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Molecular Phylogeny and Taxonomy of the Butterfly Subtribe Scolitantidina with Special Focus on the Genera Pseudophilotes, Glaucopsyche and Iolana (Lepidoptera, Lycaenidae)

Vladimir A. Lukhtanov * and Anastasia V. Gagarina

Department of Karyosystematics, Zoological Institute, Russian Academy of Sciences, Universitetskaya Nab. 1, 199034 Saint-Petersburg, Russia
* Correspondence: lukhtanov@mail.ru

Simple Summary: The Palearctic butterfly genera Pseudophilotes, Glaucopsyche and Iolana have attracted the attention of many entomologists because their species are used as model objects for studying ecology and evolution. The genera have previously been the subjects of several taxonomic studies based on the analysis of their morphological and molecular characteristics, but none of these studies are based on complete species sampling. In our work, we used a set of mitochondrial and nuclear genes to reveal the phylogeny of these genera as well as the phylogeny of the subtribe Scolitantidina, to which these genera belong. In the genus Pseudophilotes, we identified 10 species including among them, the enigmatic Central Asian taxon P. panope, which has often been assigned to other genera. We clarified the taxonomic structure of the genus Glaucopsyche, which was found to consist of four subgenera. We confirm that the genus Iolana includes nine species distributed across the southwestern part of the Palearctic. The results obtained here will be important for the conservation of the Scolitantidina species, some of which are local and protected by national and international laws.

Abstract: The Palearctic blue butterfly genus Pseudophilotes Beuret, 1958 is not homogenous regarding the morphology of its genital structures. For this reason, some of its species have been considered to be representatives of other genera of the subtribe Scolitantidina (subfamily Polyommatinae). Here, we address these taxonomic problems by analyzing the phylogenetic relationships between the genera, subgenera, and species of this subtribe inferred via the analysis of five nuclear and two mitochondrial DNA sequences. We demonstrate that the enigmatic Asian species P. panope (Eversmann, 1851) belongs to the genus Pseudophilotes but not to Praephilotes Forster, 1938 or Palaeophilotes Forster, 1938 and does not represent the independent genus Inderskia Korshunov, 2000, as hypothesized previously. We synonymize P. svetlana Yakovlev, 2003 (syn. nov.) and P. marina Zhdanko, 2004 (syn. nov.) with P. panope. We demonstrate a deep genetic divergence between lineages that were previously considered as subspecies of the single species Iolana iolas (Ochsenheimer, 1816). As a result, we confirm the multispecies concept of the genus Iolana Bethune-Baker, 1914. We show that the Holarctic genus Glaucopsyche can be divided into four subgenera: Glaucopsyche Scudder, 1872 (=Shijimiaeoides Beuret, 1958), Apelles Hemming, 1931, Bajluana Korshunov and Ivonin, 1990, and Phaedrotes Scudder, 1876.

Keywords: Lepidoptera; Lycaenidae; Polyommatini; host plant; phylogeny; DNA barcoding

1. Introduction

The subtribe Scolitantidina Tutt, 1907 belongs to the tribe Polyommatini (subfamily Polyommatinae) and includes about 17–22 genera and about 85–100 described species distributed throughout the Holarctic and Oriental regions [1]. Eliot [2] recognized this group as a cluster of morphologically similar genera and called it “the Glaucopsyche section” (after the name of one of the genera in this group). Mattoni [3] treated it as the tribe
Scolitantidini. Hesselbarth et al. [4] divided this group into the subtribes Scolitantidina and Glaucopsychina Hemming, 1931 within the tribe Polyommatini. Subsequent studies confirmed the monophyly of this group, but the division into the subtribes Scolitantidina and Glaucopsychina was not supported [5,6]. Over the past 50 years, this subtribe has been the subject of a series of taxonomic and phylogenetic studies based on the use of morphological and molecular markers [3,5–9]. Despite this, the phylogenetic position, taxonomic status (genus-subgenus-synonymy) and species diversity of some genera within the subtribe Scolitantidina have remained unclear. In particular, this applies to the genera Pseudophilotes, Glaucopsyche and Iolana.

The genus Pseudophilotes was found not to be homogenous regarding the morphology of its genital structures and larval food plants [10–15]. For this reason, some of its species have been considered to be representatives of other genera, including Rubrapterus [10,11], Inderskia [12], Praephilotes [13], and Palaeophilotes [14,15]. The genus Pseudophilotes includes somewhere between eight and twelve species that are distributed across the temperate zone of Eurasia from the Atlantic coast in the west to East Siberia (Yakutia) in the east, and are also found locally in North Africa and the Levant [16]. This genus has attracted the attention of numerous researchers, as some of its species have been used as models in ecological [17–20] and evolutionary studies [16,21], particularly in studies of insect–plant coevolution [22,23]. Nearly all species of the genus are considered endangered or threatened and are protected by international and/or national laws [24–33]. The genus Pseudophilotes has been the subject of several taxonomical studies based on analyses of its morphology [4,7,10,12,14,15,34–36] and molecular markers [6,9,16,20,21,37]. The morphological analyses revealed an unusually high level of male genitalia variations on both intra- and inter-specific levels, resulting in descriptions of several new taxa [10,12,14,34–36]. Available multilocus molecular studies have focused on particular species and species groups within the genus Pseudophilotes [16,20,21], but none of them are based on complete species sampling. In particular, no information on molecular markers is available for the Asian species P. panope, P. svetlana, and P. marina. The genus Glaucopsyche was revealed to be a paraphyletic entity in a phylogenomic study by Ugelvig et al. [6] and a monophyletic group in a whole-genome study by Zhang et al. [9]. However, both studies [6,9] were based on an incomplete sampling of nominal genera and did not include the taxon Bajluana Korshunov and Ivonin, 1990, which is based on the little-known and morphologically distinct species, Glaucopsyche argali.

