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## A molecular phylogeny of the subfamily Polyommatinae (Lepidoptera: Lycaenidae)

### Молекулярная филогения подсемейства Polyommatinae (Lepidoptera: Lycaenidae)

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**Ключевые слова:** Lepidoptera, Lycaenidae, Polyommatinae, филогения, молекулярно-биологические и морфологические исследования.

**Abstract.** Molecular and morphological study of the subfamily Polyommatinae allows to make the following conclusions: the tribe Candalidini and the genus *Cupidopsis* should be excluded from the subfamily. The status of the tribe Niphandini should be reduced to a subtribe level. Thus, subfamily Polyommatinae consists of two tribes: Lycaenesthina and Polyommatini. Elimination of the non-taxonomic rank “section” and the combined morphological and genetic analysis make it possible to distinguish 22 subtribes within the tribe Polyommatini, which meet the requirements of monophyly: Brepheidiina, Pithecorpina, Niphandina, Danina, Azanina, Theclineshina, Lycaenopsina, Jamidina, Cacyreina, Actizerina, Uranothaumatina, Lampidina, Zizulina, Catochrysopsina, Scolitantidina, Castaliina, Oboroniina, Leptotina, Zizeeriina, Fameganina, Everina, and Polyommatina.

**Резюме.** Молекулярно-генетическое и морфологическое изучение голубянок подсемейства Polyommatinae позволяет прийти к следующим выводам: триба Candalidini и род *Cupidopsis* должны быть выведены из состава подсемейства, а триба Niphandini понижена в статусе до уровня подтрибы. Таким образом, в подсемействе Polyommatinae остаются две трибы: Lycaenesthina и Polyommatini. Упразднение внетаксономического ранга «секция» и комплексный морфолого-генетический анализ позволяют выделить в составе трибы Polyommatini следующие 22 подтрибы, отвечающие требованию монофилии: Brepheidiina, Pithecorpina, Niphandina, Danina, Azanina, Theclineshina, Lycaenopsina, Jamidina, Cacyreina, Actizerina, Uranothaumatina, Lampidina, Zizulina, Catochrysopsina, Scolitantidina, Castaliina, Oboroniina, Leptotina, Zizeeriina, Fameganina, Everina и Polyommatina.

Currently the most commonly used and adequate system of Lycaenidae is the concept of Eliot [1973]. It is based solely on an analysis of morphological characters. However, the author uses unreasonably excessive fragmentation of many genera, as well as higher-level taxa.

The author also uses such non-taxonomic concept as a section (Section sensu Eliot).

The leading role in the study of taxonomy and systematics of organisms is currently allocated to molecular genetic research. This rule undoubtedly applies to Blue butterflies, and the publication of many articles in the last decade only emphasizes that [Wiemers, 2003; Kandul et al., 2004; Lukhtanov et al., 2005; Vodolazhsky et al., 2009; Wiemers et al., 2010; Ugelvig et al., 2011; Vila et al., 2011; Talavera et al., 2013, 2015; Stradomsky, 2014]. Traditional morphological methods can not ensure the construction of a natural system of Lycaenidae. High variability of the wing pattern, structure of genitalia and other features cannot create an adequate system of Blue butterflies. However, the analysis of some morphological characters can be used as an additional criterion for the construction of the system. First of all, this holds true for the study of genitalia.

The goal of this study was an attempt to build a natural phylogeny of one of the largest subfamilies of Blue butterflies, the Polyommatinae. This subfamily contains species with the extremely pronounced heterogeneity of external morphological characteristics and variability of the genital apparatus. The specimens of the subfamily Polyommatinae were studied with the use of molecular genetic methods. Specifically, we examined the following genetic markers: sections of the mitochondrial gene Cytochrome Oxidase subunit I, the nuclear Elongation Factor 1-alpha (the nuclear coding sequence) and the nuclear noncoding sequence internal transcribed spacer 2 (the nuclear noncoding sequence). At the same time the genital structures of Polyommatinae representatives have been investigated.

### Material and methods

All specimens examined in this study are archived at the museum of the Institute of Arid Zones SSC RAS (Rostov-on-Don, Russia) as voucher specimens. Features of studied specimens are presented in the Table 1. Eighty nine species were examined.

Table 1. List of material with voucher codes and GenBank accession numbers.  
 Таблица 1. Исследованный материал: музейные номера и присвоенные номера Генбанка.

Species Вид	Voucher No. Музейный №	COI GenBank accession numbers	ITS2 GenBank accession numbers	EF-1a GenBank accession numbers
<i>Polyommatus eros</i> (Ochsenheimer, 1808)	ILL145	KF647233	KF918764	KJ671889
<i>Polyommatus daphnis</i> ([Denis et Schiffermüller], 1775)	ILL165	KF860855	KF918760	KJ671888
<i>Polyommatus yurinekrutenko</i> Koçak, 1996	ILL155	KF468767	KF468769	KF468771
<i>Aricia anteros</i> (Freyer, [1838])	ILL137	KF647244	KF894394	KJ671880
<i>Eumedonia eumedon</i> (Esper, [1780])	ILL162	KF860856	KF918765	KJ671881
<i>Kretania eurypilus</i> (Freyer, [1851])	ILL156	KF647232	KF918766	KJ671891
<i>Plebejus argus</i> (Linnaeus, 1758)	ILL157	KF860852	KF894397	KJ698660
<i>Hemiargus ceraunus</i> (Fabricius, 1793)	ILL177	KJ131014	KJ131023	KJ671870
<i>Chilades lajus</i> (Stoll, [1780])	ILL189	KJ638696	KJ638701	KJ638705
<i>Luthrodes pandava</i> (Horsfield, [1829])	ILL171	KJ131016	KJ131025	KJ671873
<i>Famegana alsulus</i> (Herrich-Schäffer, 1869)	ILL229	KP901008	KP901000	KP901004
<i>Tongeia fischeri</i> (Eversmann, 1843)	ILL174	KJ131017	KJ131026	KJ671875
<i>Cupido minimus</i> (Fuessly, 1775)	ILL164	KF860857	KF918769	KJ671886
<i>Cupido osiris</i> (Meigen, [1829])	ILL113	KC676700	KC676702	KJ671887
<i>Cupido argiades</i> (Pallas, 1771)	ILL116	KC676699	KC676701	KJ671884
<i>Cupido alcetas</i> (Hoffmansegg, 1804)	ILL142	KF647247	KF894390	KJ671883
<i>Cupido decoloratus</i> (Staudinger, 1886)	ILL143	KF647236	KF918761	KJ671885
<i>Zizeeria knysna</i> (Trimen, 1862)	ILL180	KJ131019	KJ131027	KJ671876
<i>Pseudozizeeria maha</i> (Kollar, [1844])	ILL183	KJ508010	KJ527494	KJ527486
<i>Zizina otis</i> (Fabricius, 1787)	ILL173	KJ131018	KJ131028	KJ671877
<i>Leptotes pirthous</i> (Linnaeus, 1767)	ILL160	KF860858	KF918771	KJ671872
<i>Leptotes cassius</i> (Cramer, [1775])	ILL176	KJ131015	KJ131024	KJ671871
<i>Euchrysops cnejus</i> (Fabricius, 1798)	ILL184	KJ508008	KJ527496	KJ527488
<i>Lepidochrysops peculiaris</i> (Rogenhofer, 1891)	ILL218	KP723381	KP723385	KP723383
<i>Thermoniphas alberici</i> (Dufrane, 1945)	ILL228	KP901006	KP900998	KP901002
<i>Caleta elna</i> (Hewitson, 1876)	ILL185	KJ508005	KJ527499	KJ527491
<i>Caleta roxus</i> (Godart, [1824])	ILL187	KJ508006	KJ527498	KJ527490
<i>Upolampes evena</i> (Hewitson, 1876)	ILL226	KP742775	KP742783	KP742779
<i>Psychonotis caelius</i> (C. et R. Felder, 1860)	ILL214	KP400022	KP400030	KP400026
<i>Callicita lara</i> Parsons, 1986	ILL227	KP901009	KP901001	KP901005
<i>Zintha hintza</i> (Trimen, 1864)	ILL239	KU197251	KU197253	KU197252
<i>Castalius rosimon</i> (Fabricius, 1775)	ILL194	KJ934113	KJ934118	KJ934108
<i>Tarucus balkanicus</i> (Freyer, [1844])	ILL151	KF647241	KF894398	KJ671874
<i>Glaucopsyche alexis</i> (Poda, 1761)	ILL158	KF647245	KF894392	KJ671890
<i>Turanana endymion</i> (Freyer, [1850])	ILL152	KF647234	KF918763	KJ774022
<i>Praephilotes anthracias</i> (Christoph, 1877)	ILL144	KF647243	KF894395	KJ698661
<i>Scolitantides orion</i> (Pallas, 1771)	ILL120	KC692329	KC692335	KJ774020
<i>Pseudophilotes bavius</i> (Eversmann, 1832)	ILL114	KC692327	KC692333	KJ698662
<i>Pseudophilotes vicrama</i> (Moore, 1865)	ILL119	KC692331	KC692337	KJ698663
<i>Phengaris alcon</i> ([Denis et Schiffermüller], 1775)	ILL140	KF647246	KF894391	KJ698657
<i>Phengaris arion</i> (Linnaeus, 1758)	ILL100	KC692326	KC692332	KJ698658
<i>Phengaris nausithous</i> (Bergstrasser [1779])	ILL141	KF647229	KF918770	KJ698659
<i>Catochrysops panormus</i> (C. Felder, 1860)	ILL178	KJ508007	KJ527497	KJ527489
<i>Catochrysops strabo</i> (Fabricius, 1793)	ILL190	KJ638695	KJ638700	KJ638704
<i>Zizula hylax</i> (Fabricius, 1775)	ILL182	KJ508011	KJ527493	KJ527485
<i>Lampides boeticus</i> (Linnaeus, 1767)	ILL159	KF860853	KF894399	KJ671892
<i>Phlyaria cyara</i> (Hewitson, 1876)	ILL212	KP096375	KP096393	KP096388
<i>Uranothauma delatorum</i> Heron, 1909	ILL211	KP096376	KP096394	KP096387
<i>Actizera stellata</i> (Trimen, 1883)	ILL210	KP096373	KP096391	KP096390
<i>Cacyreus marshalli</i> Butler, 1897	ILL181	KJ131012	KJ131021	KJ638703
<i>Jamides bochus</i> (Stoll, [1782])	ILL191	KJ774013	KJ774015	KJ774017

