

Muscles of the Male Genitalia in the Butterfly Subfamily Aphnaeinae (Lepidoptera, Lycaenidae)

A. I. Korzeev^{a,*} and A. A. Stekolnikov^a

^a St. Petersburg State University, St. Petersburg, 199034 Russia

*e-mail: korzeev@gmail.com

Received December 21, 2020; revised January 23, 2022; accepted January 23, 2022

Abstract—The morphology of the skeleton and musculature of the male genitalia of the subfamily Aphnaeinae (Lycaenidae), distributed mainly in the Afrotropical Region, were studied and compared with those in other ancient taxa of the family Lycaenidae. The position of Aphnaeinae in the system of the family Lycaenidae is discussed based on the analysis of new comparative morphological data and with reference to the literature data. The male genital musculature was studied for the first time in *Phasis thero* (Linnaeus, 1764), *Chrysoritis pyroeis* (Trimen, 1864), and *Aloeides pierus* (Cramer, [1779]), representing distantly related genera of Aphnaeinae. The composition and topography of their muscles, unlike those of the previously studied Middle Asian species *Cigaritis epargiros* (Eversmann, 1854), were found to correspond to the ground plan of the nymphaloid group of families (Nymphalidae + Lycaenidae) and to show archaic features as compared with those in such early branches of Lycaenidae as the subfamilies Curetinae and Riodininae. Sympleiomorphic similarity of the musculature features of Aphnaeinae and Lipteninae (Liptenini) was established. The subfamilies Aphnaeinae, Poritiinae s. str., Lipteninae, and Miletinae share such a common feature as the lower wall of the saccus elongated into a narrow caudal plate (“saccus directed caudal”). However, it remains unknown whether this character is a synapomorphy of these subfamilies or a result of parallel evolution, i.e., a homoplasy.

Keywords: Lepidoptera, Lycaenidae, Aphnaeinae, male genitalia, muscles, phylogeny

DOI: 10.1134/S0013873822010079

Detailed studies of the skeleto-muscular system of genitalia in the family Lycaenidae started only at the end of the 20th century, although the characters of genital skeleton have been successfully used in the taxonomic and phylogenetic research of Lepidoptera for nearly a century. At present, the genitalia musculature has been studied in most subfamilies of Lycaenidae: Lycaeninae, Polyommatainae, Theclinae (Kuznetsov and Stekolnikov, 1998, 2001; Stekolnikov and Kuznetsov, 2005; Stekolnikov, 2011; Stekolnikov et al., 2014; Stekolnikov and Korzeev, 2019a), and also in tropical representatives of the ancient subfamilies Riodininae, Curetinae, Lipteninae, and Miletinae (Kuznetsov and Stekolnikov, 1998; Stekolnikov and Korzeev, 2017, 2019b; Korzeev and Stekolnikov, 2021). The subfamily Aphnaeinae Distant, 1884 is mainly distributed in Africa with only small enclaves in the Oriental and Palaearctic regions.

Until recently this taxon was considered a tribe within the subfamily Theclinae (Eliot, 1973; Scott, 1985; Hepner, 2003). At the same time, a study of the skeleto-muscular system of the male genitalia in *Cigaritis epargiros* (Eversmann, 1854) (Kuznetsov and Stekolnikov, 1998), along with other features, supported the idea that the tribe Aphnaeini might be more primitive than other groups within Theclinae. The recent molecular studies (Kaliszewska et al., 2015; Espeland et al., 2018) showed this taxon to be ancient and not directly related to the subfamily Theclinae. As a result, the tribe Aphnaeini was promoted again to the subfamily Aphnaeinae (Williams, 2015), but its phylogenetic position remains obscure. For instance, in the opinion of Z.A. Kaliszewska and co-authors (Kaliszewska et al., 2015, fig. 1), the subfamily Aphnaeinae is contrasted with the phylogenetic branch consisting of two sister subfamilies

Poritiinae s. l. (Poritiinae + Lipteninae) and Miletinae; according to other data (Espeland et al., 2018, fig. 1), Aphnaeinae and Poritiinae s. l. form a sister group to the subfamily Miletinae. We hope that the study of the skeleto-muscular system would help to clarify this discrepancy and determine the similarities and differences of morphological evolution in these closely related subfamilies of Lycaenidae.

The genital apparatus of males of the subfamily Aphnaeinae includes all the main elements: the tegumen, vinculum, uncus, subunci, valvae, juxta, and aedeagus. The genital skeleton in this taxon was studied earlier, and schematic drawings of the genital structures of a number of species are available in the literature (Stempffer, 1967; Eliot, 1973; Heath, 1997). The muscles were described earlier only in *Cigaritis epargiros* (Kuznetsov and Stekolnikov, 1998, fig. 3).

MATERIALS AND METHODS

We have studied the skeleton and muscles of the male genitalia in three species of the subfamily Aphnaeinae: *Phasis thero* (Linnaeus, 1764), *Chrysoritis pyroeis* (Trimen, 1864), and *Aloeides pierus* (Cramer, [1779]). Specimens of the first two species were fixed in 70% ethanol immediately upon collection in nature. The male genital muscles of *A. pierus* were studied in a single dry specimen. In addition, data on the muscle topography of the previously studied Middle Asian species *Cigaritis epargiros* (Eversmann, 1854) (Kuznetsov and Stekolnikov, 1998) were included in comparative analysis.

The genital apparatus of the butterflies preserved in 70% ethanol was extracted with forceps and cleaned of fat tissue. Then elements of the genitalia together with their muscles were removed one after another, using forceps and a scalpel, in order to determine the attachment sites of the muscles. To study muscles in dry specimens, the abdomen was softened by gently boiling it first in water and then in weak alkali solution; the degree of tissue softening was controlled under a microscope with approximately 1-min intervals.

The male genitalia in most species of Lycaenidae can be most effectively dissected in the following four steps.

1. Dorsal transection of the vinculum above the insertion site of the valvar adductors *m4*, followed by dissection of the adductors themselves. This makes it possible to extract the dorsal part of the genitalia together with

the uncus depressors *m1* and the anal cone retractors *m2(10)*. If muscles *m4* are inserted on the upper portion of the vinculum or on the tegumen, the vinculum and the muscles should be transected simultaneously.

2. Extraction of the aedeagus with forceps after longitudinal dissection of the tegumen and transection of the phallic muscles *m6(5)* and *m7(6)*.

3. Visual detection of the insertion sites of the phallic muscles *m6(5)* and *m7(6)*, and also the valvar adductors *m4* and the ventral valvar abductors *m8(3)*, on the lower part of the genitalia after removal of the aedeagus.

4. Examination of the valvar muscles *m4* and *m5(7)* by gradually removing the median membrane of the valva. The position of the muscles should be preliminarily studied with a microscope in transmitted light.

If the genitalia are strongly modified, the dissection procedure may have to be emended.

None of the studied species has a protected status in the Republic of South Africa (Henning et al., 2009).

