

New data on taxonomy and systematics of the genus *Diamesa* Meigen (Diptera: Chironomidae: Diamesinae) from Tien Shan and Pamir Mountains, with description of two new species

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ABSTRACT

An illustrated morphological description of two new species of the genus *Diamesa* Meigen, 1835 are provided from Tien Shan and Pamir mountains: *D. akhrorovi* sp. nov., as male adult; *D. alibaevae* sp. nov., as male adult and pupa. DNA barcoding results of the two new described species with comparative database on some related *Diamesa* species are also given. The two new described species key both near *D. planistyla* Reiss, 1968, which was known from Nepal and redescribed here from Tien Shan. The diversity of *Diamesa* genus using three mitochondrial (COI, COII, 16S rRNA), two nuclear (18S rRNA, EF-1a) markers and estimating phylogenetic relationships is also highlighted. Six of the studied *Diamesa* species including *D. akhrorovi* sp. nov. and *D. alibaevae* sp. nov. represent a well-supported monophyletic clades with high Bayesian posterior probability, while *D. cinerella* Meigen and *D. hamaticornis* Kieffer have a lower interspecific sequence divergence.

Key words: Chironomids; *Diamesa*; systematics; new species; barcoding; Tien Shan; Pamir.

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INTRODUCTION

The biological diversity of the Tien Shan and Pamir mountains is extremely interesting and unique, but unfortunately is still poorly investigated. Before the current investigations, only insufficient information on chironomids was possible to find throughout hydrobiological studies based essentially on larval material (Pankratova, 1950; Kurmangalieva, 1974; Akhrorov, 1975, 1988; Kustareva and Ivanova, 1980). On the basis of a first imago and larval material collected during the eighties of the last century in rivers and streams of Tien Shan and Pamir mountains, one new genus of Podonominae (*Shilovia*) and six species belonging to three genera of the subfamily Diamesinae (including three undescribed species) were found (Makarchenko and Bulgakov, 1986; Makarchenko, 1989a-b; Makarchenko and Kobayashi, 1997; Makarchenko and Makarchenko, 2000). Some years later, additional taxonomic and systematics studies on the Diamesinae subfamily have been implemented on this important area of the Palaearctic Region. Based on a large fieldwork accumulated since 2000 by Dr. Dmitry Palatov (Moscow State University) who travelled along the Pamir and Tien Shan Regions, a material composed of adults, pupae and

larvae of Diamesinae have been examined using morphological studies and DNA barcoding allowed us to generate a list of 19 taxa/species: *Boreoheptagyia* sp., *Boreoheptagyia* sp. 1, *Diamesa bertrami* Edwards, *D. cinerella* Meigen, *D. filicauda* Tokunaga, *D. hamaticornis* Kieffer, *D. khumbugelida* Saether et Willassen, *D. planistyla* Reiss, *Diamesa akhrorovi* sp. nov., *D. alibaevae* sp. nov., *D. steinboeckii* Goetghebuer, *D. zelentzovi* Makarchenko, *D. zhiltzovae* Makarchenko, *Pagastia* aff. *lanceolata* Tokunaga, *Pagastia* sp. 1 Makarchenko, *Pagastia* sp. 2, *Pseudodiamesa* sp. (gr. *branickii*), *Pseudodiamesa* sp. (gr. *nivosa*), and *Shilovia rara* Makarchenko (currently transferred to Diamesinae subfamily).

Molecular lower-level phylogenetic research and DNA barcoding within family Chironomidae is considered as a beneficial approach for resolving the taxonomic status and evolution of chironomids (Ekrem *et al.*, 2007, 2010a, Montagna *et al.*, 2016, Makarchenko *et al.*, 2017). Multilocus approach using mitochondrial and nuclear markers showed its reliability for phylogenetic analysis (Ekrem *et al.*, 2010a; Cranston, 2010, 2012).