The genus Iolana is distributed throughout countries surrounding the Mediterranean Sea, in the Levant, Iran, Central Asia, northern Pakistan and northern India. It is represented by a number of allopatric, clearly closely related, but morphologically well-differentiated taxa. These taxa are considered as (i) subspecies of the same species, (ii) representatives of the two species I. iolas and I. gigantea, or (iii) seven to nine independent species [4,13]. These three taxonomic hypotheses have never been tested using molecular markers.

Here, we address these taxonomic problems by analyzing the phylogenetic relationships between the species of the subtribe Scolitantidina inferred via an analysis of the nuclear genes ribosomal subunit 28S (28S), histone 3 (H3), elongation factor 1α (EF1α) and wingless (wg), the non-coding nuclear internal transcribed spacer 2 (ITS2), and two mitochondrial genes, cytochrome oxidase I and II (COI and COII).

2. Materials and Methods

2.1. Taxon Sampling

According to Eliot [2], Mattoni [3], Ugelvig et al. [6], and Korshunov and Ivonin [36], the following genera should be included in the subtribe Scolitantidina (=Glaucopsyche section sensu Eliot, 1973):

- Apelles Hemming, 1931 (Type-species [TS]: Polyommatus melanops Boisduval, [1828]);
- Bajluana Korshunov & Ivonin, 1990 (TS: Lycaena argali Elwes, 1899);
- Caerulea Forster, 1938 (TS: Lycaena coeligena coelestis Alpheraky, 1897);
- Euphilotes Mattoni, [1978] (TS: Lycaena enoplos Boisduval, 1852);
For molecular analysis, we used representatives of all these nominal genera, except the very rare monotypic Central Asian genera Palaeophilotes, Micropsyche, Sinia and Subsolanoides. The species sampling included the type species for all studied nominal genera. For the genus Pseudophilotes, we analyzed representatives of all traditionally recognized species. The GenBank and/or BOLD accession numbers of the studied samples are presented in Table 1 and Figures 1–6. These accession numbers are searchable via GenBank (https://www.ncbi.nlm.nih.gov/genbank/, accessed on 28 November 2022) and/or BOLD (https://boldsystems.org/index.php/Public_BINSearch?searchtype=records, accessed on 28 November 2022) sites that contain information about the sequences and vouchers.