The nomenclature of muscles used herein combines two common naming systems. The part preceding the parentheses corresponds to the nomenclature of Forbes (1939), and the part in the parentheses corresponds to the numeration used earlier by the Russian researchers (Kuznetsov and Stekolnikov, 2001).

RESULTS

Phasis thero (Linnaeus, 1764)

Skeleton

Schematic drawings of the male genitalia of *Phasis thero* were published earlier by Stempffer (1967, fig. 154) and Heath (1997, figs. 92, 124).

Abdominal segment IX is represented by the annulus (Fig. 1, 1).

The tegumen (Fig. 1, 1) is dome-shaped and slightly extended anteriorly. In its distal part the convex median area is merged with the uncus, and its proximal angles continue laterally into the vinculum.

The uncus (Fig. 1, 1, 2) is a broad convex lobe with laterally rounded edges and a blunted median protrusion at the distal end. The uncus is merged with the tegumen

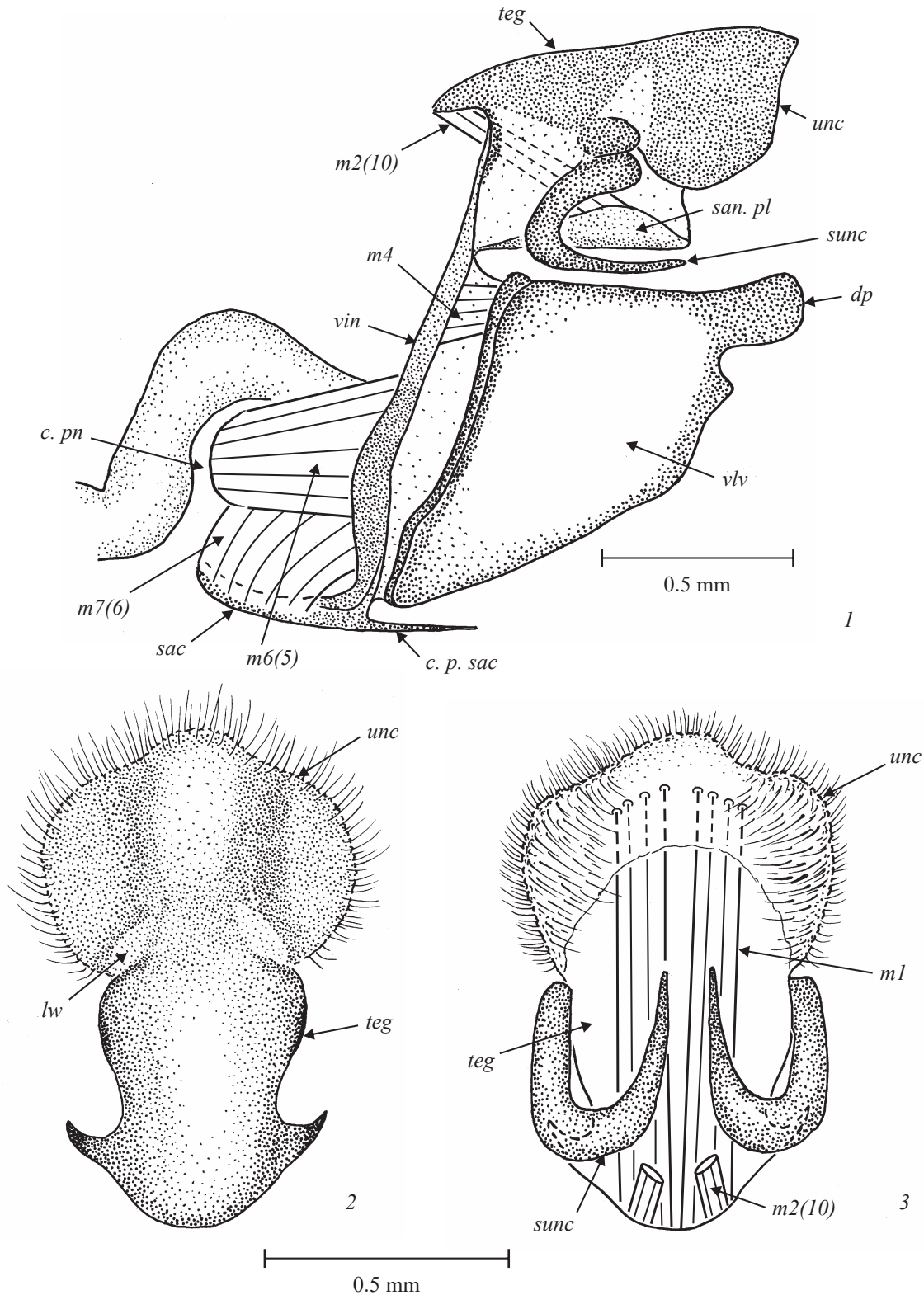


Fig. 1. *Phasis thero* (L.), skeleton and muscles of male genitalia: (1) genitalia in lateral view; (2) uncus and tegumen in dorsal view; (3) uncus and tegumen in ventral view. Designations in Figs. 1–5: *aed*, aedeagus; *anl*, anellus; *ann*, annulus; *c. p. sac*, caudal plate of saccus; *c. pn*, caecum penis; *crn*, cornuti; *dp*, dorsal process of valva; *htrs*, hemitransstilla; *jx*, juxta; *sac*, saccus; *san. pl*, subanal plate; *scl*, sacculus; *sunc*, subuncus; *teg*, tegumen; *unc*, uncus; *vin*, vinculum; *vlv*, valva; designations of muscles are given in the text.