In the present paper two new species of the genus *Diamesa* Meigen, 1835 are described based on material collected from Tien Shan and Pamir mountains: *D. akhrorovi* sp. nov., as male adult; *D. alibaevae* sp. nov.,

as male adult and pupa. DNA barcoding results of the two new described species with comparative database on some related *Diamesa* species are also given. In addition, we used five commonly molecular markers COI, COII, 16S, 18S and EF-1a for estimating phylogenetic relationships of the studied *Diamesa* species. Descriptions of other new species will be published in separate papers.

METHODS

The material was preserved in 96% ethanol for DNA-analysis and in 70% ethanol for further study of morphology and slide-mounting, following the methods by Makarchenko (1985). The pupa and adult male were associated by using of DNA barcoding. The terminology follows Sæther (1980).

Holotypes and paratypes of the new species are deposited in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS).

Total genomic DNA was extracted from thorax of the 27 individual specimens of chironomids using the Invitrogen PureLink Genomic DNA Mini Kit in compliance with the manufacturer's protocols. Primers for PCR amplification on three mitochondrial and two nuclear markers given in Tab. S1. The PCR conditions and thermal regime given in Makarchenko *et al.* (2017), but the elongation time varied in accordance with the length of the amplicons. The PCR products were bidirectionally sequenced using the BigDye Terminator v3.1 cycle kit and run on an ABI 3130xl (Applied Biosystems™). Sequences were manually assembled and edited using Finch TV and MEGA 7 (Kumar *et al.*, 2016). The obtained sequences were checked aligned at the nucleotide level using MUSCLE (Edgar, 2004). The obtained alignment has one gap in 18S rRNA. To estimation the Kimura 2 parameter (K2P) distances we use MEGA 7 (Kumar *et al.*, 2016).

Bayesian phylogenetic analyses were conducted with MrBayes v. 3.2.6 (Ronquist and Huelsenbeck, 2003). PartitionFinder 2.1.1 (Lanfear *et al.*, 2012) was used to select the best-fit partitioning scheme and models separately for each codon position of protein coding genes as well as ribosomal loci using the greedy algorithm with linked branch lengths for the corrected Akaike Information Criterion as the optimality criterion for model selection. The best models for partitions given in Tab. S2. For the protein coding markers (COI, COII, EF-1a), we were convinced that there was no influence for the tree topology by saturation in third codon positions. The constructed NJ trees for 5 markers did not reveal a significant contradiction with the final tree.

Bayesian Inference was performed with two independent runs of Metropolis-coupled Markov chain Monte Carlo analyses, with each run comprising one cold

chain and three heated chains at a default temperature of 0.1. The chains were run for 10 million generations and sampled every 100 generations. A burn-in of 100 000 generations (or 1% of the sampled trees) was used. Moreover, trace files were visually inspected in Tracer 1.7 (Rambaut *et al.*, 2018). All the obtained sequences of chironomids given in Tab. S3.

DESCRIPTIONS

Diamesa planistyla Reiss

(Fig. 1 A,C,F,G).

Diamesa planistyla Reiss, 1968: 57, Fig. 2; Serra-Tosio, 1983:17.

Material. Five adult males, Kirgizstan, Chuy Region, Alamudun district, Tien Shan Mountains, Kirghiz Range, Adygene River, altitude 2109 m above sea level, 29.V.2016, N 42°34.247', E 74°28.649', leg. D. Palatov; 2 adult males, the same data, except for left tributary of the Ala-Archa River, 29.05.2016, leg. D. Palatov; 1 adult male, Xinjiang Uygur Autonomous Region of the Republic of China, Tien Shan Mountains, Bogdo-Ula Range, Zienzan River, altitude 2443 m above sea level, 10.VII.2017, N 43°49.738', E 88°10.351', leg. D. Palatov; 1 adult male, the same data except for Urumqi City District, Sangecha River, altitude 2356 m above sea level, 13.VII.2017, N 43°40.583', E 88°17.006', leg. D. Palatov.

