Nazari (2003), Naderi (2012), Kirti (2020), and others. In males and females of all three species, the background of upper side of wings is usually white. In *Pieris brassicae*, the background of the underside of wings is white, but in the two other species, little differences can be seen in the underside of wings. For example, in *Pieris pseudorapae*, the apex of the forewing and the whole of hindwing are bright sulfur yellow, and in *Pieris rapae* the apex of the forewing and the whole of hindwing is light yellow and covered with dust mass of gray scales. *Pieris brassicae* in both males and females is larger than the species *Pieris pseudorapae* and *Pieris rapae*. On the upper side of wings, in males of *Pieris brassicae* and *Pieris pseudorapae*, the apex of forewing has a black spot that extends to V_3 , but in *Pieris rapae* a black spot of the apex of the wing is inconspicuous and rarely exceeds V_6 , even sometimes, absent. Also, on the hindwing of *Pieris brassicae* and *Pieris pseudorapae*, a black spot is located on the S_7 of the anterior margin on the upper side that is absent in *Pieris rapae*. Also, some differences in the distribution and number of spots on upper side and underside of wings can be seen between these species that are listed in the result.

The results from the genital structure of two species *Pieris brassicae* and *Pieris rapae* are very similar to the results obtained from Kirti et al.'s study (2020). Also, in the case of species *Pieris rapae*, the results were consistent with Al-Asady and Aljaf (2015). In the study of male genitalia structure, despite many similarities, small differences can also be seen. For example, the valva in *Pieris brassicae* in the lateral edge is slightly straight and armed with a strong thorn-like appendage in the distal part of the ventral margin, while in *Pieris pseudorapae* and *Pieris rapae* thorn-like appendages are not seen. Small thorns are seen on the dorsal margin of the valva only in *Pieris pseudorapae*. In *Pieris rapae*, the valva is triangular and the apex is sharper than in the *Pieris brassicae* and *P. pseudorapae*. Uncus in *Pieris pseudorapae* is long with a very sharp tip that is larger than *Pieris brassicae* and *Pieris rapae*. Regarding other species, we did not find a documented and comparable study about genitalia structure. Shapes of wings, textures, and the color of butterflies change with a great range to such an extent that these characteristics play an important role in the distinction of species at first glance (Kaya et al., 2015). Besides, it is well known that closely-related species or species under similar selective pressures tend to show similar patterns, and those patterns are often obscured by intraspecific variations and/or seasonal forms, thus adding an element of confusion in identification. Several of these species are only distinguished by genitalic preparations (Dias, Casagrande, & Mielke, 2010). In this regard, the

genital structure has been one of the most important sources of characteristic information in Lepidoptera systematics to distinguish between species (Atay, 2012). The shape, structure, and metric dimensions of such parts of male genitalia organs as the valve, uncus, juxta, vinculum, and aedeagus are the major parameters (Onder, 1998; Kiyak, 2000). With all these interpretations, the species reported in our work were all identifiable in terms of wing morphology. The reason for studying genitalia is to increase our knowledge in this field so that it can be used in future taxonomic studies. The study will contribute to the collection of data for genital organs of butterflies in western Mazandaran which has been missing so far.

Altitude is a variable frequently related to changes in species richness and abundance. In general, a negative correlation of altitude was observed with abundance or species richness. Although each family shows a different rate of decline, in the Pieridae family, change in richness is almost imperceptible as the altitude increases (Meléndez-Jaramillo et al., 2019). The results of our study are consistent with Meléndez-Jaramillo et al.'s study in that height has no significant effect on the number of species. Therefore, it can be suggested that vegetation and perhaps temperature and humidity are more determining factors in the abundance and richness of species of butterflies Pieridae than altitude in these study areas.

In a study on the butterfly fauna of northern and northwestern Iran in summer 2007 by Lehmann and Zahiri (2011), 12 species of the family Pieridae were identified, of which only four species *Aporia crataegi*, *Pontia (daplidice)*, *edusa Colias crocea*, and *Gonepteryx rhamni* were reported in the Yush region in western Mazandaran province and the rest of the species belonged to Azerbaijan, Ardabil and Tehran. Studies conducted by other researchers such as Nazari, Naderi, and others led to the presentation of a variety of species of this family in northern Iran. But in these studies, the stations were scattered and cover the whole north of Iran and did not deal exclusively with the west of Mazandaran province. Therefore, the accurate faunistic study of butterflies requires coherent planning and many stations must be explored one by one. It is suggested that these studies should be performed in the center and east of Mazandaran province and also on all families of the butterflies.

It is hoped that these results, in addition to their shortcomings, increase our knowledge of the butterfly fauna in this area. Our studies will continue and in future, we will try to study more species of Lepidoptera from different families based on the male and female genitalia structure which will certainly be very useful to researchers in the systematic and phylogenetic studies of these insects. Further studies of Lepidoptera distributions are indeed needed to evaluate the biodiversity in this area before ecosystems are destroyed by various factors.

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