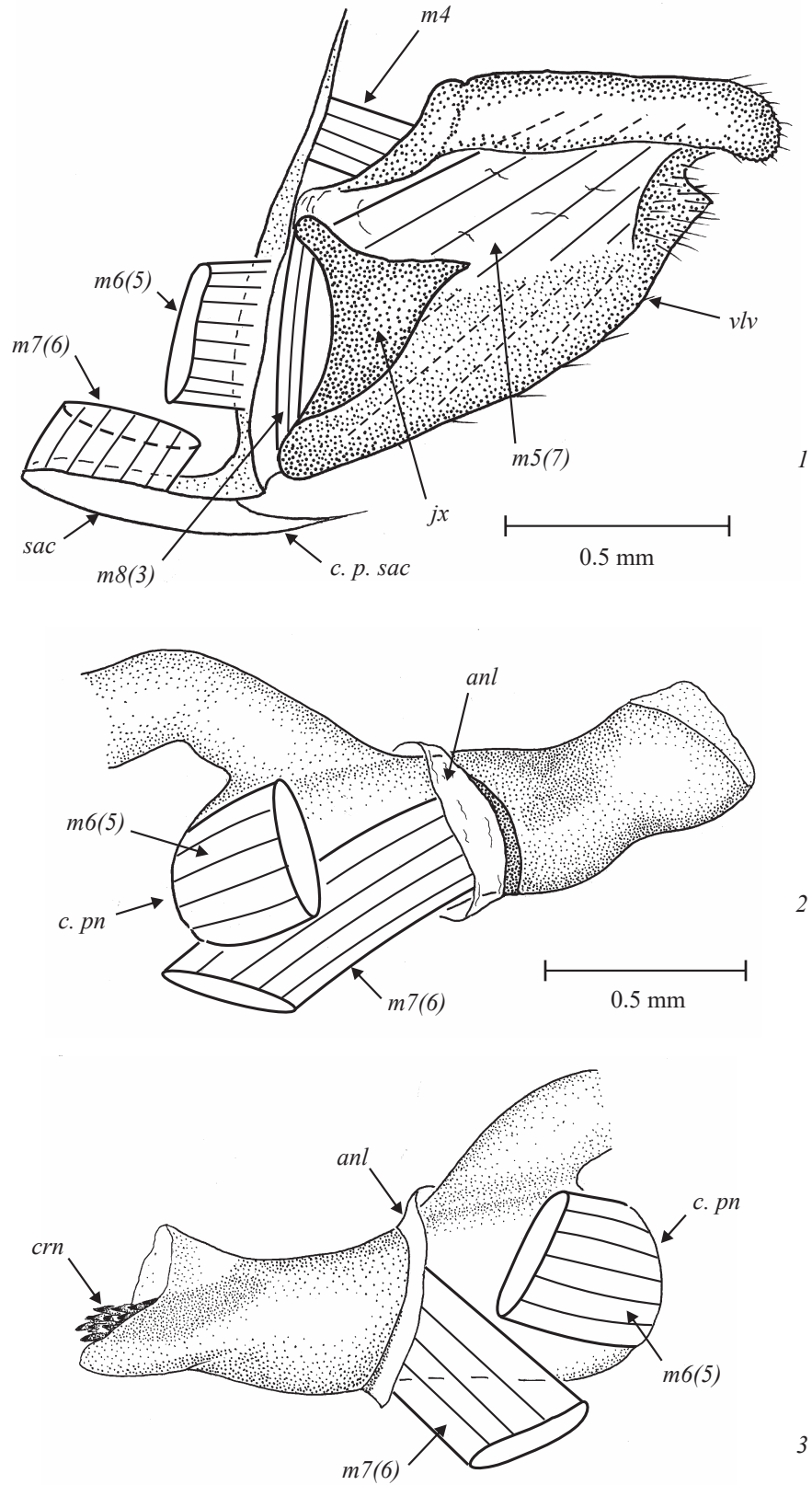


Fig. 2. *Phasis thero* (L.), skeleton and muscles of male genitalia: (1) right valva, view of the median surface; (2) aedeagus in right-side view; (3) aedeagus in left-side view.

medially but separated from it by membranous areas laterally.

The subunci are falciform and articulated with the tegumen near the basal angles of the uncus (Fig. 1, 1, 3).

The anal cone (Fig. 1, 1) has an elongate cymbiform subanal plate.

The vinculum (Fig. 1, 1) is narrow and merged dorsally with the tegumen, while ventrally it continues into a flat triangular saccus. The ventral surface of the saccus extends caudally to form the caudal plate, corresponding to the "saccus directed caudal" (see Eliot, 1973).

The valvae are wide, trapezoidal, with strongly sclerotized lateral walls (Fig. 1, 1). The median walls of the valvae (Fig. 2, 1) are membranous but include a vast sclerotized zone at the costal margin and the sacculi. The costal margin of the valvae distally forms rounded dorsal outgrowths, and its proximal region extends into the lobe of the hemitransstilla. The valvae are articulated with the vinculum along nearly its entire length by a relatively wide articular membrane. The sacculi are positioned close together at their base.

The juxta (Fig. 2, 1) consists of a pair of flat triangular lobes, positioned lateral of the aedeagus at the base of the valvae.

The aedeagus (Fig. 2, 2, 3) is large and divided in the interzonal area by a transverse sclerotized fold into two approximately equal parts: the internal subzone and the external suprazone. The subzone has a massive caecum penis. The ductus ejaculatorius enters the subzonal part of the aedeagus dorsally. The suprazone bears large acicular cornuti on the vesica.

Muscles

Seven pairs of muscles were found: *m1*, *m2(10)*, *m4*, *m5(7)*, *m6(5)*, *m7(6)*, and *m8(3)*.

Muscles *m1* are depressors of the uncus (Fig. 1, 3). They extend as flat and wide ribbons from the anterior margin of the tegumen into the uncus and are inserted on its membranous ventral wall close to its distal margin.

Muscles *m2(10)* are retractors of the anal cone (Fig. 1, 1, 3). They originate on the anterior margin of the tegumen ventral to the basal attachment sites of the uncal depressors and extend to the lateral margins of the subanal plate in its distal region.

Valvar muscles (Fig. 1, 1; Fig. 2, 1).

Muscles *m4* are adductors of the valvae; they extend from the dorsal part of the vinculum to the lobes of the hemitransstilla.

Muscles *m5(7)* are longitudinal intravalvar muscles. They occupy almost the entire cavity of the valvae, extending from the sacculi and the articular margin of the valvae to the bases of their dorsal outgrowths.

Muscles *m8(3)* are abductors of the valvae; they extend from the ventrolateral angle of the sacculi base to the dorsolateral angles of the juxta. Contraction of these muscles probably contributes to slight lateral abduction of the valvae.

Muscles of the aedeagus (Fig. 1, 1; Fig. 2, 2, 3).

Muscles *m6(5)* are protractors of the aedeagus. They extend from the lateral arcs of the vinculum to the caecum penis.

Muscles *m7(6)* are retractors of the aedeagus. They originate from the entire dorsal surface of the saccus and are inserted ventrally and laterally on the interzonal area of the aedeagus.

Chrysoritis pyroeis (Trimen, 1866)

Skeleton

The genitalia of the genus *Chrysoritis* Butler, [1897] were illustrated by Stempffer (1967, fig. 159) by the example of the type species *Ch. oreas* (Trimen, 1891). Schematic drawings of some genital elements of many species of the subfamily Aphnaeinae, including *Ch. pyroeis* studied by us, were published earlier by Heath (1997).

The tegumen (Fig. 3, 1–3) is dome-shaped and merged with the uncus into a single structure. Proximally the tegumen forms a median tubercle continuing into the lateral parts of the vinculum.

The uncus (Fig. 3, 1–3) is lobe-shaped, with rounded lateral parts extending ventrally. The caudal margin of the uncus has a very short and blunt median protrusion. Although the uncus and tegumen are merged medially, their lateral parts are separated by membranous areas.