Remarks. *D. planistyla* Reiss was described by Reiss (1968) from the Himalayas based on a single male specimen which did not have antennae. Later, Serra-Tosio (1983) redescribed this species from the same area on two male specimens, specifying that the antennae were with numerous but shortened setae, and gonostylus had only one, but not two, preapical megaseta. Also, the author noted that the preapical part of the gonostylus had a dark sclerotized protrusion. Serra-Tosio believed that a gonostylus with two megaseta is an aberration. We do not hold this opinion, because all males collected from the Tien Shan, which we refer to *D. planistyla*, have gonostylus with two megaseta, as in the description of Reiss (1968).

Comparing the Reiss and Serra-Tosio descriptions with our data on *D. planistyla* and new species close to it, we had concluded that Serra-Tosio most likely dealt with the one of new species we found. After receiving additional material from the Himalayas in the near future, we hope to solve the arisen taxonomic problem both at the morphological and molecular-genetic levels.

Taking in consideration the above data, we found it expedient to give the redescription of *D. planistyla* male according to our material.

Adult male (n=4). Total length: 3.6-4.3 mm. Total length/wing length 0.97-1.16. Colouration: body and legs brown to dark brown; wings grey to dark grey.

Head. Eyes reniform, hairy; macrotrichiae 16-20 μm long, protruding beyond facets. Temporal setae including (from one side): 2 coronals, 3-4 frontals, 12-13 orbitals, 6-12 verticals and 2-6 postorbitals. Clypeus with 4-6 setae. Antenna with 13 flagellomeres, developed or little reduced plume (Fig. 1 A); basal part of flagellomeres 1-12 and last flagellomere covered with setae 116-316 μm long and 48-168 μm long respectively; apex of ultimate

flagellomere with 3-3 setae 20-28 μm long. Length (in μm) of flagellomeres 1-13: 86-104, 36-40, 40-48, 36-48, 40-44, 36-44, 40-52, 48-52, 52-64, 40-52, 44-64, 40-64, 500-648; AR 0.94-1.02. Length (in μm) of palpomeres 1-5: 32-44, 84-140, 148-208, 116-132, 64-204; 3rd palpomere with sensilla capitata, diameter of 18-20 μm . Length of flagellomeres 1-13 /length of palpomeres 1-5: 1.81-1.86. Head width/palpomeres 1-5 length: 0.86-1.29.

