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Speciation and distribution diffusion of the genus *Aporia* (Hübner, 1819) originated from Kashmir/India,Pakistan

(Lepidoptera, Pieridae)

by

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Abstract: Studies by Kanoh et al. (2016) have speculated that *Aporia leucodice* (Eversmann, 1843) and *A. soracta* (Moore, 1857) speciated from *Aporia nabellica* (Boisduval, 1836) in Kashmir from the continuity variation of wing markings and ♂ genitalia beyond species. In this study, the distribution, geographical variation of wing markings and ♂ genitalia were researched in more detail. The process of speciation by distributed diffusion is supported by our results.

Introduction: The genus *Aporia* (Hübner, 1819) is widely distributed in the region and is diversified into 33 species (Della Bruna et al., 2013). Excluding higher latitudes, most of the species of *Aporia* are distributed in subalpine regions.

Kanoh et al. (2017) studied the distribution and variations of wing markings of various *Aporia* species and considered that, as the distribution area grew wider, the continuous process of speciation left behind a geographical cline, with characteristic variations for each region. This cline occurred as the distribution diffusion (radiation) continued beyond species and the tendency of continued speciation in the direction from species with a dark wing upperside and markings on the deep yellow underside of the hindwing becoming simplified or faded out and ultimately disappearing altogether or being so faint that the wing is almost white. Examination of the morphology of the uncus of the ♂ genitalia suggests that speciation by distribution diffusion (radiative speciation) of the genus *Aporia* started from the Hengduan Mountains and Kashmir.

In this study, the distribution and ♂ genitalia of species-populations originating in Kashmir were studied in detail, and the correlation between distribution diffusion and speciation was considered.

Materials and methods: Three species of *Aporia*, namely *A. nabellica* (Boisduval, 1836), *A. leucodice* (Eversmann, 1843) and *A. soracta* (Moore, 1857) were predicted by Kanoh et al. (2017) to have differentiated since originating from Kashmir, and a distribution conceptual diagram was prepared on the basis of specimen labels using specimens owned by the Research Institute of Evolutionary Biology (RIEB) and the authors. Data from several sorces, including Katayama (2012, 2015), Della Bruna et al. (2013), Tshikolovets et al. (2014), Robin et al. (2013), and Sakai (1981) were used to show the distribution pattern.

Preparing the ♂ genitaia for examinatyion involved excising the tail part of the specimen, which was then soaked in an aqueous solution of 10% potassium hydroxide at ambient temperature for two days, washed in water, and observed by using a binocular stereoscopic microscope. For the transverse plane of the ♂ genitalia, the uncus was observed from the front and an image was drawn by focusing the microscope on the area considered to reflect the most distinctive part of the middle area.

The process of speciation due to distribution diffusion was predicted according to these distribution patterns, together with comparison of the wing markings and the continuity of morphology of the genitalia in numerous ♂ specimens.

Kanoh et al. (2017) considered that the morphology of the uncus of the ♂ genitalia of *A. nabellica* (Bdv.), *A. leucodice* (Eversm.) and *A. soracta* (Moore) as well as *A. agathon* (Gray, 1831) and *A. phryxe* (Boisduval, 1836) in the same habitat, conformed to the "water droplet" shape. This differs from the present results which reveal a "fan shape" for the uncus. However, it was suggested that the species originated from the Hengduan Mountains and so could be a different strain. These were not included in the present study.

Results and Discussion: Distribution diagram studied here is shown in fig. 1, and a dispersion route map also showed in fig. 2. Regarding habitat environment, the south side of Hindu Kush Mountains was a damp environment and the north side was dry. Representative scenery of both sides was shown in figs. 3A and B. While specimens observed ♂ genitalia were shown in fig. 4, and observation diagram of ♂ genitalia were shown in figs. 5. Moreover, the list characteristics of genitalia summerized were shown in table 1.

