A new species and new subspecies of *Eudiaphora* Dubatolov, 1990 (Lepidoptera, Erebidae, Arctiinae) from Tien-Shan and Pamir ranges

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**Abstract**

*Eudiaphora* Dubatolov, 1990 was described as a monotypic genus for *E. turensis* (Erschoff, 1874) with three subspecies. Here, we describe one more subspecies, *E. t. nozimdjoni* Spitsyn, Bolotov & Kondakov ssp. nov., from the Pamir Mountains (Tajikistan). Furthermore, we found an additional species in this genus, *E. tienshanensis* Spitsyn, Bolotov & Kondakov sp. nov. from the Tien-Shan Mountains (Kyrgyzstan). The valid status of both novel taxa is confirmed using a phylogenetic approach. An identification key for all taxa in this genus is provided.

**Key words:** Central Asia, DNA barcoding, integrative taxonomy, Kyrgyzstan, Pamir, Tajikistan, Tien-Shan.

**Introduction**

The genus *Eudiaphora* Dubatolov, 1990 contains *E. turensis* (Erschoff, 1874) with three its subspecies: nominative, *E. t. kuhitangica* Dubatolov, 2004, and *E. t. kopetdagica* Dubatolov, 2004 (see Dubatolov 2004, Dubatolov and de Vos 2010). Three other taxa, i.e. *Diaphora turensis maracandica* Seitz, 1910, *D. afghanistanensis* Daniel, 1966 and *Eudiaphora turensis f. yuldashevi* Murzin, 2003, were considered synonyms of the nominative subspecies (Dubatolov 1996, 2004).

In this study, we describe one more species and one more subspecies of *Eudiaphora* from the Central Asian mountain ranges using an integrative approach combining morphological, biogeographic and molecular evidence.

**Material and methods**

This study is based on the materials from the collection of the Russian Museum of Biodiversity Hotspots (RMBH) of the Federal Center for Integrated Arctic Research of the Russian Academy of Sciences, Arkhangelsk, Russia. The images of specimens were taken with a Canon EOS 80D camera (Canon, Tokyo,
The genitalia were boiled in 9% KOH during 3-4 min, cleaned from abdominal segments, and mounted on a temporal slide for photographing. The photos of genitalia were obtained using two research stereomicroscopes (SteREO Discovery.V8 and AXIO Zoom.V16, Carl Zeiss, Germany). After this procedure, the genitalia were placed into a small tube with an ethanol-glycerin solution that was pinned to the specimen.

Total DNA was extracted from a single leg of each dry specimen according to a standard phenol/chloroform procedure (Sambrook et al. 1989). The mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified and sequenced using primers LCO1490 (Folmer et al. 1994) and LepR (Hajibabaei et al. 2006). The PCR mix contained approximately 200 ng of total cell DNA, 10 pmol of each primer, 200 μmol of each dNTP, 2.5 μl of PCR buffer (with 20 mmol MgCl₂), 0.8 units Taq DNA polymerase (SibEnzyme Ltd., Russia), and H₂O was added for a final volume of 25 μl. Temperature cycling was as follows: 95°C (5 min), 30-33 cycles of 95°C (50 sec), 48°C (50 sec), 72°C (50 sec) and a final extension at 72°C (5 min). The sequencing was carried out using an ABI PRISM® BigDye™ Terminator v. 3.1 reagents kit. Reaction products were analysed using an automatic sequencer ABI PRISM® 3730 (Thermo Fisher Scientific, USA). The resulting sequences were checked manually using a sequence alignment editor BioEdit v. 7.2.5 (Hall 1999). Additional COI sequences were sampled from the NCBI GenBank database, including 28 sequences of the Arctiinae species and a sequence of Theretra natashae Cadiou, 1995 as an outgroup.

The sequence alignment was performed using the MUSCLE algorithm implemented in MEGA7 (Kumar et al. 2016). The phylogenetic analyses were carried out with W-IQ-TREE (Nguyen et al. 2015) through an online web server (http://iqtree.cibiv.univie.ac.at) (Trifinopoulos et al. 2016). We coded three partitions based on the codon position (three codons of the COI gene). The best-fit evolutionary models (1st codon: TNe+G4; 2nd codon: HKY+F; 3rd codon: TIM+F+G4) were selected using Model Finder based on Bayesian Information Criterion (BIC) (Kalyaanamoorthy et al. 2017). Bootstrap support (BS) values were estimated by means of an ultrafast bootstrap (UFBoot2) approach (Hoang et al. 2018). We used W-IQ-TREE, because this software was found to achieve the best-observed likelihoods compared with other fast likelihood-based phylogenetic programs (Zhou et al. 2017).