Table 1 (continuation).  
Таблица 1 (продолжение).

Species Вид	Voucher No. Музейный №	COI GenBank accession numbers	ITS2 GenBank accession numbers	Ef-1a GenBank accession numbers
<i>Jamides celeno</i> (Cramer, [1775])	ILL188	KJ638697	KJ638702	KJ638706
<i>Eicochrysops hippocrates</i> (Fabricius, 1793)	ILL209	KP096374	KP096392	KP096389
<i>Udara dilecta</i> (Moore, 1879)	ILL192	KJ698654	KJ698655	KJ698656
<i>Celastrina argiolus</i> (Linnaeus, 1758)	ILL154	KF647242	KF894396	KJ671882
<i>Celastrina morsheadi</i> (Evans, 1915)	ILL179	KJ131013	KJ131022	KJ671869
<i>Acytolepis puspa</i> (Horsfield, [1828])	ILL175	KJ131011	KJ131020	KJ671868
<i>Lycaenopsis haraldus</i> (Fabricius, 1787)	ILL200	KM211592	KM211585	KM211592
<i>Orthomiella pontis</i> (Elwes, 1887)	ILL199	KM211582	KM211584	KM211590
<i>Una usta</i> (Distant, 1886)	ILL217	KP400024	KP400032	KP400028
<i>Petrelaea dana</i> (de Nicéville, [1884])	ILL205	KM596826	KM596830	KM596828
<i>Azanus jesous</i> (Guérin-Méneville, 1849)	ILL208	KP053283	KP053287	KP053285
<i>Danis danis</i> (Cramer, [1775])	ILL204	KM586801	KM586809	KM586805
<i>Nacaduba berenice</i> (Herrich-Schäffer, 1869)	ILL198	KJ934115	KJ934120	KJ934110
<i>Ionolyce helicon</i> (Felder, 1860)	ILL216	KP901007	KP900999	KP901003
<i>Prosotas dubiosa</i> (Semper, [1879])	ILL193	KJ774014	KJ774016	KJ774018
<i>Prosotas pia</i> Toxopeus, 1929	ILL197	KJ934117	KJ934122	KJ934112
<i>Niphanda fusca</i> (Bremer et Grey, 1853)	ILL166	KJ508009	KJ527495	KJ527487
<i>Pithecopis dionisius</i> (Boisduval, 1832)	ILL206	KM596827	KM596831	KM596829
<i>Pithecopis phoenix</i> (Röber, 1886)	ILL221	KP400021	KP400029	KP400025
<i>Brephidium exilis</i> (Boisduval, 1852)	ILL207	KP053284	KP053288	KP053286
<i>Anthene emolus</i> (Godart, [1824])	ILL186	KJ508004	KJ527500	KJ527492
<i>Cupidesthes mimetica</i> (Druce, 1910)	ILL202	KM586799	KM586807	KM586803
<i>Lycaena alciphron</i> (Rottemburg, 1775)	ILL129	KC660003	KC660005	KJ671878
<i>Lycaena candens</i> (Herrich-Schäffer, [1844])	ILL136	KF647239	KF918756	KJ671879
<i>Lycaena virgaureae</i> (Linnaeus, 1758)	ILL135	KF647226	KF918778	KM211593
<i>Favonius quercus</i> (Linnaeus, 1758)	ILL161	KF860859	KF918772	KJ774019
<i>Thecla betulae</i> (Linnaeus, 1758)	ILL122	KC676696	KC676698	KJ774021
<i>Neolycaena rhymnus</i> (Eversmann, 1832)	ILL099	JF810412	JF813098	KM211591
<i>Satyrium pruni</i> (Linnaeus, 1758)	ILL124	JX112880	JX122755	KM211588
<i>Satyrium w-album</i> (Knoch, 1782)	ILL125	JX112882	JX122756	KM211586
<i>Satyrium acaciae</i> (Fabricius, 1787)	ILL123	JX112886	JX122754	KM211589
<i>Satyrium spini</i> (Fabricius, 1787)	ILL126	JX112881	JX122757	KM211587
<i>Hypothecle astyla</i> (C. et R. Felder, 1862)	ILL225	KP742774	KP742782	KP742778
<i>Cupidopsis cissus</i> (Godart, [1824])	ILL213	KP742772	KP742780	KP742776
<i>Cupidopsis iobates</i> (Hopffer, 1855)	ILL220	KP742773	KP742781	KP742777
<i>Candalides helenita</i> (Semper, [1879])	ILL203	KM586798	KM586806	KM586802
<i>Spalgis lemolea</i> Druce, 1890	ILL215	KP400023	KP400031	KP400027

Parameters for methods of DNA extraction were described previously [Vodolazhsky, Stradomsky, 2008].

We amplified DNA 5' section of the mitochondrial gene Cytochrome Oxidase subunit I (COI), the nuclear Elongation Factor 1-alpha (Ef-1a) and the nuclear noncoding sequence internal transcribed spacer 2 (ITS2) on the Mastercycler gradient (Eppendorf). The following cycling protocols were used: an initial 4 min denaturation at 95° C and 40 cycles of 30 s denaturation at 95° C, 30 s annealing at 53° C and 60 s extension at 72° C.