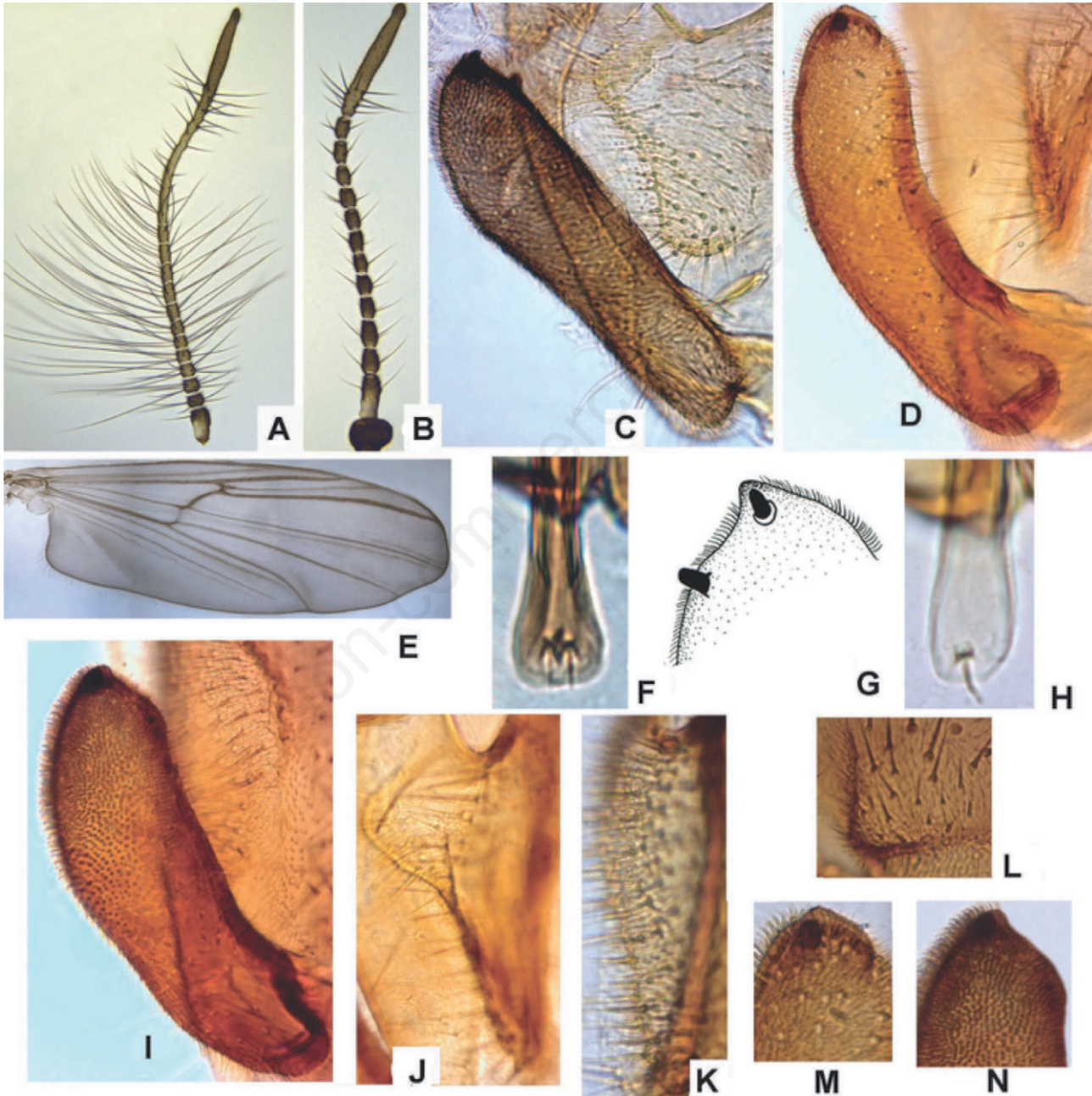


Fig. 1. Adult male of *Diamesa planistyla* Reiss (A,C,F,G), *Diamesa akhrorovi* sp. nov. (B,D,E,J,M) and *D. alibaevae* sp. nov. (H,I,K,L,N). A,B) antenna; C,D,I) gonostylus; E) wing; F,H) terminal setae of anal point; G) apical part of gonostylus; J,K) inferior volsellae; L) angular protuberance of gonocoxite; M,N) distal part of gonostylus.

Thorax. Anteprenotum with 6-8 lateral setae; dorsocentrals 9-12; prealars 5-6; scutellars 16-18 (in 2 rows).

Wing. Length 3.3-3.9 mm, width 1.0-1.2 mm. R and R₁ with 20-35 setae, R₄₊₅ with 6-10. Length RM/length MCu: 2.6-2.8. Anal lobe little reduced, truncated. Squama with 23-30 setae.

Legs. BR₁ 1.9-2.6, BR₂ 1.4-2.0, BR₃ 1.8-2.0. Spur of fore tibia 64-80 µm, spurs of middle tibia 48 µm and 48-60 µm, spurs of hind tibia 52-60 µm and 72-100 µm long. Hind tibial comb composed of 16 setae. Pulvilli absent. Claws falcated, with pointed apex. Lengths and proportions of leg segments as in Tab. 1.