**Table 1.** List of the studied samples and obtained sequences.

| Species | BOLD/Field ID | GeneBank | Gene | Country | Locality |
|---------|---------------|----------|------|---------|----------|
| **Glaucopsyche alexis var. aeruginosa** | BPALB161-16 | OP712325 | COI | Israel | Hermon |
| | BPALB162-16 | OP712326 | COI | Israel | Hermon |
| | BPAL2627-14 | OP712327 | COI | Israel | Beit Jan |
| | BPAL2522-14 | OP712328 | COI | Israel | Nahal Trivon |
| | BPAL3276-16 | OP712334 | COI | Kazakhstan | Alatau, Kolbai |
| | BPAL3274-16 | OP712332 | COI | Kazakhstan | Kolbai |
| | BPAL3275-16 | OP712333 | COI | Kazakhstan | Kolbai |
| | BPAL3408-16 | OP712338 | COI | Kazakhstan | Kyzylagash |
| **Glaucopsyche argali** | LOWAM265-11 | OP712339 | COI | Kazakhstan | Kurtshum Mts, Salkyn-Cheku |
| | LOWAM268-11 | OP712340 | COI | Kazakhstan | Salkyn-Cheku |
| | LOWAM267-11 | OP712341 | COI | Kazakhstan | Salkyn-Cheku |
| | LOWAM266-11 | OP712342 | COI | Kazakhstan | Salkyn-Cheku |
| **Glaucopsyche laetifica** | BPAL3283-16 | OP712335 | COI | Kazakhstan | Ili valley, Kotkal |
| | BPAL3284-16 | OP712336 | COI | Kazakhstan | Kotkal |
| | BPAL3285-16 | OP712337 | COI | Kazakhstan | Kotkal |
| Species                  | BOLD/Field ID    | GeneBank   | Gene | Country  | Locality              |
|--------------------------|------------------|------------|------|----------|------------------------|
| *Glaucopsyche melanops*  | BPAL3540-16      | OP712329   | COI  | Morocco  | Agadir 30.90 N 7.24 W  |
|                          | BPAL3541-16      | OP712330   |      | Morocco  | Agadir                 |
|                          | BPAL3546-16      | OP712331   |      | Morocco  | Agadir                 |
| *Iolana alfieri*         | BPAL2358-14      | OP712343   | COI  | Israel   | Avdat                  |
|                          | BPAL2524-14      | OP712348   |      | Israel   | Avdat                  |
|                          | BPAL2525-14      | OP712349   |      | Israel   | Avdat                  |
|                          | BPAL2902-15      | OP712350   |      | Israel   | Har-A-Negev            |
|                          | BPAL2359-14      | OP712352   |      | Israel   | Avdat                  |
| *Iolana andreasi*        | LOWAM286-11      | OP712351   | COI  | Iran     | Shahkuh                |
| *Iolana andreasi khayyami*| BPAL2452-14     | OP712346   | COI  | Iran     | Tehran, Polur          |
|                          | BPAL2453-14      | OP712347   |      | Iran     | Tehran, Polur          |
| *Iolana kermani*         | BPAL2450-14      | OP712344   | COI  | Iran     | Kerman, Kuh-e-Segoh    |
|                          | BPAL2451-14      | OP712345   |      | Iran     | Kerman                 |
| *Praephilotes anthracias*| BPAL3279-16      | OP712323   | COI  | Kazakhstan | Matai               |
|                          | BPAL3280-16      | OP712324   |      | Kazakhstan | Matai               |
| *Pseudophilotes abencerragus*| BPAL525-18     | OP644300   | COI  | Israel   |
|                          | BPAL526-18       | OP644301   |      | Israel   |
|                          | BPAL527-18       | OP644302   |      | Israel   |
|                          | BPAL528-18       | OP644303   |      | Israel   |
|                          | BPAL3567-16      | OP644314   |      | Morocco   | 32.5853 N 6.05611 W   |
| *Pseudophilotes bavius*  | BPALB030-16      | OP644305   | COI  | Russia   | Bashkortostan, 54.89 N 53.646 E |
|                          | L2-14            | OP679877   | ITS2 | Kazakhstan | Koibyn               |
|                          | L2-15            | OP679878   |      | Kazakhstan | Koibyn               |
|                          | L2-16            | OP679879   |      | Kazakhstan | Koibyn               |
|                          | L2-14            | OP681138   | Wingless | Kazakhstan | Koibyn               |
|                          | L2-14            | OP681135   | EF1α | Kazakhstan | Koibyn               |
|                          | L2-15            | OP681136   |      | Kazakhstan | Koibyn               |
|                          | L2-16            | OP681137   |      | Kazakhstan | Koibyn               |
|                          | L2-14            | OP678972   | 28S  | Kazakhstan | Koibyn               |
|                          | L2-15            | OP678973   |      | Kazakhstan | Koibyn               |
|                          | L2-16            | OP678974   |      | Kazakhstan | Koibyn               |
| *Pseudophilotes panope*  | BPAL512-18       | OP644294   | COI  | Kazakhstan | Koibyn               |
|                          | BPAL513-18       | OP644295   |      | Kazakhstan | Koibyn               |
|                          | BPAL514-18       | OP644296   |      | Kazakhstan | Koibyn               |
|                          | BPAL515-18       | OP644297   |      | Kazakhstan | Koibyn               |
|                          | BPAL516-18       | OP644298   |      | Kazakhstan | Koibyn               |
|                          | BPAL517-18       | OP644299   |      | Kazakhstan | Koibyn               |
|                          | BPAL601-19       | OP644310   |      | Kazakhstan | 25 km NE Atyrau       |
|                          | BPAL602-19       | OP644311   |      | Kazakhstan | 21 km NE Atyrau       |
|                          | BPAL603-19       | OP644312   |      | Mongolia  | Nuru                  |
|                          | BPAL3287-16      | OP644315   |      | Kazakhstan | Koibyn               |
| *Pseudophilotes vicrama* | BPAL553-18       | OP644304   | COI  | Israel    |
|                          | BPAL284-17       | OP644306   |      | Tajikistan |                 |
|                          | BPAL331-17       | OP644307   |      | Tajikistan |                 |
|                          | BPAL359-17       | OP644308   |      | Tajikistan |                 |
|                          | BPAL470-17       | OP644309   |      | Israel    |
| *Pseudophilotes jacuticus*| BPAL605-19      | OP644313   | COI  | Russia    | Yakutia, Yakutsk      |
Figure 1. BI tree of the subtribe Scolitantidina based on mitochondrial genes (COI + COII dataset). Posterior probabilities are indicated at the nodes.
3.2. Nuclear Tree

On the nuclear tree (Figure 2), the clades representing the genera Turanana, Phengaris + Caerulea, and Glaucopsyche (including Shijimiaeoides divina) received good support. The genus Pseudophilotes was found to be monophyletic and divided into two subgenera: Pseudophilotes s. str. and Rubrapterus. The subgenus Rubrapterus was found to include two monophyletic species: P. (R.) fatma and P. (R.) bavius. The nuclear data supported the monophyly of the subgenus Pseudophilotes; however, within this subgenus the phylogeny was not resolved. Only three species of the subgenus Pseudophilotes were found to be supported monophyletic entities (P. abencerragus, P. marina and P. barbagiae).