We used the following PCR primer pairs: forward,

5'-TAG CGA AAA TGA CTT TTT TCT A-3' (reserve forward 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') with reverse, 5'-TTG CTC CAG CTA ATA CAG GTA A-3' (reserve reverse 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') were used to amplify COI. Ef-1a was amplified with forward, 5'-TAC CAT CGA GAA GTT CGA GAA G-3' (reserve forward 5'-TGA AGG CCG AAC GTG AAC GTG G -3') and reverse, 5'-GCC ACC CCT TGA ACC AGG GCA T-3'. ITS2 was amplified with forward, 5'-GGG CCG GCT GTA TAA AAT CAT A-3' (reserve forward 5'-ACT CCT GTC TGA GGG CCG

GCT G-3') and reverse, 5'-AAA AAT TGA GGC AGA CGC GAT A-3' (reverse reverse 5'-TGA GGC AGA CTC GAT ATC CGT C-3') [Stradomsky, Fomina, 2013; Stradomsky, 2014].

Amplified fragments were separated using an automated sequencing machine (Applied Biosystems 3500).

The analysis of primary nucleotide sequences was made with the help of the application BioEdit Sequence Alignment Editor, version 7.0.5.3 [Hall, 1999].

Summary COI-Ef-1a-ITS2 nucleotide sequences were treated quantitatively using MEGA5 [Tamura et al., 2011] methods Minimum-Evolution (ME) and Maximum Likelihood (ML) and were represented as ME- и ML-cladograms.

## Results and discussion

This study based on a complex analysis of three genetic markers of blues butterflies from 4 tribes and 30 sections (sensu Eliot) of subfamily Polyommatainae, as well as an outgroup which includes some members of the subfamilies Lycaeninae, Theclinae and Miletinae. We used nucleotide sequences that are associated with various types of the evolutionary process as a marker: mitochondrial gene COI, the nuclear gene encoding a protein Ef-1a, as well as nuclear nucleotide sequence noncoding a protein ITS2, which is largely nondependent on external factors selection.

Obtained ME- and ML-cladograms (Figs 1, 2) have the maximum similarity, except for a insignificant displacement of some small clades. It should be noted that the obtained dendrograms have significant similarity with the ML-phylograms of tribe Polyommataini, which is based on the analysis of species and genes which are in many ways rather different [Vila et al., 2011: Fig. 2]. This coincidence suggests that the relationship reflected in the cladogram is very close to the natural relationship of taxa in the subfamily Polyommatainae.

In the first instance we consider an outgroup which includes representatives of subfamilies Lycaeninae, Theclinae and Miletinae. It should be noted that in this group there are some taxa previously attributed to the subfamily Polyommatainae, namely, representatives of the tribe Candalidini (*C. helenita*) and the section *Cupidopsis* sensu Eliot (*C. cissus* and *C. iobates*) of the tribe Polyommataini.

A comparison of the morphology of genitalia of *C. helenita*, *S. lemolea* (Miletinae) and *H. astyla* (Theclinae) (Figs 3–5) demonstrates their undeniable similarities. In addition, the structure of male fore tarsus in Candalidini is different from that of Polyommatainae. Consequently, the molecular genetic and some morphological characteristics suggest the need for an exclusion of tribe Candalidini from the subfamily Polyommatainae.

Comparison of genitalia of *C. cissus* and *H. astyla* (Figs 5–8) shows the uniformity of their structure in a generalized plan of the lateral view (Figs 5, 7), as well as of separate structures, for example, uncus and gnathos in ventral view (Figs 6, 8). We can note a homogeneous structure of the lobes of uncus *C. cissus* and *H. astyla*, which form rounded diamond-shaped outgrowths with

torsion on the apex and long sickle-shaped branches of gnathos.

We should also note that the butterflies of the genus *Cupidopsis* Karsch, 1895 have 10 veins on the forewing, which is characteristic only for some genera of the subfamily Theclinae, including the genus *Hypothoecla* Semper, 1890.

Thus, the results of molecular genetic analysis, as well as some significant morphological characteristics, indicate that the species of the genus *Cupidopsis* (and, consequently, *Cupidopsis* section sensu Eliot), most likely do not belong to the subfamily Polyommatainae.

All the other taxa of the subfamily Polyommatainae, except those mentioned above, form a monophyletic group. The first of the isolated clades of the subfamily includes genera *Cupidesthes* Aurivillius, 1895 and *Anthene* Doubleday, 1847, which constitute the tribe Lycaenesthini. Members of the tribe are still close to the species of the outgroup based on morphological features, which is especially characteristic for the genus *Cupidesthes*. The genitalia of representatives of the genera *Cupidesthes* (subfamily Polyommatainae) and *Satyrrium* Scudder, 1876 (subfamily Theclinae) are very similar in the lateral and in the ventral view (Figs 9–12). They have an expressed saccus, wide domed dorsal structures, a long thin aedeagus with adjacent small elongated valvae, long widely rounded branches of gnathos, and small ventrally oriented lobes of uncus.

The next three sister clades are two sections and one tribe sensu Eliot: *Brephidium* section, *Pithecus* section and the tribe Niphandini sensu Eliot. Since all of these clades are at least equivalent, it is necessary to set a lower status for the tribe Niphandini and set it as a subtribe Niphandina, placed in the tribe Polyommataini. In addition, there is a need to clarify a taxonomic status of two sections: *Brephidium* section and *Pithecops* section sensu Eliot, thus setting subtribe Brephidiina and Pithecopina also within the tribe Polyommataini.

The analysis of genitalia of the genera representing these subtribes, namely *Brephidium* Scudder, 1876, *Pithecops* Horsfield, [1828] and *Niphanda* Moore, [1875] (Figs 13, 14, 16) indicates that all of them have an original, distinct structure. This fact also substantiates an independent status of the designated subtribes. Genitalia in the genus *Brephidium* are especially unique. They are characterized by very little vulva, thin vinculum and large tegument with thick spinous hairs and processes. On the contrary, the genitalia of the tribe Niphandini are very primitive. They have a dome-shaped dorsal structure with little lobes of uncus and crescent-shaped branches of gnathos similar to those of genera *Cupidesthes* and *Satyrrium* (Figs 9–12).

Further, we distinguish two sister clades on both cladograms. The first clade includes *Danis* section and *Nacaduba* section sensu Eliot or *Danis* section sensu Eliot, *Nacaduba* section sensu Hirowatari [Hirowatari, 1992] and *Prosotas* section sensu Hirowatari. At the same time, this clade does not include the genus *Psychonotis* Toxopeus, 1930, which is traditionally included in *Danis* section. In connection with monophyletic type of this clade there is a need to designate subtribe Danina.