The subunci (Fig. 3, 1, 3) are articulated with the tegumen. The branches of the subunci extend from their

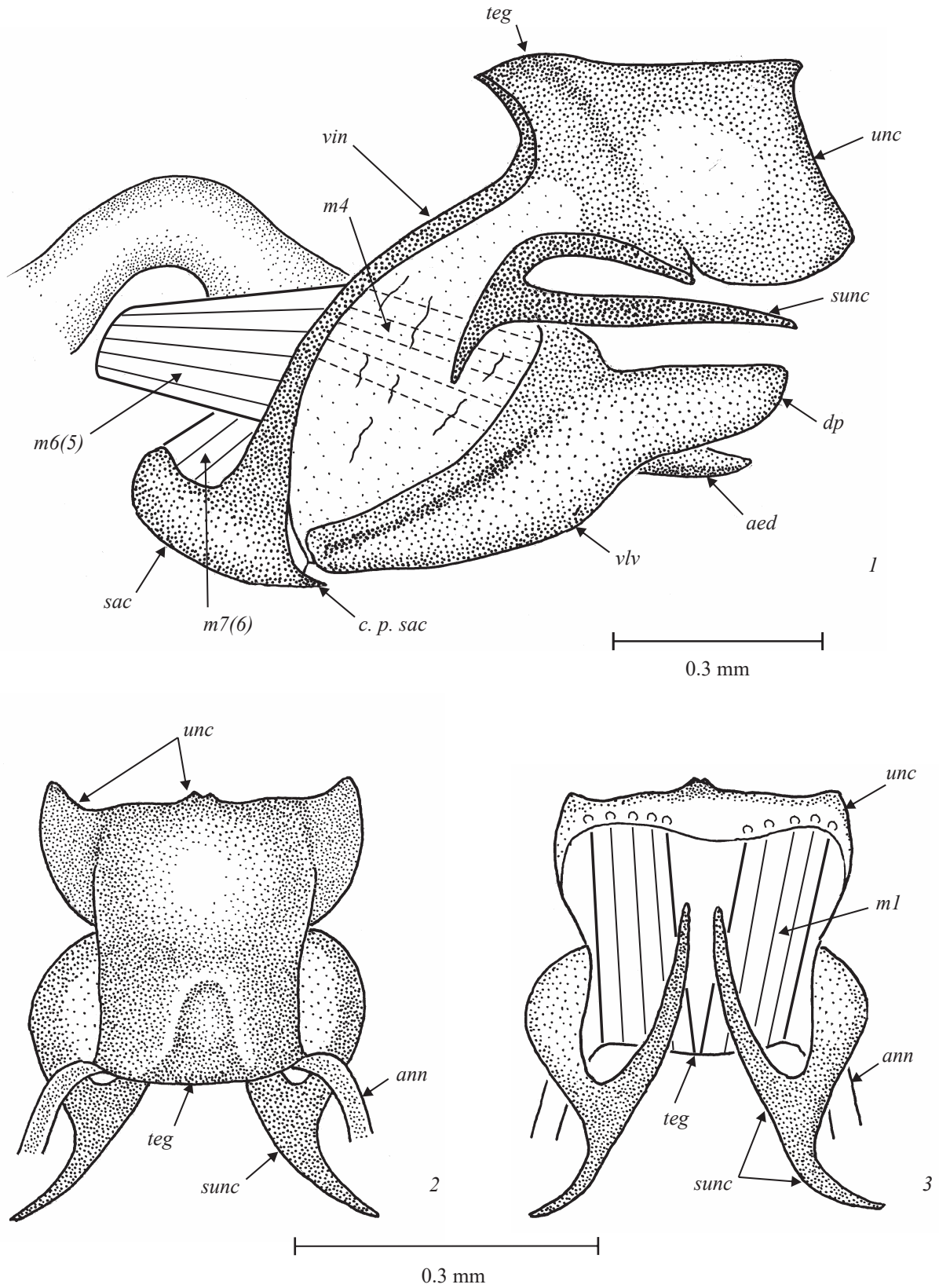


Fig. 3. *Chrysoritis pyroeis* (Trimen), skeleton and muscles of male genitalia: (1) genitalia in lateral view; (2) uncus and tegumen in dorsal view; (3) uncus and tegumen in ventral view.

base ventrally and continue distally into straight caudal outgrowths. At the bases of the straight parts of the subunci there are shorter processes directed ventrally.

The anal cone has a cymbiform subanal plate.

The vinculum is merged with the tegumen (Fig. 3, 1). Its narrow ribbon-shaped lateral parts ventromedially form the massive saccus with a slightly bifurcated cranial end, while the ventromedian saccus wall extends caudally into a short pointed caudal plate of the saccus.

The valvae are oval, with strongly sclerotized lateral walls and a long dorsal outgrowth (Fig. 4, 1). On their median wall, the base of the costal margins is dilated near the hemitransstilla (Fig. 4, 1). The valvae are articulated with the vinculum by a broad membrane narrowing ventrally to a narrow stripe near the saccus, so that the valvae look "suspended" from the annulus. The valvae are movable, although the bases of the sacculi are closely approximate.

The juxta (Fig. 4, 1) consists of a pair of flat, strongly sclerotized triangular lobes positioned between the bases of the median valvar walls and the aedeagus.

The aedeagus is cylindrical, with a well-developed caecum penis (Fig. 4, 2). The ductus ejaculatorius enters the subzonal part of the aedeagus dorsally.

Muscles

The composition of the muscles is the same as in *Phasis thero*.

Muscles *m1* are depressors of the uncus (Fig. 3, 3), connecting the anterior margin of the tegumen with the membranous ventral wall of the uncus close to its distal margin.

Muscles *m2(10)* are retractors of the anal cone, extending from the anterior margin of the tegumen to the lateral margins of the subanal plate in its distal region.

The valvar muscles are represented by three pairs: *m4*, *m5(7)*, and *m8(3)* (Fig. 3, 1; Fig. 4, 1):

Muscles *m4* are adductors of the valvae. These are thick muscles extending from the lateral part of the vinculum to the lobes of the hemitransstilla.

Muscles *m5(7)* are longitudinal intravalvar muscles, extending from the sacculi to the distal region of the

valvae and inside their dorsal outgrowths. Their contraction results in median bending of these outgrowths.

Muscles *m8(3)* are ventral abductors of the valvae, extending from the base of the sacculi to the dorsal angles of the juxta.

Muscles of the aedeagus (Fig. 3, 1; Fig. 4, 2).

Muscles *m6(5)* are protractors of the aedeagus, extending from the lateral arcs of the vinculum in their ventral part to the caecum penis.

Muscles *m7(6)* are retractors of the aedeagus, originating from the entire dorsal surface of the saccus and inserted ventrally and laterally on the interzonal area of the aedeagus.

Aloeides pierus (Cramer, [1779])

Skeleton and Muscles

The genital skeleton of this species was illustrated in detail by Heath (1997, figs. 44, 119, 157) and earlier, together with that of two other species of this genus, by Stempffer (1967, fig. 155). *Aloeides pierus* differs slightly from the other two studied species of Aphnaeinae in the shape of the aedeagus and valvae, but the three species do not differ in the composition and attachment sites of the muscles (Fig. 5, 1, 2).