Figure 2. BI tree of the subtribe Scolitantidina based on nuclear sequences (28S + H3 + EF1-a + wingless + ITS2 dataset). Posterior probabilities are indicated at the nodes.
No serious topology conflict was found between the mitochondrial and nuclear trees. Therefore, the mitochondrial and nuclear data were combined resulting in a mixed matrix [49], in which both the DNA barcodes of multiple species and specimens and the multi-locus data of representative taxa were represented (Table S1). This led to a noticeable increase in the resolution of the resulting phylogram (Figure 3). The following genera and suprageneric groups were identified as monophyletic: Phengaris (including Maculinea), Phengaris + Caerulea, Philotiella, Euphilotes, Philotiella + Euphilotes (Figure 3), Turanana (including Otnjukovia), Pseudophilotes (Figure 4), Turanana + Pseudophilotes (Figure 3), Glaucaopsycha, Iolana (Figure 5), Praephilotes, Phaedrotes, and Scolitantides (Figure 3).

Figure 3. BI of the subtribe Scolitantidina based on concatenation of nuclear and mitochondrial genes (28S + H3 + EF1-a + wingless + ITS2 + COI + COII dataset). Posterior probabilities are indicated at the nodes. (1), (2), (3), and (4) are the four supported main lineages within the subtribe Scolitantidina.
Figure 4. Fragment of the concatenated (28S + H3 + EF1-a + wingless + ITS2 + COI + COII) BI tree. The subgenus *Pseudophilotes* (*Pseudophilotes*) (=Inderskia, *syn. nov.*.) is shown. Posterior probabilities are indicated at the nodes.
Figure 5. Fragment of the concatenated (28S + H3 + EF1-a + wingless + ITS2 + COI + COII) BI tree. The genus *Glaucopsyche* is shown. A is the subgenus *Apelles*. Posterior probabilities are indicated at the nodes.
2.2. DNA Studies

The nuclear DNA sequences 28S, ITS2, EF1-α and wg were obtained from the department of Karyosystematics (Zoological Institute RAS, St. Petersburg) using the primers and protocols described in [16]. Standard COI barcodes (partial sequences of the cytochrome c oxidase subunit I gene) were obtained from the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using their standard high-throughput protocol described by deWaard et al. [38]. The pictures, and collection data of these specimens have been deposited and can be freely downloaded from the BOLD Public Data Portal (http://www.boldsystems.org/index.php/databases, accessed on 28 November 2022). Information about the obtained sequences is presented in Table 1.

For the analyses we used our own sequences as well as published sequences (nuclear sequences 28S, H3, EF1-α, wingless, and ITS2 and mitochondrial genes COI and COII) extracted from GenBank [6,9,16,20,21,39–43] (Table 2). The GenBank/BOLD/museum accession numbers of the analyzed sequences are presented in Figures 1–6. Two taxa (Lampides boeticus and Phylaria cyara) belonging to the Lampides and Phylaria sections sensu Elliot, 1973 were used to root the tree. The nuclear ribosomal 28S rRNA gene fragment and the nuclear ITS2 sequences were aligned with the software MAFFT v7.245, using the iterative refinement method G-INS-i [44] via the MAFFT online server (http://mafft.cbrc.jp/alignment/server/, accessed on 28 November 2022). As the ITS2 region consists of highly variable sections, its alignment remained partly ambiguous. We therefore used the software Aliscore v.2.0 (The Leibniz Institute for the Analysis of Biodiversity, Bonn, Germany) [45] to

Figure 6. Fragment of the concatenated (28S + H3 + EF1-α + wingless + ITS2 + COI + COII) BI tree. The genus Iolana is shown. Posterior probabilities are indicated at the nodes.
identify the ambiguously aligned or randomly similar sections within the ITS2 alignment as described previously [16]. Other sequences were aligned using BioEdit software [46] and were edited manually. Nucleotide substitution models for each dataset were estimated based on the Bayesian information criterion using jModeltest, version 2 [47]. The best fitting models were as follows: GTR + G + I for 28S, GTR + G + I for COI, GTR + G for H3, K2 + G + I for EF1a, K2 + G for wg, GTR + G + I for COII and K2 + G for ITS2.