Analysis of the genital structure of the genera

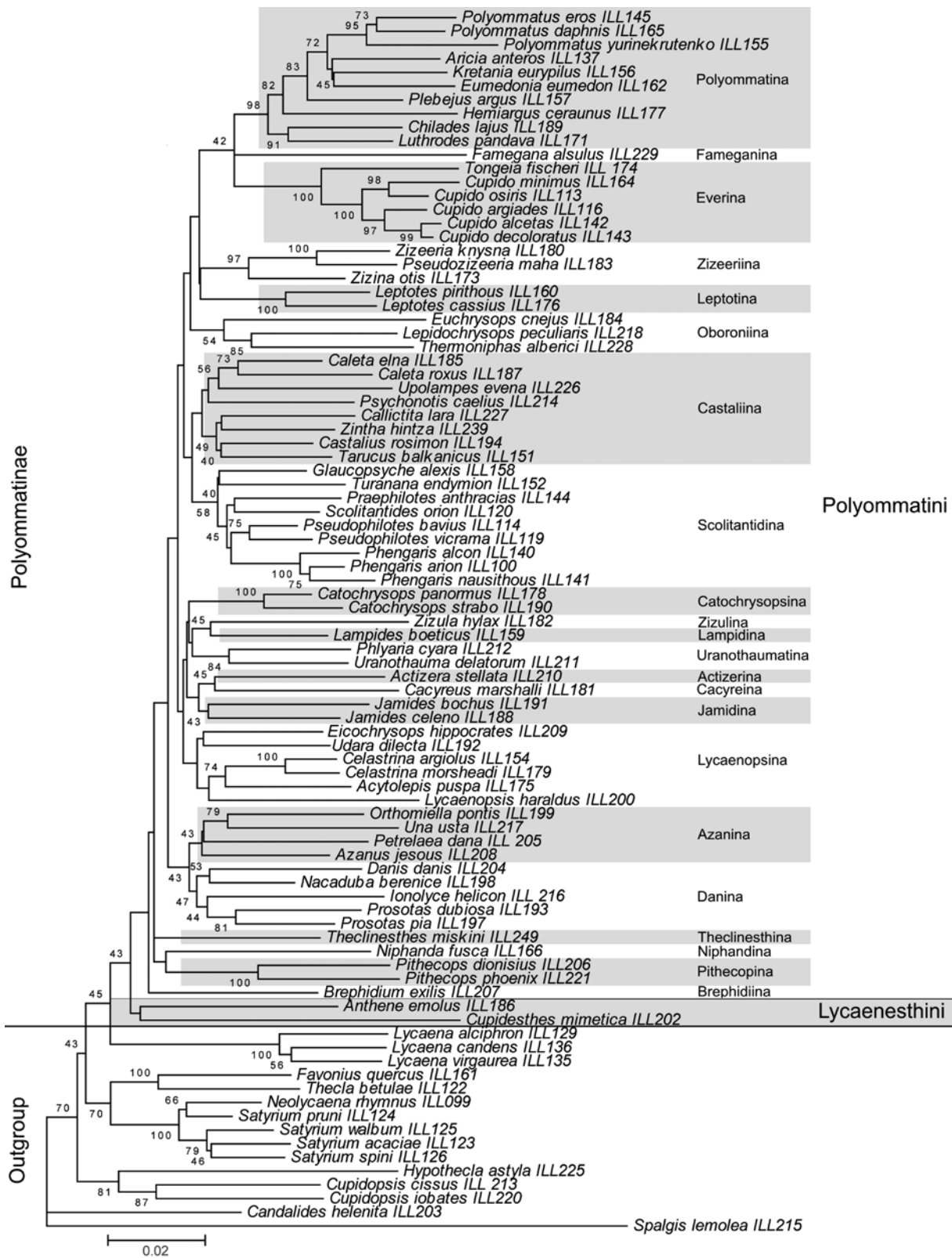


Fig. 1. Lycaenidae: ME-cladogram based on the Minimum Evolution method of analysis of distances for COI, Ef-1a and ITS2 DNA sequences.

Рис. 1. Lycaenidae: ME-клатограмма, построенная методом Минимальной Эволюции на основе последовательностей ДНК COI, Ef-1a и ITS2.

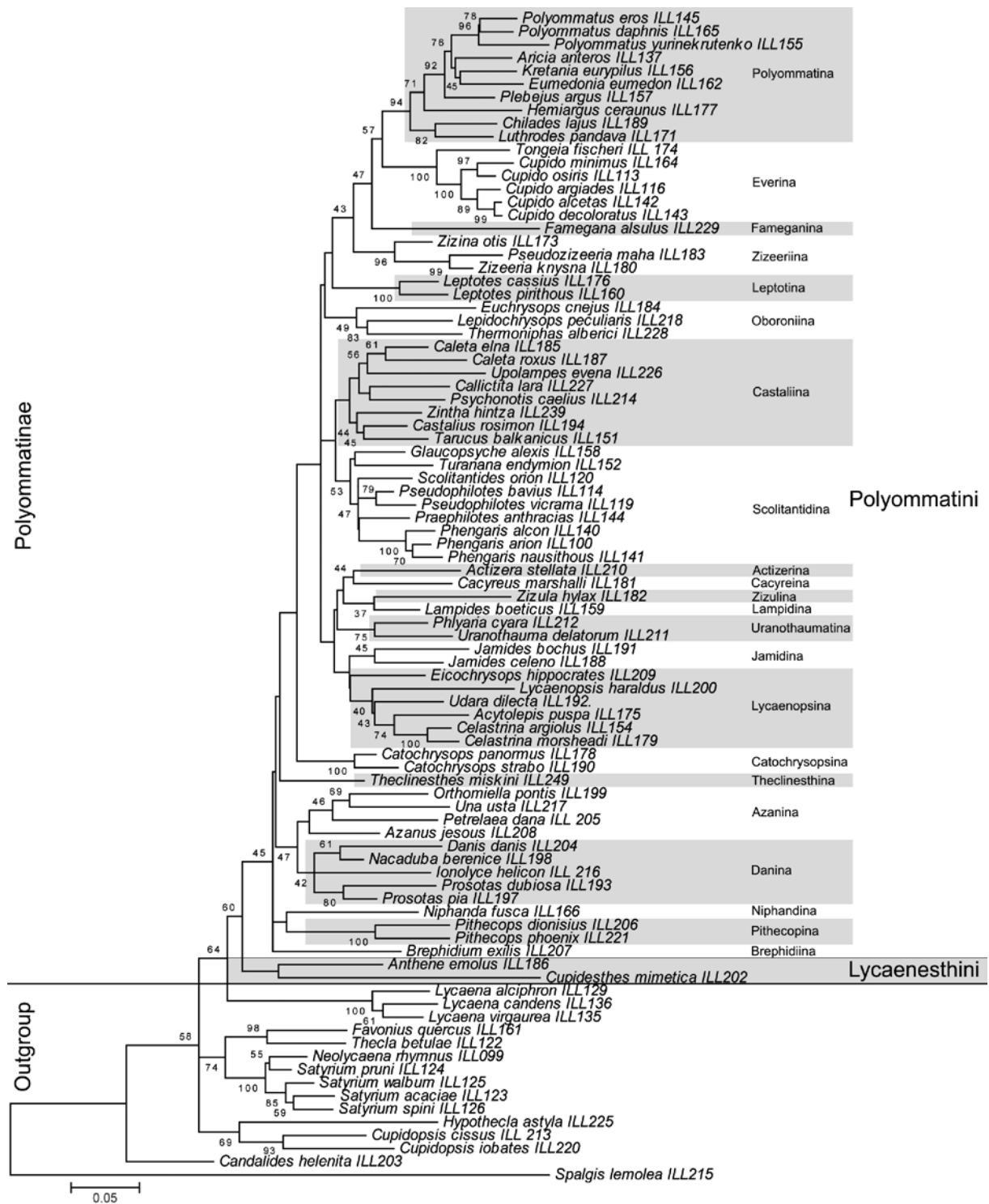


Fig. 2. Lycaenidae: ML-cladogram based on the Maximum Likelihood method of analysis of distances for COI, Ef-1a and ITS2 DNA sequences.

Рис. 2. Lycaenidae: ML-клатограмма, построенная методом Максимального Правдоподобия на основе последовательностей ДНК COI, Ef-1a и ITS2.

*Danis* Fabricius, 1807, *Nacaduba* Moore, [1881], *Ionolyce* Toxopeus, 1929 and *Prosotas* Druce, 1891 shows that the male genitalia in these taxa have large wide aedeagus, very convex tegumen, well expressed branches of gnathos, moderately narrow valva (Figs 17–20). At the same time

genitalia of the genus *Psychonotis* are clearly different: tegumen is not convex, little expressed branches of gnathos, valva is very wide (Fig. 46). Thus, the members of the genus *Psychonotis* clearly stand out from the subtribe Danina by structure of the genitalia.

Sister clade comprises the following sections sensu Eliot: *Azanus*, *Petrelaea* and *Una*. An obviously monophyletic type of clade allows us to combine these sections into subtribe Azanina. Male genitalia in genera *Azanus* Moore, [1881], *Petraea* Toxopeus, 1929 and *Orthomiella* de Nicéville in Marshall et de Nicéville, 1890 (Figs 21, 24, 27) are elongated, flattened, with a long thin aedeagus. The lobes of uncus in these genera in the ventral projection have a triangular shape (Figs 22, 25, 28). Valvae tend to expand from *Azanus* to *Orthomiella* (Figs 23, 26, 29). Genitalia of the species of the genera *Petrelaea*, *Orthomiella* and *Una* de Nicéville in Marshall et de Nicéville, 1890 have a small saccus.