Cigaritis epargiros (Eversmann, 1854)

Skeleton and Muscles

Cigaritis epargiros differs from all other species of the subfamily (Kuznetsov and Stekolnikov, 1998) in the presence of an additional muscle *m32*, occupying the vinculo-valvar position. Otherwise, the musculature and skeleton of the male genitalia of *C. epargiros* and the rest of Aphnaeinae are similar.

DISCUSSION

Ancestral Type of Musculoskeletal System of Genitalia in Aphnaeinae

The male genitalia in the subfamily Aphnaeinae have a relatively uniform morphology (Heath, 1997), and all their elements are included in the ground plan of the nymphaloid group of families (Kuznetsov and Stekolnikov, 2001). These are, in particular, a dome-shaped

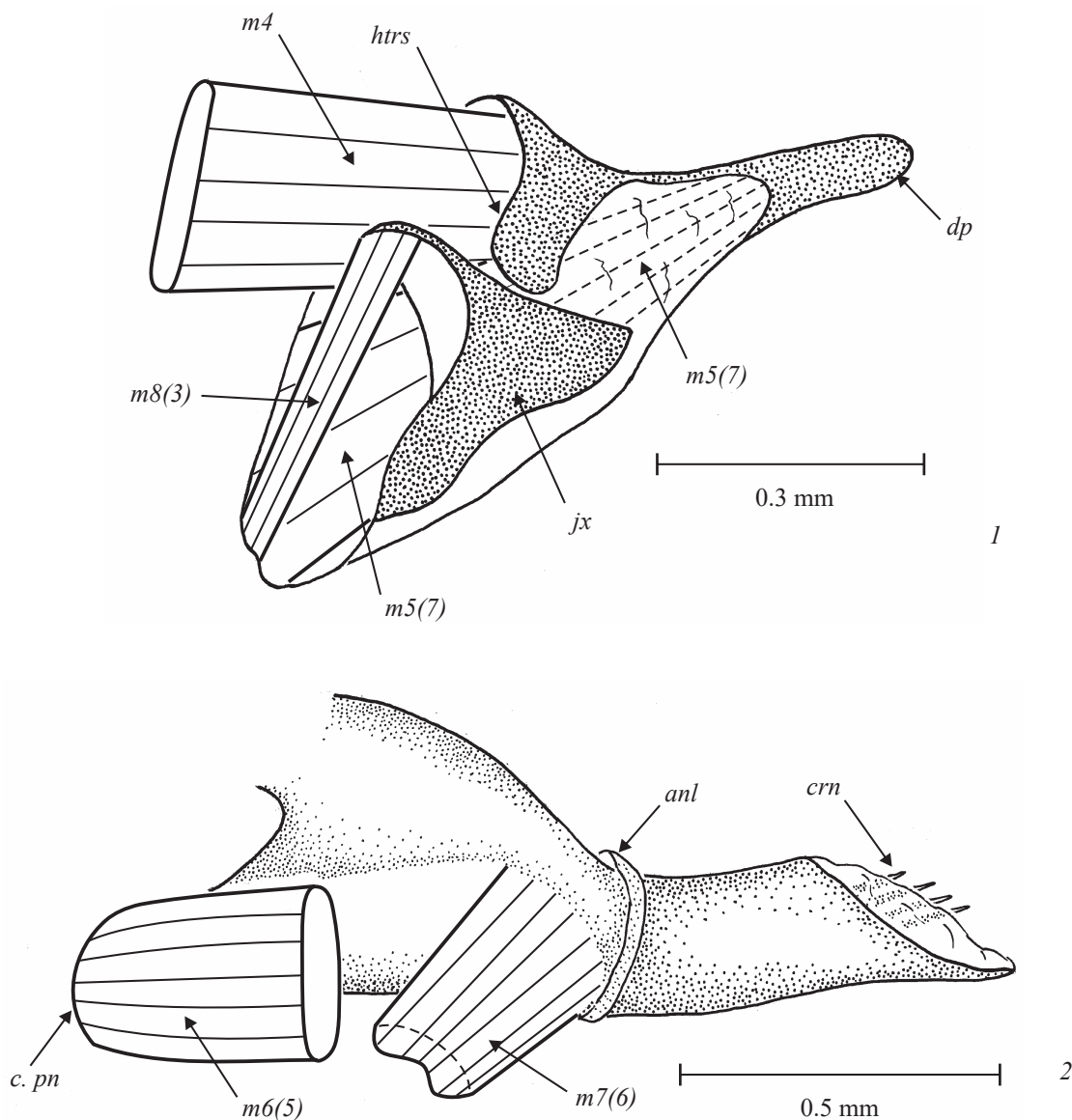


Fig. 4. *Chrysoritis pyroeis* (Trimen), skeleton and muscles of male genitalia: (1) right valva, view of the median surface; (2) aedeagus in right-side view.

tegumen, a unilobate uncus in some species, falciform subunci, a well-developed saccus, a distinct sacculus, a rigidly sclerotized juxta, and a tubular aedeagus with a caecum penis. Thus, the genital skeleton is less modified in Aphnaeinae than in more ancient taxa of Lycaenidae: Curetinae and Riodininae (Stekolnikov and Korzeev, 2017), in which the anellus is differentiated and the valvae are modified, especially in Curetinae.

The muscles found in Aphnaeinae (with the exception of *m32*), namely *m1*, *m2(10)*, *m4*, *m5(7)*, *m6(5)*, *m7(6)*,

and *m8(3)*, belong to the ground plan of Ditrysiina and occupy the positions characteristic of ancestral groups (Kuznetsov and Stekolnikov, 2001). The uncus depressors *m1* extend from the tegumen to the base of the uncus. Muscles *m2(10)* connect the tegumen with the anal cone. The valvar adductors *m4* extend from the lateral part of the vinculum to the valvae. The intra-valvar muscles *m5(7)* are longitudinal, while *m8(3)* connect the valvae with the juxta (a state characteristic of Lycaenidae). The phallic muscles *m6(5)* extend from the lateral part of the vinculum to the caecum penis, and