Molecular Operational Taxonomic Units (MOTUs) were obtained using the single rate Poisson tree processes (sPTP) model (Kapli et al. 2017) and the Bayesian Poisson tree processes (bPTP) model (Zhang et al. 2013) for single-locus species delimitation. A phylogenetic input tree was obtained from the IQ-TREE analysis (see above). The genetic divergence values (uncorrected p-distances) between COI haplotypes were computed in MEGA7 (Kumar et al. 2016).

Results

Three different taxa of the genus Eudiaphora were collected from high altitude ranges in Tajikistan and Kyrgyzstan (Figs 1-2). The series of seven male specimens collected from Khorog town, Pamir Range, Tajikistan (border with Afghanistan) can be assigned to Eudiaphora turensis turensis by the marking patterns and male genitalia structure (Figs 1A, 2A-2C). In contrast, a single male specimen collected from the Surkhob River valley in Tajikistan differs from the nominative subspecies by a creamy-white wing coloration, a broad postdiscal band on the forewing connecting with the discal spot, an elongated, narrow valva tapering distally, without clear separation on the narrow distal and broad proximal parts, a narrow, strongly sclerotized juxta, and a W-shaped saccus (Figs 1B, 2D-2F). Another male specimen collected from the Sary-Chelek Region of the Tien-Shan Range in Kyrgyzstan is similar to Eudiaphora turensis turensis by the marking patterns but strongly differs from it by the male genitalia structure, in which it has a short and broad juxta, a U-shaped saccus expanding proximally like a wineglass, and a valva with a strongly elongated narrow distal part (Figs 1C, 2G-2I).

Our phylogenetic analysis reveals that the genus Eudiaphora represents a monophyletic entity (BS = 100%) (Fig. 3). Eudiaphora turensis turensis and the Surkhob Valley specimen seem to be sister lineages with moderate support (BS = 87%). The Tien-Shan specimen appears to be the most divergent lineage within the genus. The bPTP species-delimitation model indicates that Eudiaphora turensis turensis and the Surkhob Valley specimen most likely belong to the same species, while sPTP species-delimitation model suggests that they may represent two separate species-level units (Table 1). Both these models reveal that the Tien-Shan specimen can be considered a separate species (Table 1). While the level of genetic divergence between

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**Eudiaphora turensis turensis** and the Surkhob Valley specimen is relatively low (COI p-distance = 0.9%), the Tien Shan specimen shares a larger genetic divergence from these taxa corresponding to interspecific differences (COI p-distance = 2.3-2.9%).

Based on the results outlined above, we consider the specimens from the Surkhob Valley and Tien Shan to be taxa new to science, i.e. *Eudiaphora turensis nozimdjonii* ssp. nov. and *Eudiaphora tien Shanensis* sp. nov., respectively.

### Table 1. Molecular diagnoses of the *Eudiaphora* taxa

| Taxon                                | Reference COI sequences (voucher no.) | Mean COI p-distance from the nearest neighbor, % | The nearest neighbor | Support of MOTUs by different versions of the PTP models | Fixed nucleotide differences based on the COI sequence alignment of congeners |
|-------------------------------------|--------------------------------------|-------------------------------------------------|---------------------|--------------------------------------------------------|--------------------------------------------------------------------------------|
| E. turensis turensis                | MK887193 (Sph806); MK887195 (Sph808) | 0.9                                             | E. turensis turensis | No; Yes                                                 | 180 C, 354 G, 369 G, 427 C, 486 T                                            |
| E. turensis nozimdjonii ssp. nov.   | MK887192 (holotype Sph0805)          | 0.9                                             | E. turensis turensis | No; Yes                                                 | 66 G                                                                            |
| E. tien Shanensis sp. nov.          | MK887194 (holotype Sph0807)          | 2.3                                             | E. turensis nozimdjonii ssp. nov. | Yes; Yes                                               | 90 G, 168 C, 201 T, 216 G, 237 T, 385 C, 402 G, 462 C, 478 T, 513 T, 543 C, 567 T, 618 C, 639 T |