Aporia nabellica (Boisduval, 1836)

Aporia nabellica (Bdv.) is considered to have the oldest type of morphology (Kanoh et al., 2017), with a dark upperside to the wing and V-shaped markings on the deep yellow underside of the hindwing. *Aporia nabellica* (Bdv.) has a belt-shaped distribution pattern from Kashmir to Uttar Pradesh in north west India (fig. 1). The wing markings of *A.*

n. hesba (Evans, 1912), a taxon distributed in Chitral, Pakistan (type locality), has the most pronounced regression. This area, the taxon is living in, is the most western part of its distribution. The characteristics of the ♂ genitalia of *A. n. hesba* (Evans) are shown in fig. 5. If compared to *A. n. nabellica* (Bdv.), the tip of the valva is more exserted, and the inner hole of the valva is smaller and closer to the base. *Aporia n. hesba* (Evans) has a shorter and wider juxta. the morphology of the protrusion of the central part of the tegumen is also different. All other characteristics are similar between the two subspecies.

Aporia leucodice (Eversmann, 1843)

Aporia leucodice belucha (Marshall, 1883) had a belt-shaped distribution along the southern slope of Hindu Kush Mountains (fig. 1) affecting the damp coniferous forests (fig. 3A). Subspecies distributed from the mountain range to the dry environment on the north side (fig. 3B), affected Afghanistan, Tadzhikistan, Kyrgyzstan, Kazakhstan, and north-east Iran. All subspecies were small in size.

The ♂ genitalia of *A. leucodice* (Eversm.) are shown in fig. 5. Three subspecies were observed at this time. *Aporia l. leucodice* (Eversm.) and *A. l. illumina* (Grum-Grishimailo, 1890) were very similar to each other, but *A. l. belucha* (Marshall) was different from the other two subspecies with a wider juxta with square-shaped tip; when looking from the tegumen side the shape was the "saddle" type, and looked similar to *A. nabellica hesba* (Evans). This similarity can be clearly seen in table. 1. Wing markings of *A. l. belucha* (Marshall) and *A. nabellica* (Bdv.) showed a deep yellow hindwing underside and V-shaped markings from cell 2 to cell 8, these were closer to the outer margin than the central part, and were similar to each other (fig. 4). From this, a correlation between *A. nabellica hesba* (Evans) and *A. leucodice belucha* (Marshall) was concluded. In addition, *A. l. leucodice* (Eversm.) and *A. l. illumina* (Gr.-Gr.) do not have a longitudinal groove in the middle of the dorsal side of the uncus, whilst, when viewing the tegumen from the side, the centre was the "mountain" type and the juxta has pointed tip. These characteristics were thought to have changed during distribution diffusion to the north side of Hindu Kush Mountains.

Wing markings of *A. leucodice* (Eversm.) have a tendency to fade out, the yellow on the hindwing underside becomes lighter, and the V-shaped markings close to the cell, as its habitat becomes farther away from Kashmir, considered to be the starting point of distribution diffusion (Kanoh et al., 2017). *Aporia leucodice aryana* (Wyatt & Omoto, 1966) is distributed to north side of Hindu Kush Mountains, east of Afghanistan. *Aporia leucodice* (Eversm.), *A. l. aryana* (Wyatt & Omoto) lives in areas closest to Kashmir, considered to be the starting point of distribution diffusion. However, yellow on the hindwing underside is light and the V-shaped markings are close to the cell (fig. 4). This is the final trait of distribution diffusion. Consequently, *A. l. aryana* (Wyatt & Omoto) is presumed to be a subspecies which differentiated from *A.l. illumina* (Gr.-Gr.), and not directly from *A. l. belucha* (Marshall).

Hence, as shown in fig. 2, *A. l. belucha* (Marshall) did not directly cross the Hindu Kush Mountains, but is thought to have gone northward to low-altitude areas on the eastern side of the mountains; *A.l. illumina* (Gr.-Gr.) might be differentiated from this population in Tajikistan and Kyrgyzstan and from there then differentiated to *A. l. aryana* (Wyatt & Omoto) and separately spreading in the direction of the Tianshan Mountains to the Afghanistan side by distribution diffusion. Moreover, another population might have developed in northern Iran* from the distribution diffusion of *A. l. illumina* (Gr.-Gr.) to the west. Among the populations from Kazakhstan on the north side of Hindu Kush Mountains to Iran, *A. l. illumina* (Gr.-Gr.) is thought to have old morphology close to *A. l. belucha* (Marshall), since it has mostly dark yellow on the hindwing underside and the V-shaped markings continuing from cell 2 to cell 8 from the centre are closer to the outer margin than other subspecies (fig. 4). This also supports the prediction of distribution diffusion as described above.