*Theclinessthes* section sensu Eliot is a sister to the subtribe Danina and Azanina clade. The clade slightly changes its position depending of the type of cladograms (ME or ML), but it is always an independent clade. Accordingly, it is possible to establish the monophyletic subtribe Theclinesstina. Male genitalia of the genus *Theclinessthes* Röber, 1891 have a peculiar structure (Fig. 15): vinculum is very wide, lobes of uncus and branches of gnathos are directed ventrally.

The subtribe Lycaenopsina was designated previously [Stradomsky, 2014; Talavera et al., 2015]. The only question is the scope of taxa included in it. According to Talavera et al. [2015] as well as our ML-cladogram, monophyletic clade combines *Lycaenopsis*, *Eicochrysops* and *Jamides* sections sensu Eliot. However, ME-cladogram defines the *Jamides* section in the capacity of sister to subtribe Lycaenopsina, and in the case of their joining there will be a paraphyletic taxon. In addition, the analysis of genitalia reveals that male of genera *Eicochrysops* Bethune-Baker, 1924, *Lycaenopsis* C. & R. Felder, [1865], *Acytolepis* Toxopeus, 1927 (Figs 30–32), *Celastrina* Tutt, 1906 have a vinculum with wide wing-shaped expansion on the apex, the lobes of uncus are oriented caudally, and the branches of gnathos are mostly reduced to some extent. At the same time, genitalia of the members of *Jamides* section have a different shape (Fig. 33): vinculum has no widening, branches of gnathos are well developed, lobes of uncus are directed ventrocranial. Therefore, there is a need to allocate Jamidina to a separate subtribe.

The following small related subtribes present exclusively African taxa. The members of subtribes Cacyreina (*Cacyreus* section sensu Eliot) and Actizerina (*Actizera* section sensu Eliot) have peculiar genitalia (Figs 34, 35). Each subtribe includes only one section sensu Eliot. The genitalia of the genus *Actizera* Chapman, 1910 have very long, apically pointed lobes of uncus and small branches of gnathos. The lobes of uncus in the genus *Cacyreus* Butler, 1897 are small, conical, have extra long styloid process, branches of gnathos are long. There is a need to include two sister clades into the subtribe Uranothaumatina: *Uranothauma* section sensu Eliot and *Phlyaria* section sensu Eliot. While the genitalia of genera *Uranothauma* Butler, 1895 and *Phlyaria* Karsch, 1895 in the lateral projections (Figs 38, 40) have some minor differences, the similarity of genitalia of species of these sections in the caudal projection (Figs 39, 41) is obvious.

Sister subtribes Lampidina (one species, one genus) and Zizulina (two species, one genus) related to African

subtribe are very small specieswise and each one includes only one section sensu Eliot. Male genitalia of the genus *Lampides* Hübner, [1819] (Fig. 36) are essentially similar to male genitalia of *Glaucoopsyche* section (Figs 42–44), except short branches of gnathos. At the same time, genitalia in the genus *Zizula* Chapman, 1910 is very peculiar (Fig. 37): aedeagus has a deep cleavage laterally, valve has numerous bristles and processes of a specific shape.

Catochrysopsina stands as a very separate subtribe. It includes a single *Catochrysops* section sensu Eliot. Male genitalia of the genus *Catochrysops* Boisduval, 1832 are very primitive (Fig. 45) and similar to male genitalia of the genera *Cupidesthes* and *Satyrium* (Figs 9, 11).

Two large sister clades which comprise a significant number of genera are subtribes Scolitantidina and Castaliina. The subtribe Scolitantidina includes only one *Glaucoopsyche* section sensu Eliot and was designated previously [Stradomsky, 2014]. It was explored in detail by molecular methods [Ugelvig et al., 2011]. It should be noted that the male genitalia in this subtribe are quite homogeneous (Figs 42–44): gnathos has long branches, apex is directed caudally, lobes of uncus are short, aedeagus is short, thick, with blunt apex.

Subtribe Castaliina contains a large number of sections: *Castalius* section sensu Eliot, *Zintha* section sensu Eliot, *Callictita* section sensu Eliot, *Upolampes* section sensu Eliot, and the genus *Psychonotis*, previously included in the *Danis* section. Genitalia in the subtribe Castaliina look heterogeneous (Figs 46, 49, 52, 55, 58), but there is a need to note the following factors. Located basally in subtribe, the genus *Psychonotis* has small folds on valva (Fig. 47), which are greatly expanded in the genus *Zintha* Eliot, 1973 (Fig. 53) or even form a separate structure in genera *Tarucus* (Fig. 55), *Castalius* (Fig. 59) and *Callictita* Bethune-Baker, 1908. Furthermore, the valvae of the genera *Psychonotis* (Fig. 47), *Caleta* Hiwatari, 1992 (Fig. 50), *Zintha* (Fig. 53), *Tarucus* (Fig. 56) have long pointy processes. Also the juxtas of members of all these genera (Figs 48, 51, 54, 57, 60) have a tendency to expand from moderate (*Psychonotis*) to wide wing-shaped (*Tarucus*) branches. Thus, noteworthy is a complication of the structure of the ventral parts in male genitalia of all members of the subtribe Castaliina.

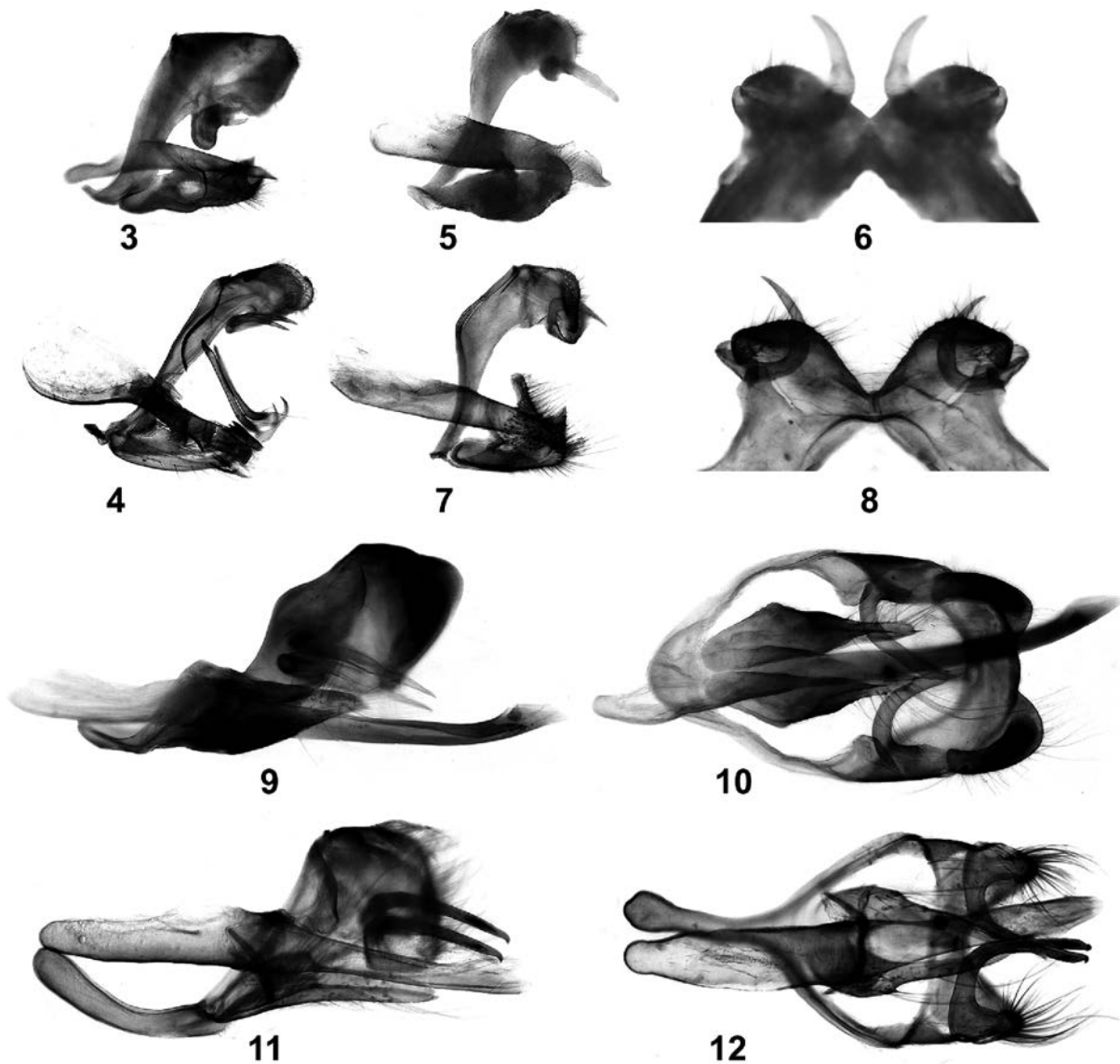
The subtribe Oboroniina comprises *Euchrysops* section sensu Eliot. Male genitalia in the subtribe are characterized by high affinity (Figs 61–63). They have long narrow valva, very long branches of gnathos and very massive aedeagus.