Table 2. Fragments of DNA sequences used for phylogenetic analysis.

| Sequence | Total Length, bp | Number of Variable Sites | Number of Parsimony Informative Sites |
|----------|------------------|--------------------------|---------------------------------------|
| COI      | 1497             | 454                      | 353                                   |
| COII     | 679              | 184                      | 116                                   |
| EF1a     | 1161             | 238                      | 157                                   |
| H3       | 327              | 57                       | 42                                    |
| ITS2     | 449              | 104                      | 81                                    |
| wg       | 369              | 120                      | 67                                    |
| 28S      | 820              | 93                       | 65                                    |

The Bayesian analyses (Bayes inference, BI) were performed for each individual data set (28S, COI, H3, EF1-a, wg, COII, and ITS2) using the program MrBayes 3.2 [48] and the best fitting models. Two runs of 10,000,000 generations with four chains (one cold and three heated) were performed. The consensus of the obtained trees was visualized using FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 28 November 2022). These analyses revealed no significant gene tree–species tree conflicts in the data. Then, the genes were concatenated. In doing so, we were based on the evidence that combining multiple mitochondrial DNA barcodes with multilocus nuclear data for representative major taxa can significantly improve the resolution of phylogenetic analysis [49]. The concatenated alignment is presented in the Supplementary Materials (Table S1). The BI analysis of the concatenation 28S + COI + H3 + EF1-a + wg + COII + ITS2 was performed using partition of the data by gene.

2.3. Genus and Subgenus Concepts

We have previously argued that a genus-rank taxon must meet four criteria: (1) monophyly, (2) morphological discreteness, (3) conformity to a certain evolutionary age interval, and (4) conformity to historical nomenclatural traditions (stability and preservation of traditionally recognized taxa) [50]. While the first, second, and fourth criteria seem to be almost universally accepted, the use of criterion three (correspondence of the genus to a certain evolutionary age) is less obvious, and the evolutionary ages of traditionally accepted genera in different groups of living organisms vary greatly. Therefore, in this paper, we used three parameters as a genus criterion. Two of them are obligatory: (1) the monophyly and (2) the morphological discreteness from other genera. One was optional (the group was traditionally considered as a genus). We interpreted the existence of reasonable doubts about the monophyly of a genus as being in favor of dividing the group into two or more undoubtedly monophyletic entities.

As a subgenus, we considered a lineage that was also monophyletic and morphologically discrete but for which there was no tradition to consider it as a genus. Usually such a lineage (subgenus) in combination with other lineages (subgenera) forms a traditionally accepted genus. An additional (though not obligatory) reason for giving a lineage the status of a subgenus was the presence of a previously described available name for it.

2.4. Methodology of Molecular Taxonomy: Genomics, Phylogenomics, DNA-barcoding, and Mixed (Phylogenomics + Barcoding) Approaches

We live in a time when works based on genome-wide data are beginning to appear in insect taxonomy [e.g., 8,9], but at the same time, articles based on multilocus data (phy-
logenomic approach) [e.g., 6,16,20] or on single mitochondrial gene COI (DNA-barcoding approach) [39,40] still dominate. It seems to us that in between these methodologies is the mixed approach proposed by Talavera et al. [49] who demonstrated that DNA barcodes combined with multilocus data of representative taxa could generate reliable, higher-level phylogenies. This approach is indispensable for poorly studied groups and allows us to combine the suitable length of concatenated sequences for representative (“skeleton”) taxa with the completeness of species sampling.

3. Results

3.1. Mitochondrial Tree

Phylogenetic trees based exclusively on mitochondrial genes performed poorly, resulting in numerous polytomies (Figure 1). However, there were nodes that had good support. Thus, within the genus Glaucaopsyche, the clade Glaucaopsyche lycormas + Shijimiaeoides divina was identified. The genus Pseudophilotes was monophyletic and divided into two subgenera Pseudophilotes sensu stricto and Rubrapterus. The P. panope complex of the genus Pseudophilotes (P. panope + P. marina + P. svetlana) was monophyletic and isolated from other species of the genus. Pseudophilotes abencerragus and P. barbagiae were sister species.

3.2. Nuclear Tree

On the nuclear tree (Figure 2), the clades representing the genera Turanana, Phengaris + Caerulea, and Glaucaopsyche (including Shijimiaeoides divina) received good support. The genus Pseudophilotes was found to be monophyletic and divided into two subgenera Pseudophilotes sensu stricto and Rubrapterus. The subgenus Rubrapterus was found to include two monophyletic species: P. (R.) fatma and P. (R.) bavius. The nuclear data supported the monophyly of the subgenus Pseudophilotes; however, within this subgenus the phylogeny was not resolved. Only three species of the subgenus Pseudophilotes were found to be supported monophyletic entities (P. abencerragus, P. marina and P. barbagiae).

3.3. Concatenated Tree

No serious topology conflict was found between the mitochondrial and nuclear trees. Therefore, the mitochondrial and nuclear data were combined resulting in a mixed matrix [49], in which both the DNA barcodes of multiple species and specimens and the multilocus data of representative taxa were represented (Table S1). This led to a noticeable increase in the resolution of the resulting phylogram (Figure 3). The following genera and suprageneric groups were identified as monophyletic: Phengaris (including Maculinea), Phengaris + Caerulea, Philotyiella, Euphilotes, Philotyiella + Euphilotes (Figure 3), Turanana (including Otnjukovia), Pseudophilotes (Figure 4), Turanana + Pseudophilotes (Figure 3), Glaucaopsyche (Figure 5), Iolana (Figure 6), Praephilotes, Phaedrotes, and Scolitantides (Figure 3).