Aporia soracta (Moore, 1857)

Aporia soracta (Moore, 1857) is considered to be a type of the subspecies of *A. leucodice* (Eversm.) according to Della Bruna et al. (2013). However listed as a different species by Katayama (2012).

Described as fig. 1, *A. soracta* (Moore) showed distribution from the northern part of Pakistan to western Nepal. This region includes the southern slopes of the Himalaya Mountains, and is a damp environment. *Aporia soracta* (Moore) is larger and has longer forewing and hindwing with various wing markings; it is considered reasonable to treat this taxon as an independent species. As shown in fig. 1, *A. s. soracta* (Moore) is distributed from northern Pakistan to north-western India and *A. s. sara* (Evans, 1932) is distributed from north-western India to the west side of Nepal. Markings of *A. s. soracta* (Moore) showed a range of variation, but are close to no markings. On the other hand, *A. s. sara* (Evans) has clear markings even in western Nepal, which is the end of distribution of *A. soracta* (Moore). Kanoh et al. (2017) theorised that differentiation of *A. soracta* (Moore) occurred from an population which moved to the southern slopes of the Himalaya Mountains, from *A. nabellica* (Bdv.) through *A. l. belucha* (Marshall), similar to *A. leucodice* (Eversm.). In addition, the wing markings of each type of *Aporia* continues to fade as the differentiation process progressed due to distribution diffusion into various areas. However, within *A. soracta* (Moore), the nomino-

*) Tshikolovets et al. (2014) named the population in Iran as *A. l. leucodice* (Eversm.). However, the type locality of *A. l. leucodice* (Eversm.) is the Tarbagatai Mountains east of Kazakhstan, and the eastern limit of distribution of *A. leucodice* (Eversm.). Since the population of *A. l. leucodice* (Eversm.) in northern Iran is the western limit of distribution, review of the nomenclature of *A. l. leucodice* (Eversm.) is needed. Consequently, it is listed as "ssp.?" in figs. 1, 2.

typical subspecies is distributed in areas close to Kashmir, predicted as the origin of distribution diffusion, and has a greater degree of fading in its markings compared with *A. s. sara* (Eversm.), which is at the limit of distribution diffusion. This is thought to be caused as the flow of distribution diffusion spread to the Nepal side once and then returned to the Pakistan side through various causes such environmental changes, as shown in fig. 2. As shown in fig. 5, the top of the juxta has a square tip in *A. sara* (Evans), but *A. s. soracta* (Moore) has a pointed tip. Since juxta of *A. nabellica* (Bdv.) also has a square-shaped tip, *A. s. sara* (Eversm.) is considered to have an older morphology than *A. s. soracta* (Moore). In both of these two subspecies, two specimens had a longitudinal groove in the center of back side of the uncus. The juxta has a protrusion zone around the center which is forked into two (table 1). This characteristic is not observed in the other species, and is considered to be the common morphology in the species.

In the classification of the genus *Aporia*, Della Bruna et al. (2013) showed that morphology of the tip of the uncus of ♂ genitalia is important. Kanoh et al. (2017) indicated that in addition to the tip of the uncus, the shape when seen from the side and the transverse plane view are important in predicting genetic relationships in *Aporia*. In this study, the uncus of *A. nabellica* (Bdv.), *A. leucodice* (Eversm.), and *A. soracta* (Moore) were all observed to be long-triangular shape when viewed from the back side. The tip of the uncus is a tapered cone shape and the transverse plane is fan shaped. These common characteristics show that the three types share the same source species group. Furthermore, the clear longitudinal groove along the center backside of the uncus is not observed in other species of genus *Aporia* (Kanoh et al. 2017), and is thought to be a basic characteristic of specie populations originating Kashmir. Results of detailed observations of the distribution and ♂ genitalia at this time could confirm the conclusionof Kanoh et al. (2017) that after *A. nabellica* (Bdv.) in Kashmir differentiated to *A. l. belucha* (Moore), further differentiation to other subspecies of *A. leucodice* (Eversm.) occurred to the north of Hindu Kush Mountains as distribution diffusion occurred. On the other hand, *A. soracta* (Moore) differentiated from an population distributed and diffused from *A. nabellica* (Bdv.) in the southern slopes of the Himalaya Mountains.