### Taxonomy

**Family** Erebidae Leach, [1815]  
**Subfamily** Arctiinae Leach, [1815]  
**Tribe** Arctiini Leach, [1815]

**Genus** Eudiaphora Dubatolov, 1990  
Type species: *Spilosoma turensis* Erschoff, 1874

**Eudiaphora turensis turensis** (Erschoff, 1874)  
= *Spilosoma turensis* Erschoff (1874): 33.  
= *Eudiaphora turensis turensis* (Erschoff, 1874). – Dubatolov (2004): 152.  
= *Diaphora turensis maracandica* Seitz (1910): 92 (type locality: “Turkestan” [Uzbekistan, Samarkand]).  
= *Diaphora afghanistanensis* Daniel (1966): 163 (type locality: “Afghanistan, Hazaradjat, Koh-i-Baba, Pandjao Umgebung, 2500 m” [environs of Punjab town, Baba Range, Afghanistan, approx. 34.3869° N, 67.0243° E]).  
= *Eudiaphora turensis* f. *yuldashevi* Murzin (2003): 131 (type locality: environs of Bakanas village, middle reaches of the Ili River, Kazakhstan, approx. 44.8130° N, 76.2835° E).

**Figs 1A, 2A-C.**

**Type.** Lectotype male (designated by Dubatolov, 1994) in the Zoological Institute (ZISP) of the Russian Academy of Sciences, Saint Petersburg, Russia (Dubatolov 2004).

**Type locality.** “In desertis Kisil-Kum et inter Ulus et Djam” [Uzbekistan, in arid areas of the Kyzylkum Desert and between Ulus village (39.5667° N, 66.3667° E) and Dzam town (39.4325° N, 66.4096° E)].

**Material examined.** 7♂♂. Tajikistan (border with Afghanistan), Pamir Range, Khorog town, botanical garden, altitude 2249 m, 37.4803° N, 71.5997° E, 25-28 June 2018, Spitsyn leg.
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**Reference COI barcode sequences.** GenBank accession numbers MK887193 and MK887195 (voucher numbers Sph806 and Sph808, respectively).

**Differential diagnosis.** This subspecies could be distinguished from the other taxa in the genus by combination of the following characters: wing coloration light brown or gray; postdisal band is narrow and is clearly separated from the apex of the central cell, not connecting with the discal spot; valva elongated, broad, weakly sclerotized, with clear separation on the narrow distal and broad proximal parts; length of the narrow distal part of valva is shorter than the length of its broad proximal part; saccus U-shaped, with its branches being parallel to each other.

**Molecular diagnosis.** The nominative subspecies differs from the other congeners with available sequences by five diagnostic nucleotide substitutions in the COI gene fragment (Table 1). The COI p-distance between the nominative subspecies and *Eudiaphora turensis nozimdjoni* ssp. nov. is 0.9%.

**Distribution.** The nominative subspecies is thought to be widespread in Uzbekistan, Afghanistan, Kazakhstan, Kyrgyzstan, Turkmenistan, Tajikistan, China, and Mongolia (Dubatolov 2004).

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**Eudiaphora turensis kopetdaghica Dubatolov, 2004**

**Type.** Holotype male in the Siberian Zoological Museum of the Institute of Animal Systematics and Ecology, Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia).

**Type locality.** Southern Turkmenistan, Central Kopet Dag Range, 15 km W of Firjuza town, Dushak Mountain, near meteorological station, altitude 2150 m.

**Differential diagnosis.** This subspecies could be distinguished from the other taxa in the genus by combination of the following characters: wing coloration dark brown; narrow postdisal band on the forewing is clearly separated from the veins M3 and CuA bifurcation near the apex of the central cell; length of the narrow distal part of valva is shorter than the length of its broad proximal part.

**Distribution.** Putative endemic lineage to the Kopet Dag Range, southern Turkmenistan.

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**Eudiaphora turensis kuhitangica Dubatolov, 2004**

**Type.** Holotype male in the Siberian Zoological Museum of the Institute of Animal Systematics and Ecology, Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia).

**Type locality.** Southeastern Turkmenistan, Koytendag Range, Koytendag Nature Reserve, foothill of the Aýrybaba Mountain, plateau with *Juniperus polycarpos* var. *seravschanica* (Kom.) Kitam., altitude 2800 m.

**Differential diagnosis.** This subspecies could be distinguished from the other taxa in the genus by combination of the following characters: wing coloration dark brown; postdisal band is two times wider than that in *E. turensis kopetdaghica* but less contrast; this band is located just near the veins M3 and CuA bifurcation close to the apex of the central cell; length of the narrow distal part of valva is not shorter than the length of its broad proximal part.

**Distribution.** Putative endemic lineage to the Koytendag Range, southeastern Turkmenistan.

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**Eudiaphora turensis nozimdjoni Spitsyn, Bolotov & Kondakov ssp. nov.**

Figs 1B, 2D-F, 4.