4. Discussion

Our analysis revealed four supported main lineages within the subtribe Scolitantidina: (1) Phengaris + Caerulea; (2) Euphilotes + Philotyiella; (3) Pseudophilotes + Turanana, and (4) Scolitantides + Philotes + Praephilotes + Iolana + Glaucaopsyche. This result is consistent with the previous molecular data [6] but does not support the division of the studied group into the subtribes Scolitantidina and Glaucaopsychina [4]. Within lineage (1) we found a pattern that was previously [5–7,51] described: the nominal genus Maculinea was nested within the genus Phengaris. The genus Phengaris (including Maculinea) was a sister of Caerulea. Within lineage (2), the sublineages Euphilotes and Philotyiella were found to be closely related and weakly differentiated taxa. Euphilotes and Philotyiella were described by Mattoni as two distinct genera [3]. Zhang et al. [8] downgraded Philotyiella to the rank of a subgenus of Euphilotes because their COI barcodes differed by only 3.3%. Our data also showed that Philotyiella was better treated as a subgenus than a genus.
Lineage (3) included two sister genera: Turanana and Pseudophilotes (6, 9, our data). Phylogenomic data for Otnjukova [5,6] and genomic data for Micropsyche [9] demonstrated that these taxa were junior subjective synonyms of Turanana.

The genus Pseudophilotes is divided into two subgenera: Pseudophilotes sensu stricto and Rubraperis. Within the subgenus Pseudophilotes, one of the most controversial points is the phylogenetic position of the species \( P. \) barbagiae, endemic to Sardinia. In the work of Todisco et al. [37] and Bartońová et al. [21], it was shown that, according to mitochondrial data, this was a sister species of \( P. \) abencerragus, which is distributed across North Africa, the Iberian Peninsula, and the Levant. At the same time, according to the combined nuclear–mitochondrial data [16], \( P. \) barbagiae was found to be included in the same clade as the European species \( P. \) panoptes and \( P. \) baton. Our analysis, as well as the data of Wiemers et al. [52], tends to support the sister relationship between \( P. \) barbagiae and \( P. \) abencerragus. The position of \( P. \) barbagiae on the phylogenetic tree is essential for deciding whether the species originated from Africa or from Europe, but it should be recognized that this issue has not yet been resolved. In the situation of apparent mitonuclear discordance, genome-wide data may be needed to resolve this problem.

Pseudophilotes panope, described by E. Eversmann from NW Kazakhstan, is one of the rarest and most enigmatic species of the subtribe Scolitantidina. Researchers previously attributed it to the genera Pseudophilotes, Praephilotes, Paleophilotes, Inderska, or considered it as a species whose genus was unknown [3,53]. The obtained nuclear and mitochondrial molecular data indicated the undoubted proximity of this taxon to species of the subgenus Pseudophilotes (Pseudophilotes), resulting in the synonymy: Pseudophilotes Beuret, 1958 (=Inderska Korshunov, 2000, \textit{syn. nov.}). Pseudophilotes panope has long been known in western Kazakhstan [15] and has only recently been found in eastern Kazakhstan (described as Paleophilotes [sic] \textit{marina} Zhdanko, 2004) and Mongolia (described as \textit{Pseudophilotes svetlana} Yakovlev, 2003). A detailed analysis of the external morphology, male genitalia and ecological preferences of populations belonging to \( P. \) panope, \( P. \) marina and \( P. \) svetlana was carried out by Morgun [15]. This author concluded that “all populations are the forms of one species with slightly different phenotypes, which may be due to adaptation (e.g., color, type of soil in inhabited biotopes, altitude above sea level)”. Tshikolovets et al. [53,54] downgraded \( P. \) marina and \( P. \) svetlana to subspecies of \( P. \) panope. Our study revealed identical DNA barcodes in the populations from west and east Kazakhstan and Mongolia. Based on this, we propose a synonymy: \( P. \) panope Eversmann, 1851 (=svetlana Yakovlev, 2003, \textit{syn. nov.}; =marina Zhdanko, 2004, \textit{syn. nov.}).

An interesting feature of \( P. \) panope is its monophagy on Astragalus lasiophillus Ledebur (Fabaceae), whereas caterpillars of other species of the genus are predominantly associated with the plants of the family Lamiaceae [3–8,14–22]. Association with \textit{Astragalus lasiophillus} has been also confirmed by us for the east Kazakhstan population of the species via observation of oviposition (Figure 7). A possible clue to this unusual feature is that another species of the genus, \( P. \) abencerragus, can also facultatively feed on plants of the family Fabaceae [22]. Feeding on legumes (Fabaceae) is probably an ancestral trait of Polyommatini butterflies [22]; this trait was either lost in the Pseudophilotes lineage but reappeared in \( P. \) panope as a reversion, or it was maintained in \( P. \) panope when the ancestor of the remaining Pseudophilotes switched to Lamiaceae.