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Table 1: Characteristics of ♂genitalia in *Aporia soracta* (MOORE, 1857), *A. nabellica* (BOISDUVAL, 1836), *A.leucodice* (EVERSMANN, 1843)

Species name	Subspecies name	Uncus		Tegumen		Juxta		
		Fan shape in transverse plane	Longitudinal groove in dosal side	"Saddle" type	"Mountain" type	Square- shaped tip	Pointed- shaped tip	Protrusion zone around center
Aporia soracta (MOORE)	ssp. *soracta* (MOORE,1857)	○	○	○			○	○
	ssp. *sara* (EVANS, 1932)	○	○	○		○		○
A. nabellica (BDV.)	ssp. *nabellica* (BDV., 1836)	○	○	○		○		
	ssp. *hesba* (EVANS, 1912)	○	○	○		○		
A. leucodice (EVERSM.)	ssp. *belucha* (MARSHALL, 1883)	○	○	○		○		
	ssp. *illumina* (GR.-GR., 1890)	○			○		○	
	ssp. *eucodice* (EVERSM., 1843)	○			○		○	

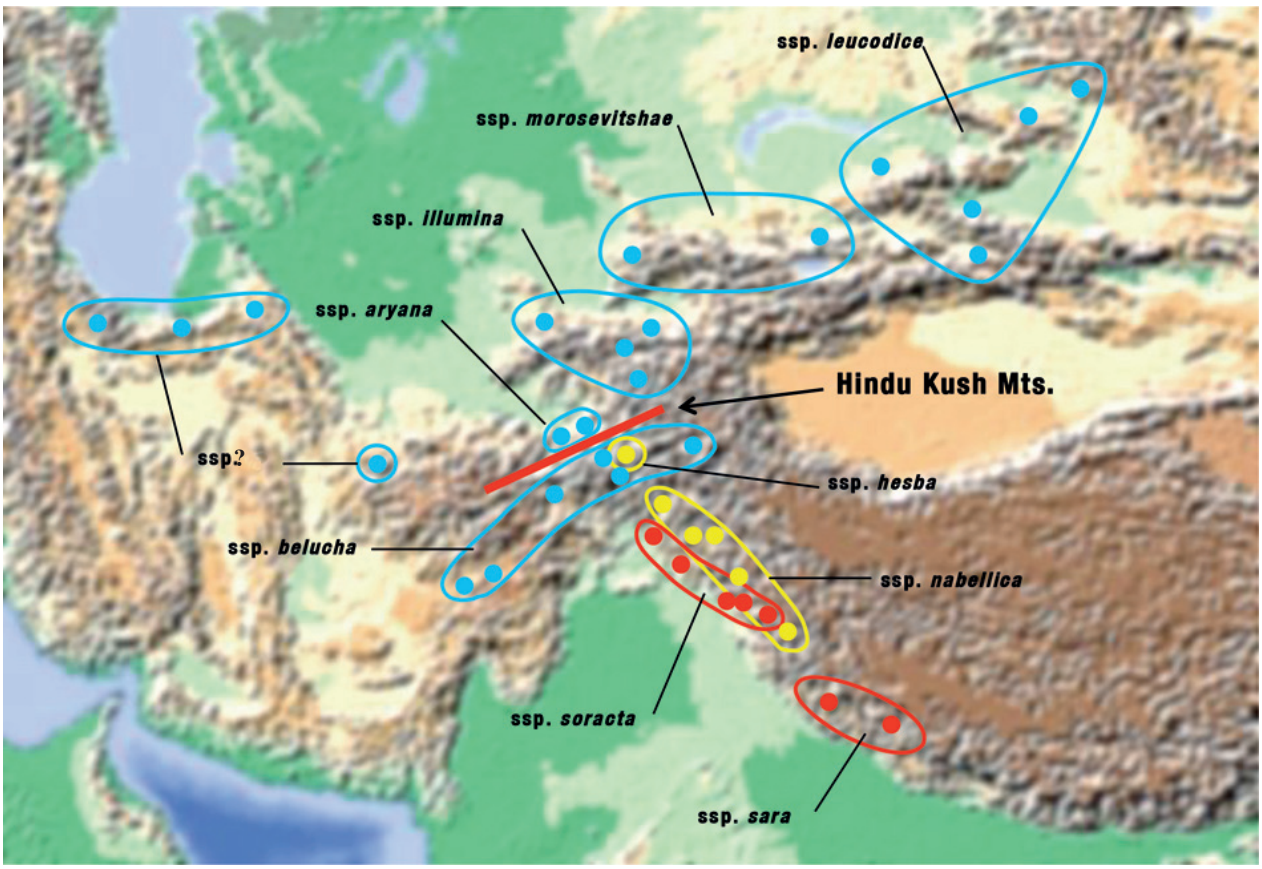

Fig. 1: Distribution map of *Aporia nabellica* (Bdv., 1836), *A. leucodice* (Eversm., 1843) and *A. soracta* (Moore, 1857).
●: *A. nabellica* (Bdv.); ●: *A. leucodice* (Eversm.); ●: *A. soracta* (Moore)