The subtribe Leptotila contains only *Leptotes* section sensu Eliot. This subtribe was designated previously [Talavera et al., 2013]. The main feature of the genus *Leptotes* Scudder, 1876 is aedeagus apically splitted in the ventral view (Figs 66, 67).

The clade that is a sister to the subtribe Leptotina unites members of *Zizeeria* section sensu Eliot and can be designated as a subtribe Zizeeriina. Male genitalia in genera *Zizeeria* Chapman, 1910 and *Zizina* Chapman, 1910 (Figs 64, 65) are very peculiar: aedeagus is very swollen, valvae have numerous long setae, branches of juxta and gnathos are elongated and thin.

Three sister clades, apical in the subfamily Polyommattinae and monophyletic, represent three





Figs 3–12. Genitalia of Lycaenidae species (3–5, 7, 9, 11 – lateral view; 6, 8 – uncus and gnathos; 10, 12 – ventral view).

Рис. 3–12. Гениталии представителей Lycaenidae (3–5, 7, 9, 11 – боковая проекция; 6, 8 – ункус и гнатос; 10, 12 – вентральная проекция).

3 – *Spalgis lemolea*; 4 – *Candalides helenita*; 5–6 – *Hypothecla astyla*; 7–8 – *Cupidopsis cissus*; 9–10 – *Satyrium pruni*; 11–12 – *Cupidesthes thyrsis*.

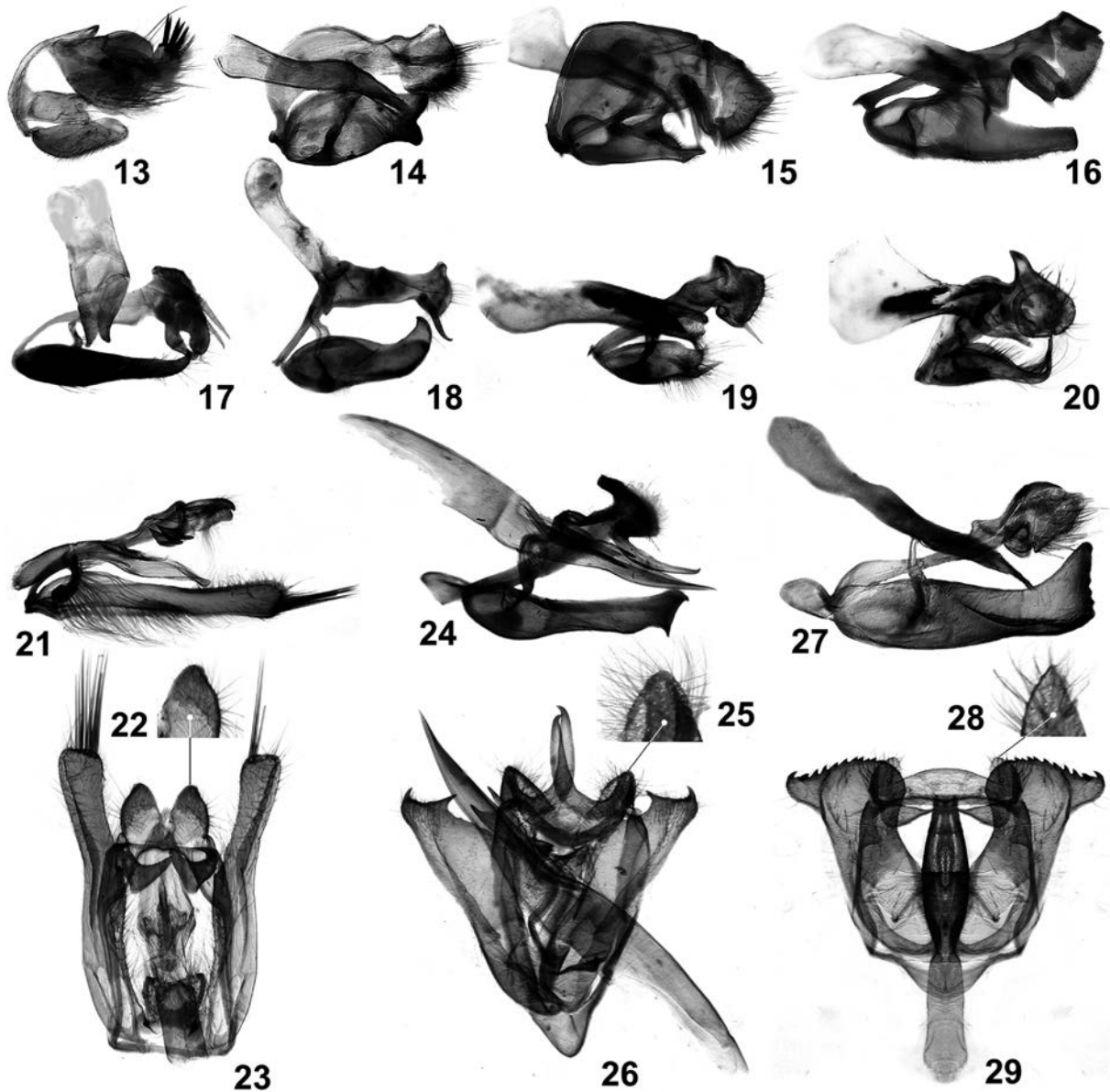
subtribes: Fameganina and previously named Everina [Talavera et al., 2015] and Polyommata [Talavera et al., 2013]. They include three sections sensu Eliot: *Famegana* section, *Everes* section and *Polyommatus* section respectively.

The close connection between these subtribes is confirmed by the details of the structure of the male genitalia. Genitalia of the three taxa look different in the lateral view (Figs 68, 71, 74). However, study of the dorsal structures (eg. in genera *Famegana* Eliot, 1973, *Cupido* Schrank, 1801 and *Plebejus* Click 1780) suggests that the structures of lobes of uncus are similar to the subtribes Fameganina (Fig. 69) and Polyommata (Fig. 75). The uncuses are represented by binate elongated lobes. On the contrary, the unpaired uncus in the subtribe Everina (Fig. 72). At the same time, the branches of gnathos do not

reach uncus in ventral view in genitalia of *Famegana* and *Cupido* (Figs 69, 72). A specimen of the genus *Plebejus* has branches of gnathos located directly under the lobes of uncus (Fig. 75).