Our study demonstrated that within the subgenus Pseudophilotes, only three species, \( P. \) panope, \( P. \) abencerragus, and \( P. \) barbagiae, were clearly differentiated with respect to DNA barcodes and other studied molecular markers (Figures 1–4). As for the species complex \( P. \) baton, \( P. \) panoptes, \( P. \) vicrama, \( P. \) sinaicus, and \( P. \) jacuticus, as noted earlier, they share the same or similar DNA barcodes (Figure 4) despite their morphological differences [21]. With the data available, it is impossible to decide whether this complex represents completely separated species with secondary contacts, stages of an incomplete speciation, or a single polymorphic species [21]. In our opinion, in accordance with the principle of nomenclatural stability and preservation of traditionally recognized taxa, \( P. \) baton, \( P. \) panoptes, \( P. \) vicrama, \( P. \) sinaicus, and \( P. \) jacuticus should be interpreted as species until further evidence is obtained.
in favor of or against their species status. In any case, we must state that molecular (based on DNA barcodes) identification of the species *P. baton*, *P. panoptes*, *P. vicrana*, *P. sinaicus*, and *P. jacuticus* seems to be problematic.

![Image](https://example.com/image.png)

**Figure 7.** Habitat (a) and larval foodplant (*Astragalus lasiophillus*) (b) of *P. panope* in Dzhungarian Alatau, E. Kazakhstan. Photo: V.Lukhtanov.

Within the *Scolitantides–Glaucopsyche* lineage (3), the genus *Glaucopsyche* was revealed in our study to be a paraphyletic group, with the species *Glaucopsyche piasus* forming a separate cluster on the tree (Figure 3). However, support for major basal branches within the *Scolitantides–Glaucopsyche* lineage was low in our study; therefore, the identified paraphyly of the genus *Glaucopsyche* cannot be considered proven. The genus *Glaucopsyche* was revealed as a paraphyletic entity in a phylogenetic study by Ugelvig et al. [6] and as a monophyletic group in a whole-genome study by Zhang et al. [9]. The later authors revealed a closer relationship between *Glaucopsyche piasus* (subgenus *Phaedrotes*) and other *Glaucopsyche* species than with *Iolana*, *Praephilotes*, *Scolitantides*, and *Philotes*. Our data showed that the genus *Glaucopsyche* also included three additional sublineages, which together formed a monophyletic unity. These three lineages can be interpreted as the subgenera *Glaucopsyche* sensu stricto, *Apelles* Hemming, 1931, and *Bajluana* Korshunov and Ivonin, 1990.

Within these three later subgenera, *Bajluana* was the most differentiated with respect to male genitalia [36,55]. The subgenus *Bajluana* included one species, *Glaucopsyche* (*Bajluana*) *argali*, which is endemic to the Altai and Saur–Tarbagatai Mts. Four groups of populations of this species are known: (1) the nominotypical subspecies (*G. argali argali*, mountains surrounding the Chuya steppe in the Russian Altai), (2) subspecies *argali* *chingiz* Churkin, 2005 (the southern part of the Mongolia Altai), (3) subspecies *argali* *arkhar* Lukhtanov, 1990 (the Saur, Tarbagatai, and Monrak mountains in Kazakhstan) and (4) the southern part of the Kurchum range in the Kazakhstan Altai (Salkyn–Cheku mountain). The analysis of the DNA barcodes showed that despite the geographical isolation, the first, third, and fourth groups of populations were similar to each other. For the second group of populations, molecular data are not yet available.

*Shijimiaeoides divina* is traditionally assigned to the independent genus *Shijimiaeoides* (and sometimes also to the genus *Sinia* by mistake, see [6]). However, molecular data point to its closeness to the core species of the subgenus *Glaucopsyche* (*Glaucopsyche*). Morphologically, this species is also similar to the typical *Glaucopsyche*, especially to *G. lycormas* [56], which differs in the presence of yellow or reddish spots on the underside of the hindwings. It is obvious that the presence/absence of these yellow or reddish spots is a highly variable characteristic within the subfamily Polyommatinae even on an intra-specific level [13]. Therefore, we support the opinion [9] on the synonymy of *Glaucopsyche* Scudder, 1872 (=*Shijimiaeoides* Beuret, 1958).

The subgenus *Glaucopsyche* also includes two little-known species from Central Asia: *G. charybdis* and *G. laetifica*. Both species inhabit near-water biotopes (riverbanks) in the desert zone, and their caterpillars are associated with licorice (*Glycyrrhiza*) (Fabaceae) [57].
The species are allopatric. *Glaucopsyche charybdis* is found in the basins of the Amu Darya, Zeravshan and Syr Darya (Fergana Valley) rivers. *Glaucopsyche lactifica* is found in the basin of the river Ili and in the downstream of the Syr-Darya River. *Glaucopsyche charybdis* (hind wing underside is gray-brown) and *G. lactifica* (hind wing underside is blue-green) are morphologically well distinguishable, but their DNA barcodes turned out to be similar. From the Dzhungarian Alatau Mts in eastern Kazakhstan, the morph *G. alexis* var. *aeruginosa* is known, resembling *G. laetifica* in color. The DNA barcode data showed that the var. *aeruginosa* was a color variant of *G. alexis* and was not conspecific with *G. laetifica*.