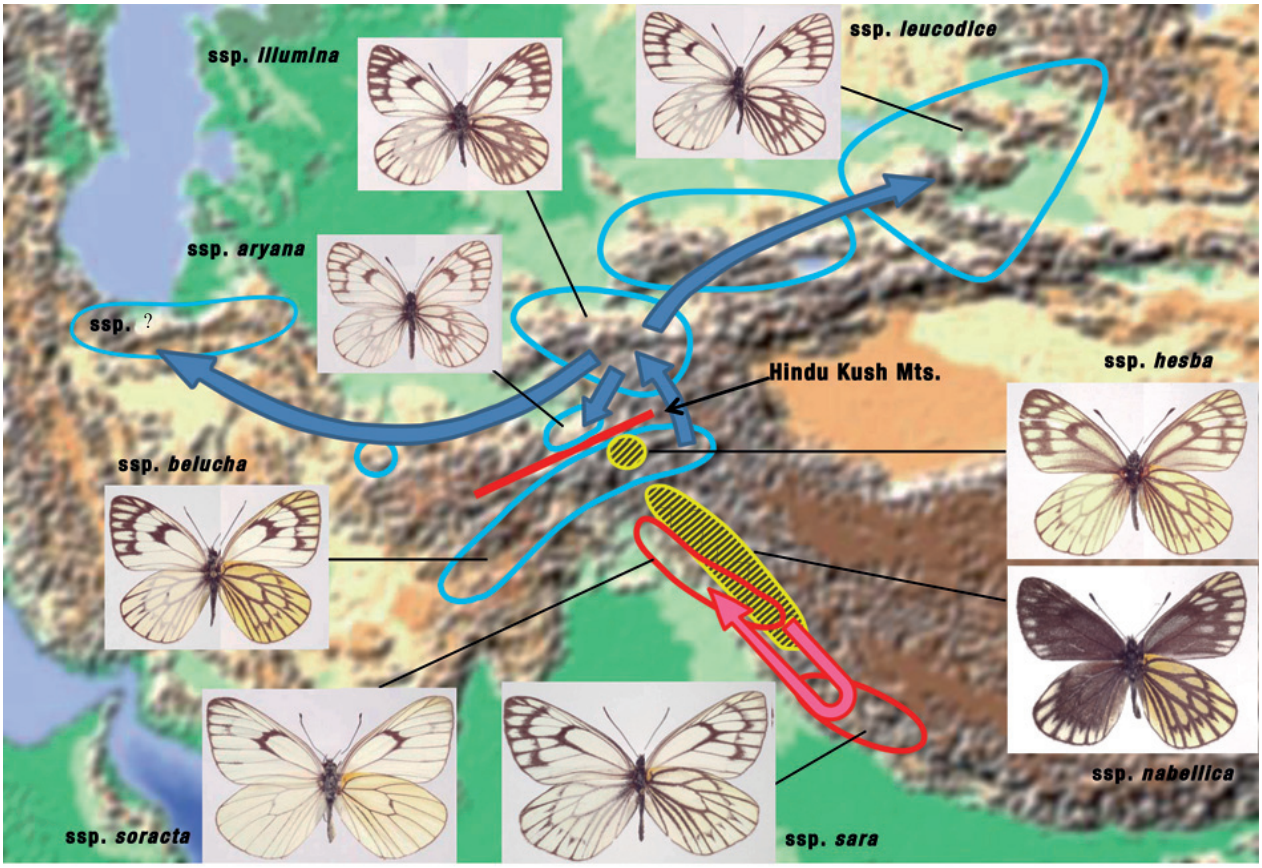

Fig. 2: Dispersion patterns and radiation route map of *Aporia nabellica* (Boisduval, 1836), *A. leucodice* (Eversmann, 1843) and *A. soracta* (Moore, 1857).
: *A. nabellica* (Boisduval, 1836) O : *A. leucodice* (Eversmann, 1843) O: *A. soracta* (Moore, 1857)

Fig. 3: Different environments in the Hindu Kush Mountains. Photos by Seiji Sakai.
A: South side of Hindu Kush Mts., Lawari Pass,Chitral, Pakistan, July 2016.
B: North side of Hindu Kush Mts., Anjuman Valley, Afghanistan, July 1971.