Valvae in these three subtribes also have certain similarities. The specimen of the genus *Plebejus* has a valve with shortened costal and caudal processes (Fig. 76). Specimens of the genus *Famegana* have greatly elongated processes (Fig. 70). Males of the genus *Cupido* have elongated, thin and crisscrossing each other processes (Fig. 73). Thus, we can see transitional forms between the subtribe in morphological features, confirming, together with the molecular genetic results, close relationship of Fameganina, Everina and Polyommata.

Thus molecular genetic and morphological study of the subfamily Polyommatae allows to make the



Figs 13–29. Genitalia of Lycaenidae species (13–21, 24, 27 – lateral view; 22, 25, 28 – right lobe of uncus; 23, 26, 29 – ventral view).

Рис. 13–29. Гениталии представителей Lycaenidae (13–21, 24, 27 – боковая проекция; 22, 25, 28 – правая лопасть ункуса; 23, 26, 29 – вентральная проекция).

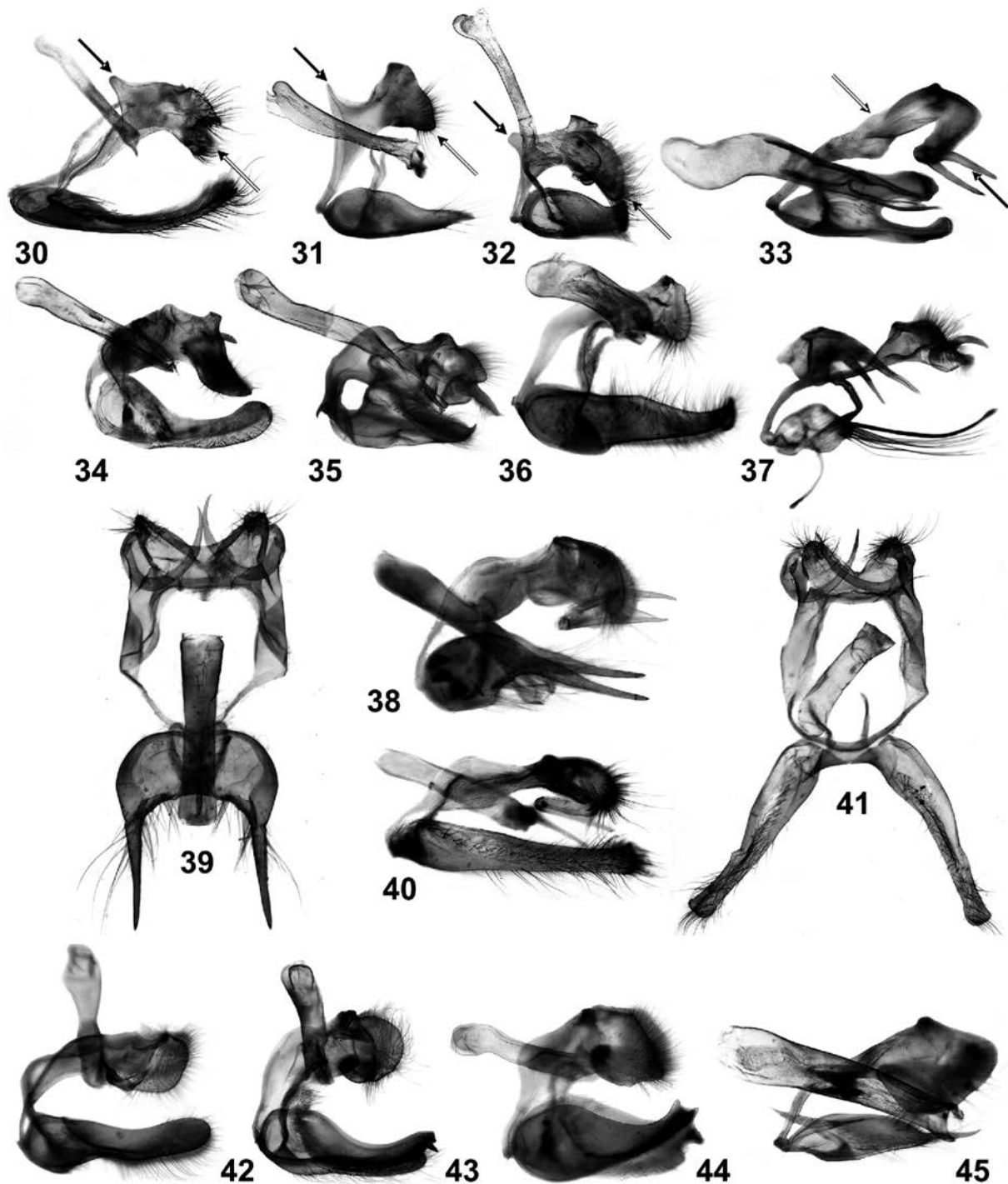
13 – *Brephidium exilis*; 14 – *Pithecopis dionisius*; 15 – *Theclinesthes miskini*; 16 – *Niphanda fusca*; 17 – *Danis danis*; 18 – *Nacaduba kurava*; 19 – *Ionolyce helicon*; 20 – *Prosotas dubiosa*; 21–23 – *Azanus jesous*; 24–26 – *Petrelaea dana*; 27–29 – *Orthomiella pontis*.

following conclusions: the tribe Candalidini and the genus *Cupidopsis* should be excluded from the subfamily. The status of the tribe Niphandini should be reduced to the subtribe level. Therefore, the subfamily Polyommatainae consists of two tribes: Lycaenesthina and Polyommataini. Elimination of a non-taxonomic rank “section” and the combined morphological and genetic analysis make possible to discriminate subtribes from the tribe Polyommataini, which meet the requirements of monophyly: Brephidiina, Pithecopina, Niphandina, Danina, Azanina, Theclinesthina, Lycaenopsina, Jamidina, Cacyreina, Actizerina, Uranothaumatina, Lampidina,

Zizulina, Catochrysopsina, Scolitantidina, Castaliina, Oboroniina, Leptotina, Zizeeriina, Fameganina, Everina and Polyommataina.

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Figs 30–45. Genitalia of Lycaenidae species (30–38, 40, 42–45 – lateral view; 39, 41 – caudal view).