**Type material.** Holotype male RMBH Sph0805: Tajikistan, Pamir Range, Surkhob River valley, ca. 8 km NW of Navobod village, altitude 1460 m, 39.0739° N, 70.0983° E, 22 June 2018, Spitsyn leg.

**Reference COI barcode sequences.** GenBank accession number MK887192 (holotype).

**Etymology.** This subspecies is named in honor of Mr. Nozimdjon Mahmudov (Dushanbe, Tajikistan) who help us to collect the holotype.

**Differential diagnosis (male).** This subspecies could be distinguished from the other taxa in the genus by combination of the following characters: wing coloration creamy-white; postdisal band is very broad and is located near the apex of the central cell connecting with the discal spot; valva elongated,
narrow, tapering distally, without clear separation on the narrow distal and broad proximal parts; juxta very narrow, strongly sclerotized; saccus W-shaped.

**Figure 1.** Male specimens of Eudiaphora spp. A E. turensis turensis (Tajikistan, Khorog) B E. turensis nozimdjoni ssp. nov. (holotype) C E. tienshanensis sp. nov. (holotype). Scale bars = 10 mm. (Photos: Vitaly M. Spitsyn).
Molecular diagnosis. This new subspecies differs from the other congeners with available sequences by one diagnostic nucleotide substitution in the COI gene fragment (Table 1). The COI p-distance between the new subspecies and *Eudiaphora turensis turensis* is 0.9%.

Description. Male. Wingspan 34 mm, forewing length 19 mm (Fig. 1B). Head creamy-white, eye black. Antenna bipectinate, black. Labial palpus long (slightly longer than twice eye diameter), dark brown, straight. Proboscis reduced. Torax creamy-white, with elongated black scales on the tegula. Abdomen dark yellow, with black band on each tergite. Legs yellow, with black apex of the femur and black spots on the tarsus. Upperside of both wings creamy-white, the hindwing is slightly lighter than the forewing; fringe broad, creamy-white. Forewing with two small black basal spots, a broad subbasal black band, a group of five discal black spots, and a broad subdiscal band. Hindwing with a comma-shaped discal black spot. Underside of both wings creamy-white, with marking patterns similar to those on their upperside. Male genitalia. Tegumen elongated, broad; uncus broadly triangular, its apex rounded (Fig. 2D). Juxta very narrow, strongly sclerotized; saccus W-shaped (Fig. 2E). Valva elongated, narrow, tapering distally, without clear separation on the narrow distal and broad proximal parts (Fig. 2D). Aedeagus long, broad, vesica with weak, rather dense granulation (Fig. 2F). Female unknown.

Distribution. Known only from the type locality: ca. 8 km NW of Navobod village, Surkhob River valley, Pamir Range, Tajikistan (Fig. 4).
Figure 3. Maximum likelihood phylogeny of the genus *Eudiaphora* (violet filling) based on the COI dataset. Numbers near nodes are BS values. Tip labels: Species name | GenBank COI acc. number | Specimen locality. Blue labels indicate the new taxa under discussion. Our bPTP species delimitation model supported most of taxa as separate species-level units with exception of the branches colored red.
**Eudiaphora tienshanensis** Spitsyn, Bolotov & Kondakov sp. nov.
Figs 1C, 2G-I, 5.

**Type material.** Holotype male RMBH Sph0807: Kyrgyzstan, Tien-Shan Range, Sary-Chelek Region, edge of mixed broadleaf forest and herb meadows, altitude 1285-1392 m, between 41.8164° N, 71.9594° E and 41.8200° N, 71.9722° E, 13 July 2018, Spitsyn leg.

**Reference COI barcode sequences.** GenBank accession number MK887194 (holotype).

**Etymology.** This species is named after the Tien-Shan Mountain Range, in which the holotype was collected.

**Differential diagnosis (male).** This species could be distinguished from the other taxa in the genus by combination of the following characters: wing coloration gray; postdiscal band is narrow and is clearly separated from the apex of the central cell, not connecting with the discal spot; juxta short, very broad, sclerotized; saccus U-shaped, expanding proximally like a wineglass; length of the narrow distal part of valva is two times longer that the length of its broad proximal part.

**Molecular diagnosis.** This species differs from the other congeners with available sequences by 14 diagnostic nucleotide substitutions in the COI gene fragment (Table 1). The COI p-distance between the new species and *Eudiaphora turensis turensis* and *E. t. nozimdjoni* ssp. nov. is 2.9 and 2.3%, respectively.