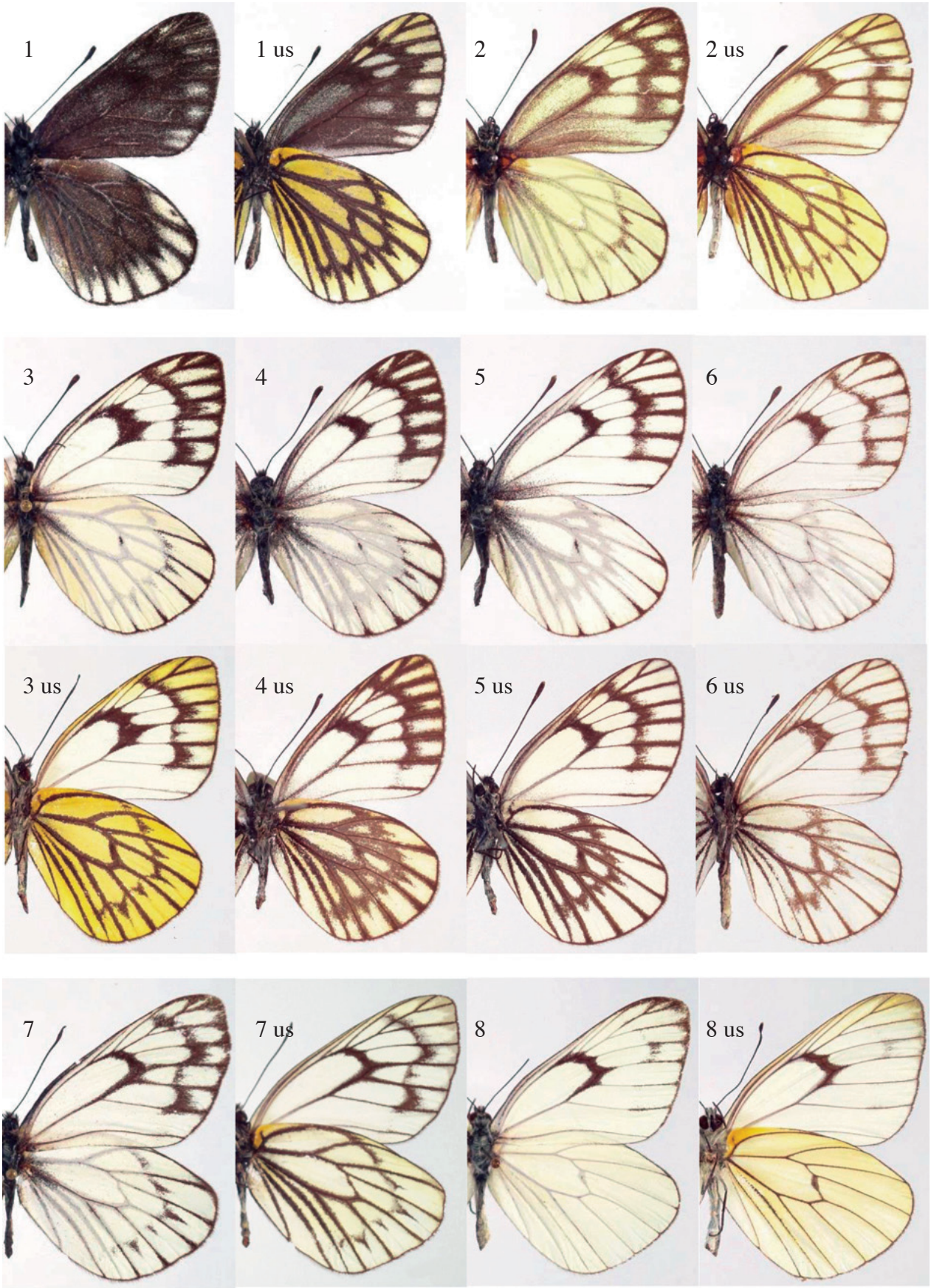

Fig. 4: *Aporia* specimens of ♂♂ used for genitalia dissection (except 6)
(1) *Aporia nabellica nabellica* (Boisduval, 1836), Gulmarg, Srinagar, Jammu and Kashmir, India. Fore wing length (FWL) = 28,3 mm; (2) *A. nabellica hesba* (Evans, 1912), North Pakistan. FWL = 28 mm; (3) *A. leucodice belucha* (Marshall, 1883), Naltar, Dist. Gilgit, Pakistan. FWL = 25,8 mm; (4) *A. leucodice illumina* (Grum-Grishimailo, 1890), Isfairam-sai Vallay, Alai Mts., Kyrgyzstan. FWL = 21,8 mm; (5) *A. leucodice leucodice* (Eversmann, 1843), Bayangol, Tian-Shan, Xinjiang Uygur, China. FWL = 22,9 mm; (6) *A. leucodice aryana* (Wyatt & Omoto, 1966), Kur Petb, Afghanistan. FWL = 20,3 mm; (7) *A. soracta sara* (Evans, 1932), Bhimtal, Uttarakhand, India. FWL = 28,4 mm; (8) *A. soracta soracta* (Moore,1857), Murree Hills, Rawalpindi, Pakistan. FWL = 28,7 mm.