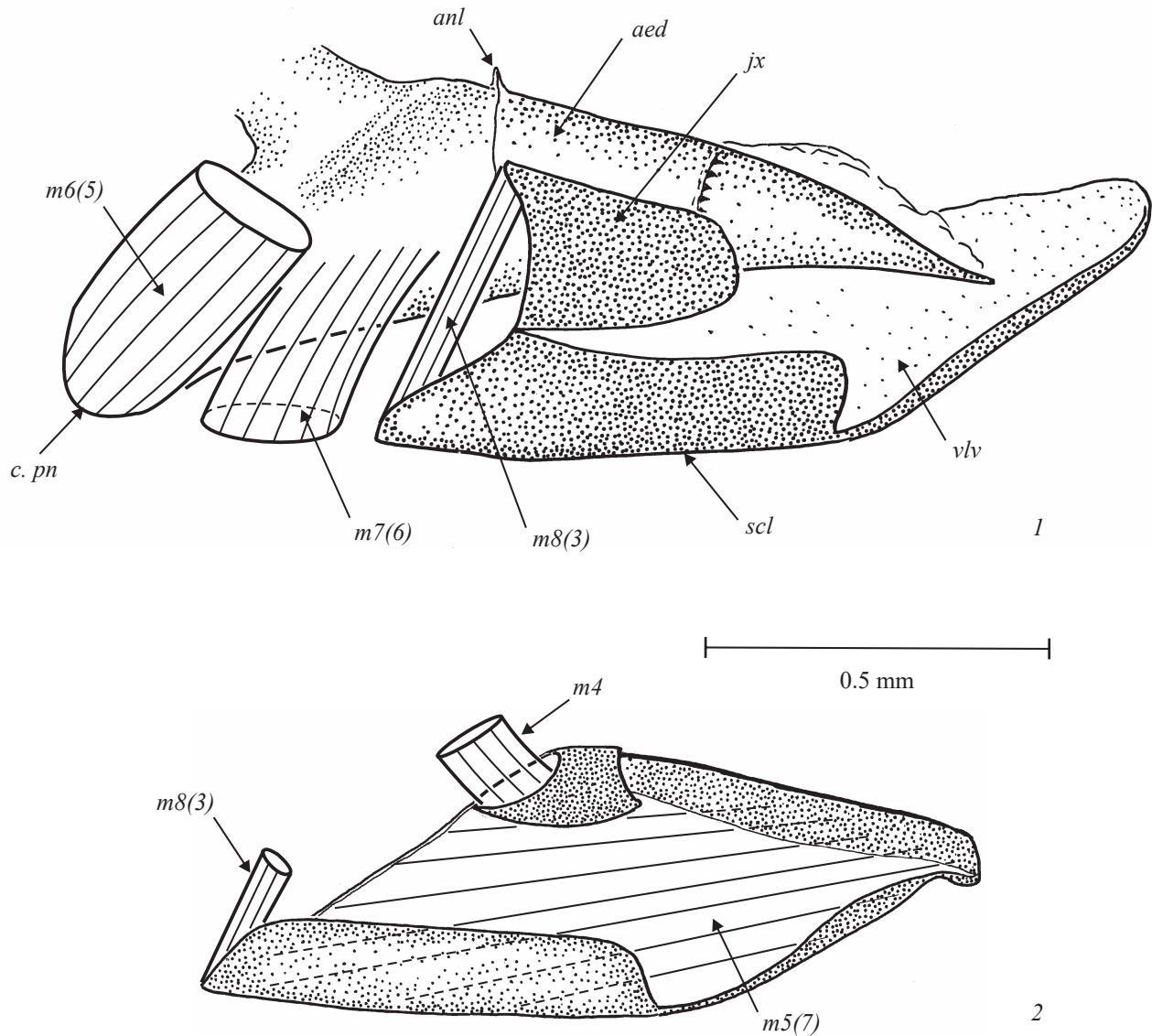


Fig. 5. *Aloides pierus* (Cramer), skeleton and muscles of male genitalia: (1) aedeagus, right valva, and juxta, view of the median surface; (2) right valva, view of the median surface.

m7(6) connect the saccus with the zonal area of the aedeagus.

Differences between the Genera of Aphnaeinae in Musculoskeletal Features of Male Genitalia

The subfamily Aphnaeinae forms a single phylogenetic lineage with Poritiinae, Lipteninae, and Miletinae (Espeland et al., 2018) and differs from these taxa in relatively uniform morphology of the male genital apparatus. Still, when the skeleton characters illustrated

by Heath (1997) are plotted on the phylogenetic tree of Aphnaeinae, certain evolutionary changes can be detected within the subfamily.

According to the proposed phylogenetic hypothesis (Boyle et al., 2015), the subfamily Aphnaeinae comprises two large clades. The basal position in one of these clades is occupied by the genera *Tylopaedia* (Trimen, 1868) and *Phasis* Hübner, [1819], having a unilobate uncus with a small median protrusion (Heath, 1997, figs. 50, 51). In other genera of this clade, namely

Trimenia Tite et Dickson, 1973, *Axiocerses* Hübner, [1819], *Aphnaeus* Hübner, [1819], and *Aloeides* Hübner, [1819], the uncus is also unilobate, with a straight or slightly convex posterior margin (Heath, 1997, figs. 44–49), or rarely with a shallow median incision, as in *Zeritis neriene* Boisduval, [1836] (Heath, 1997, fig. 41).

The other large clade comprises the genera *Crudaria* Wallengren, 1875, *Cigaritis* Donzel, 1847, *Lipaphnaeus* Aurivillius, 1916, *Chloroselas* Butler, [1886], *Chryso-ritis* Butler, [1897], and *Pseudaletis* H.H. Druce, 1888. This phylogenetic branch demonstrates evolutionary transformation of the uncus with two inconspicuous rounded lobes, as in *Crudaria* or *Chryso-ritis* (Heath, 1997, figs. 18–31), into a typical bilobate structure, as in *Cigaritis* (Heath, 1997, figs. 35–39) and closely related genera. At the same time, the genus *Pseudaletis* H.H. Druce, 1888, placed at the base of this phylogenetic branch, already has a bilobate uncus, i.e., differences in the uncus morphology have appeared independently in different genera of this clade.

The two major clades also differ in the manner of articulation of the valvae with the vinculum. The clade including *Phasis thero* is characterized by compact valvae, more or less tightly articulated with the vinculum along its entire length by a narrow articular membrane (see Fig. 1, I). The valvae of *Chryso-ritis*, *Cigaritis*, and other closely related genera are somewhat elongated, triangular, with the base of their costal margin positioned much farther from the vinculum than the base of the sacculi (Fig. 3, I). Both evolutionary trends can be traced in the schematic drawings published by Heath (1997).

Thus, within the subfamily Aphnaeinae we can distinguish two evolutionary trends of the uncus and valvae, which correspond to the divergence of the taxon (according to Boyle et al., 2015) into two major clades. The genital skeleton of *Phasis* is more primitive than that of *Chryso-ritis* and *Cigaritis*.

The uniform morphology of the genital skeleton within Aphnaeinae is consistent with very similar muscle topography in the studied species: *Phasis thero*, *Chryso-ritis pyroeis*, *Aloeides pierus*, and *Cigaritis epargiros*. The only considerable difference between these taxa is the presence of the unique paired vinculo-valvar muscles *m32* in *Cigaritis epargiros* (Kuznetsov and Stekolnikov, 1998). It was earlier suggested that these

muscles appeared as the result of splitting of the ancestral aedeagus protractors inserted on the valvae (Stekolnikov, 2008), which gave rise to two separate pairs of muscles: the aedeagus protractors *m6(5)* and the vinculo-valvar muscles. However, a serious argument against the hypothesis of *m6(5)* splitting in *Cigaritis epargiros* is the fact that neither Nymphalidae nor the related Lycaenidae demonstrate insertion of the aedeagus protractors *m6(5)* on the valvae, as in Hesperidae, Papilionidae or Pieridae. Therefore, it would be more feasible to consider the origin of *m32* as the result of splitting of the valvar adductors *m4* into two pairs of muscles. This hypothesis is supported by the vinculo-valvar position of the original *m4* and the derived muscles in *Cigaritis epargiros*. The splitting of valvar adductors is sporadically observed in some Lepidoptera, including Curetinae (Stekolnikov and Korzeev, 2017). It remains unknown whether the presence of *m32* (= *m4*) is a feature shared by all the species of the Middle Asian genus *Cigaritis* and distinguishing this genus from the African representatives of Aphnaeinae.