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**Figure 4.** Type locality of *Eudiaphora turensis nozimdjoni* ssp. nov.: ca. 8 km NW of Navobod village, 39.0739° N, 70.0983° E, Surkhob River valley, Pamir Range, Tajikistan. (Photo: Vitaly M. Spitsyn).

**Description.** Male morphology and markings. Wingspan 34 mm, forewing length 16 mm (Fig. 1C). Head dark gray, eye black. Antenna bipectinate, black. Labial palpus long (slightly longer than twice eye diameter), dark brown, straight. Proboscis reduced. Torax dark gray. Abdomen dark yellow, with black band on each tergite. Legs gray, with black apex of the femur. Upperside of both wings gray, fringe broad, gray. Forewing with a basal black spot, a discontinuous subbasal black band, a group of four discal black spots, and a discontinuous subdiscal band. Hindwing with a discal black spot. Underside of both wings gray with a discal black spot on each wing. *Male genitalia.* Tegumen elongated, broad; uncus broadly triangular, its apex rounded (Fig. 2G). Juxta short, very broad, sclerotized; saccus U-shaped, expanding proximally like a wineglass (Fig. 2H). Valva elongated, narrow, tapering distally; length of the narrow distal part of valva is
two times longer than the length of its broad proximal part (Fig. 2G). Aedeagus long, broad, vesica with weak, rather sparse granulation (Fig. 2I). Female unknown.

**Distribution.** Known only from the type locality: Sary-Chelek Region, Tien-Shan Range, Kyrgyzstan (Fig. 5).

![Figure 5. Type locality of Eudiaphora tienshanensis sp. nov.: Sary-Chelek Region, 41.8200° N, 71.9722° E, Tien-Shan Range, Kyrgyzstan. (Photo: Vitaly M. Spitsyn).](image)

**Discussion**

Our novel results indicate that the taxonomic richness of the genus *Eudiaphora* has been underestimated. *Eudiaphora turensis turensis* is thought to have an extensive range but this subspecies may actually represent a complex of cryptic taxa with more restricted distribution. *Eudiaphora turensis kopetdaghica* and *E. turensis kuhitangica* share clear morphological differences from the other taxa in this genus (Dubatolov 2004) and can therefore represent two separate species-level lineages endemic to the Kopet Dag and Koytendag ranges, respectively. Our results highlight that the genus *Eudiaphora* may be an appropriate model to estimate the role of Central Asian Mountains in insect radiation using a phylogeographic approach.

**Key to taxa of the genus Eudiaphora Dubatolov, 1990 (males)**

1. Wing coloration dark brown, size usually smaller (forewing length ≤16 mm),
   - Wing coloration light brown, gray or creamy-white, size usually larger (forewing length ≥16 mm),

2. Narrow postdisical band on the forewing is clearly separated from the veins M3 and CuA bifurcation near the apex of the central cell. Length of the narrow distal part of valva is shorter than the length of its broad proximal part.
   - Postdisical band is two times wider but less contrast. This band is located just near the veins M3 and CuA bifurcation close to the apex

E. *turensis kopetdaghica* Dubatolov, 2004 [Kopet Dag Range, southern Turkmenistan]

E. *turensis kuhitangica* Dubatolov, 2004 [Koytendag Range, southeastern Turkmenistan]
of the central cell. Length of the narrow distal part of valva is not shorter that the length of its broad proximal part.

3 Wing coloration creamy-white. Postdiscal band is very broad and is located near the apex of the central cell connecting with the discal spot. Valva elongated, narrow, tapering distally, without clear separation on the narrow distal and broad proximal parts. Juxta very narrow, strongly sclerotized. Saccus W-shaped.

– Wing coloration light brown or gray. Postdiscal band is narrow and is clearly separated from the apex of the central cell, not connecting with the discal spot. Valva with clear separation on the narrow distal and broad proximal parts. Juxta broad, with weaker sclerotization. Saccus U-shaped.

4 Juxta elongated, broad, weakly sclerotized. Saccus U-shaped, with its branches being parallel to each other. Length of the narrow distal part of valva is shorter that the length of its broad proximal part.

– Juxta short, very broad, sclerotized. Saccus U-shaped, expanding proximally like a wineglass. Length of the narrow distal part of valva is two times longer that the length of its broad proximal part.

E. turensis turensis (Erschoff, 1874) [widespread: Uzbekistan, Afghanistan, Kazakhstan, Kyrgyzstan, Turkmenistan, Tajikistan, China, and Mongolia]

E. turensis turensis nozimdjoni Spitsyn, Bolotov & Kondakov sp. nov. [Sary-Chelek Region, Tien-Shan Range, Kyrgyzstan]

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