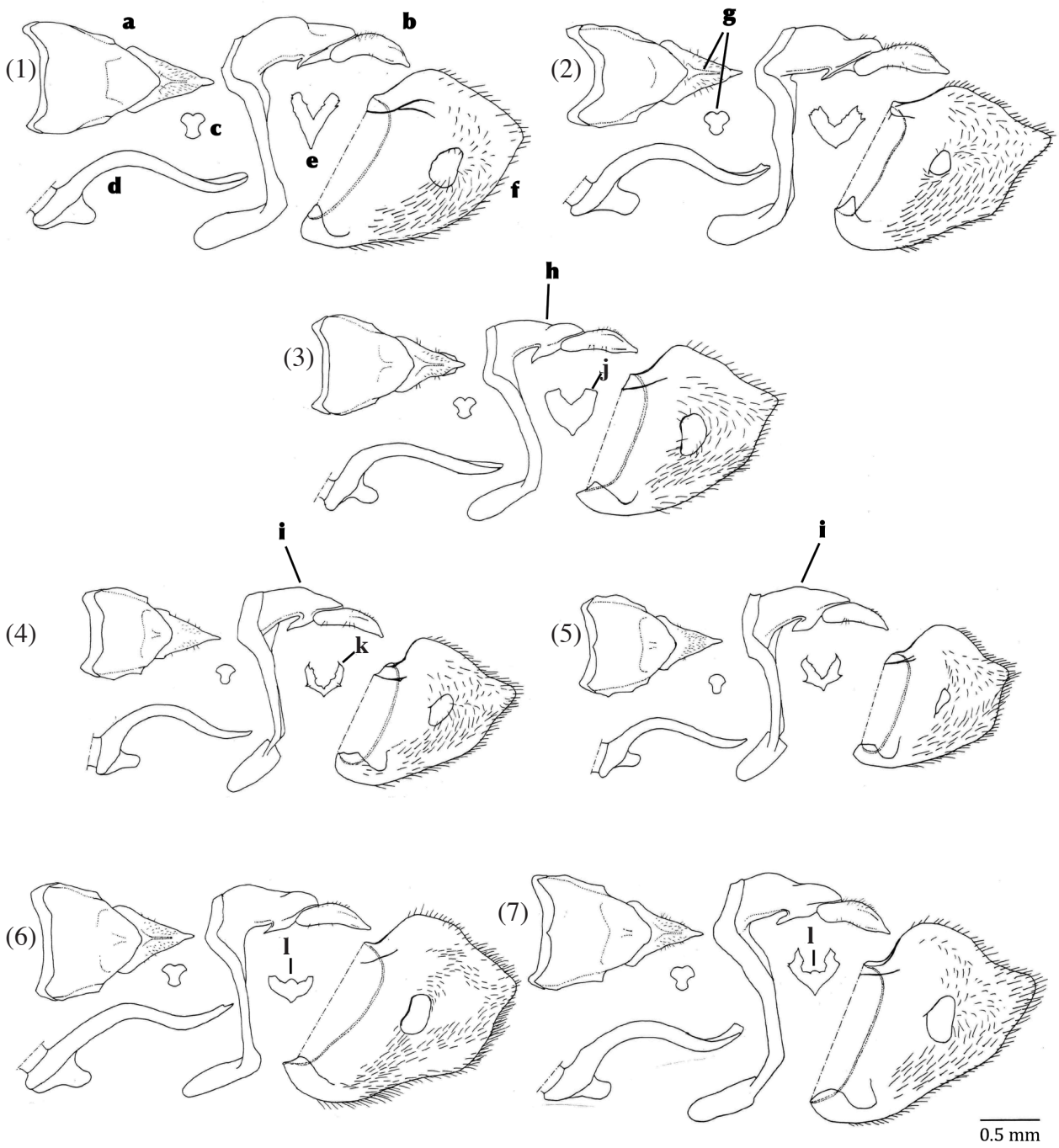

Fig. 5: ♂ genitalia of *Aporia nabellica* (Boisduval, 1836), *Aporia leucodice* (Eversmann, 1843), and *Aporia soracta* (Moore,1857)
a = Ring, dorsal view; b = Ring, lateral view; c = Transvers plane of uncus; d = Aedoeagus; e = Juxta; f = Right valva, inside view; g = Longitudinal groove; h = "Saddle" type tegumen; i = "Mountain" type tegumen; j = Square-shaped tip of juxta; k = Pointed-shaped tip of juxta; l = Protrusion zone around center in juxta.

(1) *Aporia nabellica nabellica* (Boisduval, 1836), Gulmarg, Srinagar, Jammu and Kashmir, India.
(2) *Aporia nabellica hesba* (Evans, 1912), North Pakistan.
(3) *Aporia leucodice belucha* (Marshall, 1883), Naltar, Dist.Gilgit, Pakistan.
(4) *Aporia leucodice illumina* (Grum-Grishimailo, 1890), Isfairam-sai Vallay, Alai Mts., Kyrgyzstan.
(5) *Aporia leucodice leucodice* (Eversmann, 1843), Bayan Gol, Tian Shan,Xinjiang, China.
(6) *Aporia soracta sara* (Evans, 1932), Bhimtal, Uttarakhand, India.
(7) *Aporia soracta soracta* (Moore,1857), Murree Hills, Rawalpindi, Pakistan.