Position of Aphnaeinae in the System of Lycaenidae

Aphnaeinae, Curetinae, and Riodininae. According to the results of phylogenetic analysis of Rhopalocera (Espeland et al., 2018), the subfamily Aphnaeinae together with Poritiinae s. l. (Poritiinae + Lipteninae) and Miletinae forms a large phylogenetic branch contrasted with another vast group that includes the subfamilies Lycaeninae, Theclinae, and Polyommatainae. The ancient groups Curetinae and Riodininae lie at the base of both lineages. The subfamily Aphnaeinae is a younger group that originated approximately 60 MYA, whereas Curetinae and Riodininae diverged about 80 MYA. However, comparison of the male genital skeleton and muscles of Aphnaeinae with the previously studied genitalia of Riodininae and Curetinae has shown that the genitalia of Aphnaeinae are much more primitive than those of the ancestral groups that possess several clear autapomorphies. For instance, the valvae of Curetinae are strongly differentiated and have harpae, and the anellus is modified into the dorsal fultura above the aedeagus (Stekolnikov and Korzeev, 2017). Besides, their valvar adductors have split, and the derived muscles *m4b* have changed both their attachment sites and their function: they connect the vinculum with the dorsal fultura. The subfamily Curetinae is also character-

ized by reduction of the ventral valvar abductors *m8(3)*. Two previously studied species of Riodiniinae, *Polycaena tamerlana* (Staudinger, 1886) (Kuznetsov and Stekolnikov, 1998) and *Paralaxita damajanti* (C. Felder et R. Felder, 1860) (Stekolnikov and Korzeev, 2017), revealed splitting of the aedeagus protractors *m6(5)*. Besides, the male genitalia of *Polycaena tamerlana* include the transverse intervalvar muscle *m18*; the attachment sites of the aedeagus retractors *m7(6)* have shifted from the median part of the aedeagus to the caecum penis, while the attachment sites of muscles *m4* have shifted from the valvae to the basal dorsal region of the anellus.

No synapomorphies uniting Aphnaeinae, Curetinae, and Riodiniinae have been found.

Aphnaeinae and Lipteninae. The subfamily Aphnaeinae and the sister pair Poritiinae + Lipteninae are now regarded as members of a single original clade (Espeland et al., 2018). The subfamily Aphnaeinae probably diverged in the Paleocene, about 60 MYA.

Comparison of the male genital skeleton and muscles in the ancestral tribes Epitolini and Liptenini (Stekolnikov and Korzeev, 2019b) with the corresponding features in Aphnaeinae has revealed many symplesiomorphic similarities. For instance, *Phasis* and closely related genera, similar to the ancestral Lipteninae, have an entire segment IX (an annulus), a unilobate uncus, a caecum penis, a compact juxta, and a complete set of muscles included in the ground plan of Papilionomorpha: *m1*, *m2(10)*, *m4*, *m6(5)*, *m5(7)*, *m7(6)*, and *m8(3)*. The attachment sites of all the above muscles in Aphnaeinae are typical of the ancestral groups, while in Epitolini and Liptenini some muscles have different attachment sites. As opposed to the ground plan of Papilionomorpha, the aedeagus protractors *m7(6)* in these tribes are inserted on the caecum penis (a synapomorphy of Epitolini and Liptenini). In addition, the juxta is considerably modified, and this feature seems to be related to the reduction of *m8(3)* in Epitolini (an autapomorphy of the tribe).

Thus, the subfamilies Aphnaeinae and Lipteninae are characterized by predominantly symplesiomorphic similarity of both skeleton and musculature of the male genitalia. The only synapomorphy in the genital skeleton of these taxa is the presence of a narrow caudal plate formed by the ventral wall of the saccus. Unlike Aphnaeinae, the representatives of Epitolini and Liptenini

do not have a distinct saccus, but the ventral wall of the median area of the vinculum still forms a similar pointed protrusion; this character was earlier described as “saccus, when developed, directed caudal” (Eliot, 1973).

Aphnaeinae and Miletinae. The subfamilies Aphnaeinae and Poritiinae s. l. belong to the same phylogenetic branch (Espeland et al., 2018), but Z.A. Kaliszewska and co-authors (Kaliszewska et al., 2015) considered Aphnaeinae as a group opposed to the sister pair Miletinae + Poritiinae s. l. (= Poritiinae + Lipteninae).

It is difficult to compare the skeleton and muscles of the male genitalia in Miletinae and Aphnaeinae, because within Miletinae the genital musculature has been studied only in the highly specialized tribe Miletini (Korzeev and Stekolnikov, 2021). The skeleton of the basal groups of Miletinae can be analyzed only based on the published descriptions and schematic drawings (Stempffer, 1967; Eliot, 1973; Sáfián, 2015), demonstrating the ancestral morphological type, for example, in the genera *Liphyra* Westwood, 1864 and *Auslaga* Kirby, 1890. The features of the genital skeleton in these genera correspond to the ground plan of Papilionomorpha: the presence of an annulus, a well-developed saccus, a lamellar uncus, a cylindrical aedeagus with a caecum penis, and a well-developed juxta. Correspondingly, the subfamily Aphnaeinae reveals symplesiomorphic similarity with the ancestral taxa of Miletinae in the skeletal characters. At the same time, Aphnaeinae and the ancient Miletinae, as well as the ancestral Lipteninae (Liptenini), have a single synapomorphy: the ventral wall of the saccus extended into a narrow caudal plate (Eliot, 1973).

CONCLUSIONS

Our study of the skeleto-muscular apparatus of male genitalia in three species from two distantly related phylogenetic lineages of Aphnaeinae (*Phasis thero*, *Chrysoritis pyroeis*, and *Aloeides pierus*) has shown this subfamily to be very archaic. The main features of the skeleton as well as the composition and topography of the muscles correspond to the ground plan of the nymphaloid group of families (Nymphalidae + Lycaenidae). In the genital morphology, the subfamily Aphnaeinae is more archaic than such ancient taxa of Lycaenidae as Curetinae or Riodiniinae, and almost completely corresponds to the ancestral state for the

family Lycaenidae. The only autapomorphic character of the ancient phylogenetic branch including Miletinae, Poritiinae s. str., Lipteninae, and Aphnaeinae is the ventral wall of the saccus extended into a narrow caudal plate.

At the early stage of formation of the family Lycaenidae, its morphological diversity was probably quite low, and some of its phylogenetic lineages, such as Aphnaeinae, Poritiinae s. l., Lipteninae (Liptenini), and Miletinae (Liphyrini), have retained in full the ancestral type of organization.