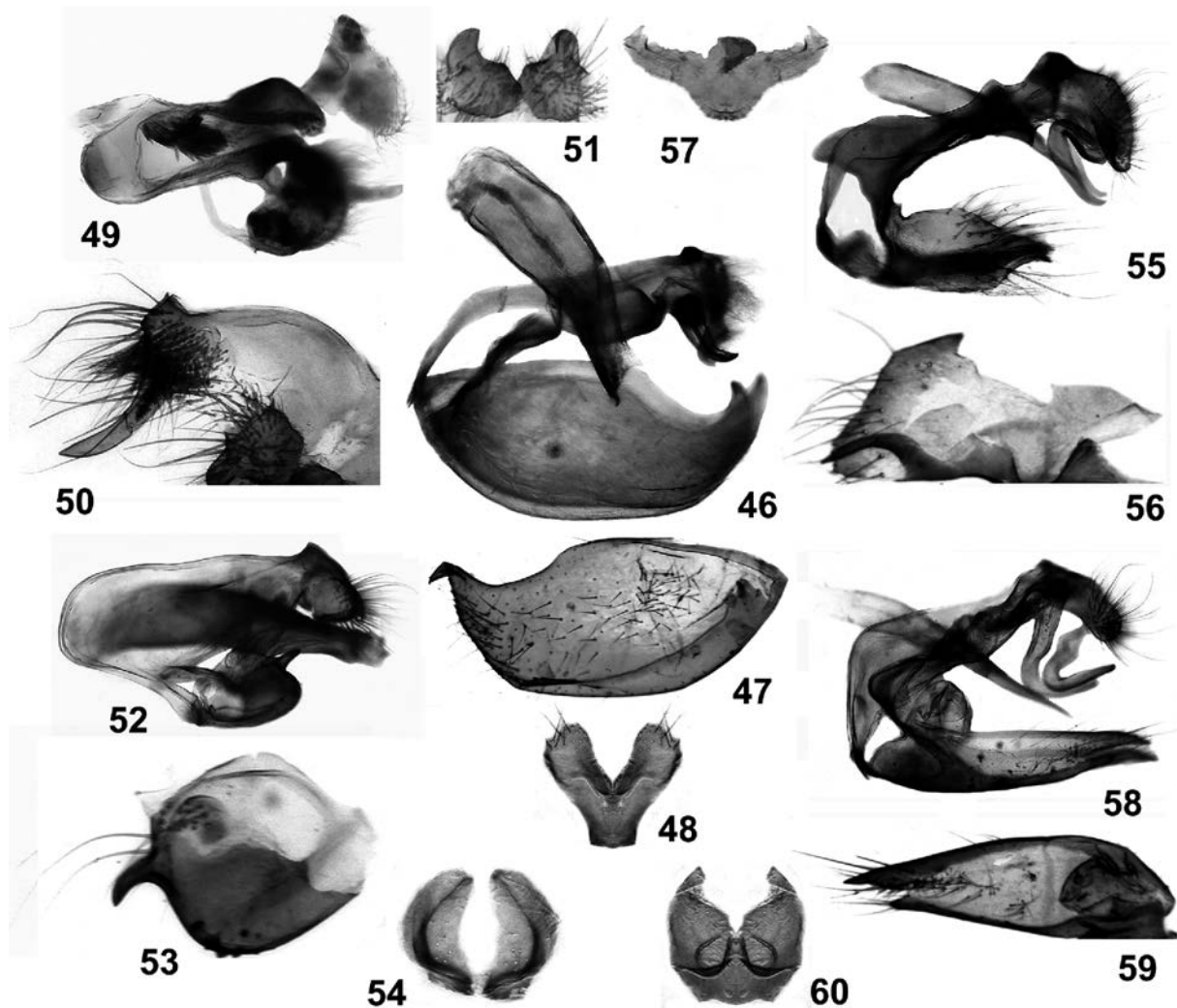
Рис. 30–45. Гениталии представителей Lycaenidae (30–38, 40, 42–45 – боковая проекция; 39, 41 – каудальная проекция).

30 – *Eicochrysops hippocrates*; 31 – *Acytolepis puspa*; 32 – *Lycaenopsis haraldus*; 33 – *Jamides celeno*; 34 – *Actizera stellata*; 35 – *Cacyreus marshalli*; 36 – *Lampides boeticus*; 37 – *Zizula hylax*; 38–39 – *Uranothauma delatorum*; 40–41 – *Phylaria cyara*; 42 – *Scolitantides orion*; 43 – *Glaucopsyche alexis*; 44 – *Phengaris arion*; 45 – *Catochrysops panormus*.

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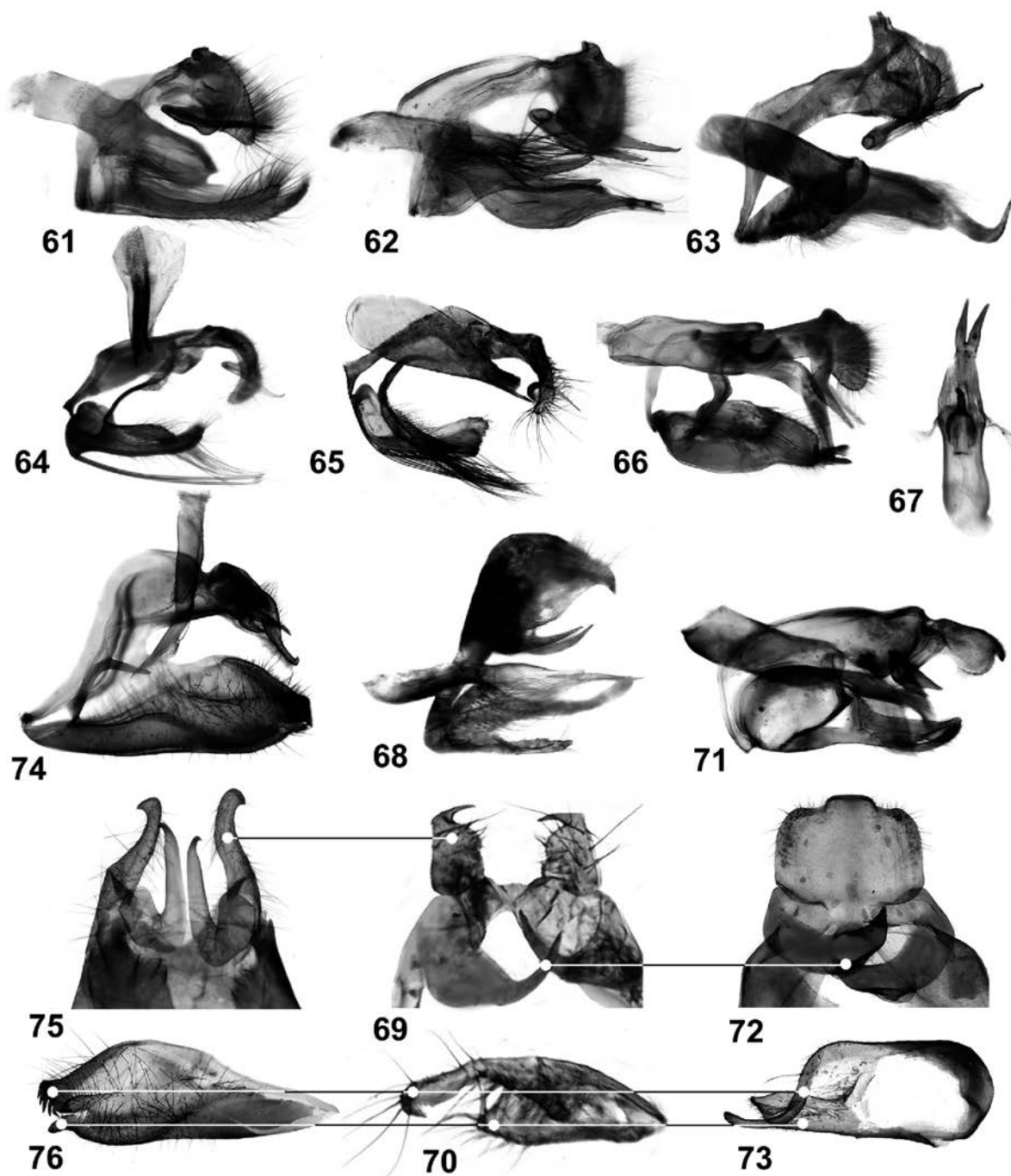
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Figs 46–60. Genitalia of Lycaenidae species (46, 49, 52, 55, 58 – lateral view; 47, 50, 53, 56, 59 – left valve, inner side; 48, 51, 54, 57, 60 – juxta).

Рис. 46–60. Гениталии представителей Lycaenidae (46, 49, 52, 55, 58 – боковая проекция; 47, 50, 53, 56, 59 – левая вальва, вид изнутри; 48, 51, 54, 57, 60 – юкста).

46–48 – *Psychonotis caelius*; 49–51 – *Caleta roxus*; 52–54 – *Zintha hintza*; 55–57 – *Tarucus balkanicus*; 58–60 – *Castalius rosomon*.



Figs 61–76. Genitalia of Lycaenidae species (61–66, 68, 71, 74 – lateral view; 67 – aedeagus; 69, 72, 75 – uncus and gnathos; 70, 73, 76 – left valve, inner side).

Рис. 61–76. Гениталии представителей Lycaenidae (61–66, 68, 71, 74 – боковая проекция; 67 – эдеагус; 69, 72, 75 – ункус и гнатос; 70, 73, 76 – левая вальва, вид изнутри).

61 – *Oboronia ornata*; 62 – *Thermoniphas alberici*; 63 – *Lepidochrysops intermedia*; 64 – *Zizina otis*; 65 – *Zizeeria knysna*; 66–67 – *Leptotes pirithous*; 68–70 – *Famegana alsulus*; 71–73 – *Cupido minimus*; 74–76 – *Plebeius argus*.