The monophyly of the genus *Iolana* and deep molecular differentiation of its species were revealed. This supports the multi-species concept of this genus [58,59] rather than a mono-species (*I. iolas* [4]) or two-species (*I. iolas* and *O. gigantea* [60]) system. There are no molecular data for two species of this genus (*I. gilgitica* and *I. arjanica*) but judging by the degree of morphological differentiation of their genitalia (59), they are good taxa of the species level. A deep differentiation between the African and Iberian populations attributed to *I. debilitata* was revealed. Perhaps they also represent different species.

5. Taxonomic Conclusions

We propose the following taxonomic arrangement of the subtribe Scolitantidina Tutt, 1907

Subtribe Scolitantidina Tutt, 1907 (= Glaucopsychina Hemming, 1931)
Genus *Euphilotes* Mattoni, [1978]
Subgenus *Euphilotes* (*Euphilotes*) Mattoni, [1978]
Subgenus *Euphilotes* (*Philotiella* Mattoni, [1978])
Genus *Phengaris* Doherty, 1891 (= *Maculinea* van Ecke, 1915)
Genus *Caerulea* Forster, 1938
Genus *Glaucopsyche* Scudder, 1872
Subgenus *Glaucopsyche* (*Glaucopsyche*) Scudder, 1872 (= *Shijimiaeoides* Beuret, 1958)
Subgenus *Glaucopsyche* (*Apelles* Hemming, 1931)
Subgenus *Glaucopsyche* (*Bajluana* Korshunov & Ivonin, 1990)
Subgenus *Glaucopsyche* (*Phaedrotes* Scudder, 1876)
Genus *Iolana* Bethune-Baker, 1914
Genus *Praephilotes* Forster, 1938
Genus *Palaeophilotes* Forster, 1938 (no molecular data available)
Genus *Scolitantides* Hübner, 1819
Genus *Turanana* Bethune-Backer, 1916 (= *Onjukovia* Zhdanko, [1997]; = *Micropsyche* Mattoni, 1981)
Genus *Pseudophilotes* Beuret, 1958
Subgenus *Pseudophilotes* (*Pseudophilotes*) Beuret, 1958 (= *Inderskia* Korshunov, 2000, syn. nov.)
Subgenus *Pseudophilotes* (*Rubrapterus* Korshunov, 1987)
Genus *Sinia* Forster, 1940 (no molecular data available)
Genus *Subsolanoides* Koiwaya, [1989] (no molecular data available)
We propose the following taxonomic arrangement of the genera *Pseudophilotes* Beuret, 1958 and *Iolana* Bethune-Baker, 1914
Genus *Pseudophilotes* Beuret, 1958
Subgenus *Pseudophilotes* (*Pseudophilotes* Beuret, 1958) (= *Inderskia* Korshunov, 2000, syn. nov.)

*P. (P.) panope* (Eversmann, 1851) (= *svetlana* Yakovlev, 2003, syn. nov.; (= *marina* Zhdanko, 2004, syn. nov.)
*P. (P.) abencerragus* (Pierret, 1837)
*P. (P.) barbagiae* De Prins & Poorten, 1982
*P. (P.) panoptes* (Hübner, [1813])
*P. (P.) baton* (Bergsträsser, [1779])
*P. (P.) vicrama* (Moore, 1865)
P. (P.) jacuticus Korshunov and Viidalepp, 1980
P. (P.) sinaicus Nakamura, 1975
  P. (P.) sinaicus sinaicus Nakamura, 1975
  P. (P.) sinaicus jordanicus Benyamini, 2000 (no molecular data available)

**Subgenus Pseudophilotes (Rubrapterus Korshunov, 1987)**

P. (R.) bavius (Eversmann, 1832)
P. (R.) fatma (Oberthür, 1890)
**Genus Iolana Bethune-Baker, 1914**
  I. iolas (Ochsenheimer, 1816)
  I. debilitata (Schultz, 1905)
    I. debilitata debilitata (Schultz, 1905)
    I. debilitata farriolsi de Sagarra, 1930
  I. lessei Bernardi, 1964
  I. alferii Wiltshire, 1948
  I. arjanica Rose, 1979 (no molecular data available)
  I. kermani Dumont, 2004
  I. andresi (Sheljuzhko, 1919)
  I. gigiltica (Tytler, 1926) (no molecular data available)
  I. gigantea (Grum-Grshimailo, 1885)

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects13121110/s1, Table S1: The concatenated alignment (28S + COI + H3+ EF1-a + wg + COII + ITS2).

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