ACKNOWLEDGMENTS

The authors are sincerely grateful to V.V. Tikhonov (Pyatigorsk, Russia) and S.I. Melnitsky (St. Petersburg, Russia) for the material provided for morphological study, and also to the anonymous reviewer for helpful comments and advice.

FUNDING

This work was supported by the Russian Foundation for Basic Research (project 18-04-00312).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

OPEN ACCESS

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission

directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

REFERENCES

- Boyle, J.H., Kaliszewska, Z.A., Espeland, M., Suderman, T.R., Fleming, J., Heath, A., and Pierce, N.E., Phylogeny of the Aphnaeinae: myrmecophilous African butterflies with carnivorous and herbivorous life histories, *System. Entomol.*, 2015, vol. 40, p. 169.
<https://doi.org/10.1111/syen.12098>
- Eliot, J.N., The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement, *Bull. Br. Mus. (Nat. Hist.) Entomol.*, 1973, vol. 28, no. 6, p. 371.
<https://doi.org/10.5962/bhl.part.11171>
- Espeland, M., Breinholt, J., Willmott, K.R., Warren, A.D., Vila, R., Toussaint, E.F.A., Maunsell, S.C., Aduse-Poku, K., Talavera, G., Eastwood, R., Jarzyna, M.A., Guralnick, R., Lohman, D.J., Pierce, N.E., and Kawahara, A.Y., A comprehensive and dated phylogenomic analysis of butterflies, *Curr. Biol.*, 2018, vol. 28, no. 6, p. 770.
<https://doi.org/10.1016/j.cub.2018.01.061>
- Forbes, W.T.M., The muscles of the lepidopterous male genitalia, *Ann. Entomol. Soc. Am.*, 1939, vol. 1, p. 1.
- Heath, A., A review of African genera of the tribe Aphnaeini (Lepidoptera: Lycaenidae), *Metamorphosis*, 1997, Suppl. 2, p. 1.
https://www.researchgate.net/publication/258121524_A_review_of_African_genera_of_the_tribe_Aphnaeini_Lepidoptera_Lycaenidae
- Henning, G.A., Terblanche, R.F., and Ball, J.B., Eds., *South African Red Data Book: Butterflies (SANBI Biodiversity Series 13)*, Pretoria: South African National Biodiversity Institute, 2009.
- Heppner, J.B., Tribal classification of the Lepidoptera, *Lepid. News*, 2003, vol. 1, p. 1.
- Kaliszewska, Z.A., Lohman, D.J., Sommer, K., Adelson, G., Rand, D.B., Mathew, J., Talavera, G., and Pierce, N.E., When caterpillars attack: Biogeography and life history evolution of the Miletinae (Lepidoptera: Lycaenidae), *Evolution*, 2015, vol. 69, no. 3, p. 571.
<https://doi.org/10.1111/evo.12599>
- Korzeev, A.I. and Stekolnikov, A.A., Male genitalia muscles of the blues' tribe Miletini (Lepidoptera, Lycaenidae: Miletinae), *Entomol. Rev.*, 2021, vol. 101, no. 1, p. 7.
<https://doi.org/10.1134/S0013873821010024>
- Kuznetsov, V.I. and Stekolnikov, A.A., Evolution of male genital skeleton and muscles in the families Riodinidae and Lycaenidae (Lepidoptera), *Entomol. Rev.*, 1998, vol. 78, no. 6, p. 691.

- Kuznetsov, V.I., and Stekolnikov, A.A., *Novye podkhody k sisteme cheshuekrylykh mirovoi fauny (na osnove funktsional'noi morfologii bryushka)* (New Approaches to the System of Lepidoptera of the World Fauna, Based on Abdominal Functional Morphology), St. Petersburg: Nauka, 2001.
- Sáfián, S., *Aslauga larseni* (Lycaenidae: Miletinae: Liphyrini), a unique new species from the Nimba Mountains, Liberia, *Metamorphosis*, 2015, vol. 26, p. 27.
- Scott, J.A., The phylogeny of the butterflies (Papilionoidea and Hesperioidea), *J. Res. Lepid.*, 1985, vol. 23, p. 241.
- Stekolnikov, A.A., *Morfologicheskie printsipy evolyutsii muskulatury nasekomykh* (Morphological Principles of Musculature Evolution in Insects), St. Petersburg: Izdat. Sankt-Peterb. Gos. Univ., 2008.
- Stekolnikov, A.A., Evolution of the skeleton and musculature of the male genitalia in the family Lycaenidae (Lepidoptera): II. Infratribe Polyommata Swainson, 1827, *Entomol. Rev.*, 2011, vol. 91, no. 1, p. 37.
<https://doi.org/10.1134/S0013873811010040>
- Stekolnikov, A.A. and Korzeev, A.I., The musculoskeletal system of male genitalia in *Curetis bulis* Westwood, 1851 (Lepidoptera, Lycaenidae: Curetinae) and *Paralaxita damajanti* (C. Felder et R. Felder, 1860) (Lepidoptera, Riodinidae: Nemeobiinae), *Entomol. Rev.*, 2017, vol. 97, no. 1, p. 1.
<https://doi.org/10.1134/S0013873817010018>
- Stekolnikov, A.A. and Korzeev, A.I., Morphology of the male genitalia in the Oriental species of the family Lycaenidae (Lepidoptera). Tribes Lycaenesthini and Polyommataini, *Entomol. Rev.*, 2019a, vol. 99, no. 3, p. 310.
<https://doi.org/10.1134/S0013873819030047>
- Stekolnikov, A.A. and Korzeev, A.I., Male genitalia muscles in the Afrotropical subfamily Lipteninae (Lepidoptera, Lycaenidae), *Entomol. Rev.*, 2019b, vol. 99, no. 9, p. 1239.
<https://doi.org/10.1134/S0013873819090021>
- Stekolnikov, A.A. and Kuznetsov, V.I., Evolution of skeleton and musculature of the male genitalia in the family Lycaenidae (Lepidoptera): I. The *Cupido*, *Glaucopteryx*, *Lycaenopsis*, and *Itylos* sections, *Entomol. Rev.*, 2005, vol. 85, no. 9, p. 1055.
- Stekolnikov, A.A., Lukhtanov, V.A., and Korzeev, A.I., Congruence between comparative morphology and molecular phylogenies: Evolution of the male genital skeletal/muscular system in the subtribe Polyommata (Lepidoptera, Lycaenidae), *Entomol. Rev.*, 2014, vol. 94, no. 2, p. 166.
<https://doi.org/10.1134/S0013873814020031>
- Stempffer, H., The genera of the African Lycaenidae (Lepidoptera, Rhopalocera), *Bull. Br. Mus. (Nat. Hist.) Entomol.*, 1967, vol. 10, suppl., p. 1.
- Williams, M.C., Classification of the Afrotropical butterflies to generic level, *Metamorphosis*, 2015, vol. 26, p. 102.