Hypopygium (Fig. 1 C,F,G). Tergite IX semicircular, posterior margin distinctly bilobed on each side of the anal point; presence of 6-13 setae. Laterosternite IX with 1-4 short setae. Anal point 120-160 µm long (free distal part), long drop-like to pear-shaped distally, apex with 1-2 thin terminal setae 8-10 µm long (Fig. 1F). Sternapodeme linearly extended transversally in its oral part; strongly projecting orally to form a pointed triangle-like expansion in its median part, 92-120 µm height. Phallapodeme consists of a large plate as figured in description of Reiss (1968, Fig. 2). Gonocoxite 420-574 µm long. Superior volsella small blade-like, covered with short setae. Inferior volsella consists of 2 distinct dorsal lobes: proximal one rounded to lobe-like, distal one elongated downwards and tapering distally. Gonostylus 260-304 µm long, uniformly elongated and straight; with two megasetae 8-12 µm long, one of which placed preapically and second located proximally at distance of 28-80 µm and close to the inner edge (Fig. 1 C,G; Reiss, 1968, Fig. 2). HR 1.47-1.75.

Pupa and larva unknown.

Distribution. The species is known from the Himalayas and the Tien Shan.

***Diamesa akhrorovi* Makarchenko et Semenchko, sp. nov.**

(Fig. 1 B,D,E,J,M; Fig. 2 A,B).

Material. Holotype: adult male, Middle Asia, Tajikistan, Gorno-Badakhshan Autonomous Region, Shughnon District, Pamir Mountains, Maisara River near of Maisara Pass, 01.VII.2016, N 37°28.981', E

72°38.348', leg. D. Palatov. Paratypes: 1 adult male, the same data as holotype.

Etymology. The new species is named in honour and memory of the Tajik hydrobiologist and researcher of the of chironomid fauna of Pamir Dr. Feruz Akhrorov.

Adult male (n=2). Total length: 3.7 mm. Total length/wing length 1.09. Colouration: body largely brown to dark brown; wings grey to dark grey; femur of all legs brown yellowish, tibia and tarsus light brown.

Head. Eyes reniform, hairy; macrotrichiae 16-20 µm long, protruding beyond facets. Temporal setae including (from one side): 2 coronals, 3-4 frontals, 12-13 orbitals, 12 verticals and 2 postorbitals. Clypeus with 8-9 setae. Antenna with 13 flagellomeres and reduced plume (Fig. 1B); basal part of flagellomeres 1-12 and last flagellomere covered with short setae 56-92 µm long and 72-132 µm long respectively; apex of ultimate flagellomere with 6 setae 20-44 µm long. Length (in µm) of flagellomeres: 140, 56, 60, 60, 60, 52, 60, 52, 62, 52, 64, 54, 280; AR 0.36. Length (in µm) of palpomeres 1-5: 52, 96, 160, 124, 164; 3rd palpomere with sensilla capitata, diameter of 24 µm. Length of flagellomeres 1-13 /length of palpomeres 1-5: 1.77. Head width/palpomeres 1-5 length: 0.97.

Thorax. Anteprenotum with 4-5 lateral setae; dorsocentrals 8; prealars 4-5; scutellars 12-14.

Wing (Fig. 1E). Length 3.42 mm, width 0.8 mm. R and R₁ with 36 setae, R₄₊₅ with 22. Length RM/length MCu: 2.8. Anal lobe little reduced, truncated. Squama with 16 setae.

Legs. Spur of fore tibia 56 µm, spurs of middle tibia 60 µm both, spurs of hind tibia 60 µm and 94 µm long. Hind tibial comb composed of 17 setae. Fore leg with 1-2 apical pseudospurs on ta₁; mid and hind legs with 9-10 pseudospurs on ta₁; ta₄ cylindrical. Pulvilli absent. Claws falcated, with pointed apex. Lengths and proportions of leg segments as in Tab. 2.

Hypopygium (Fig. 1 D,J; Fig. 2 A,B). Tergite IX semicircular, slightly narrowing distally, posterior margin distinctly bilobed on each side of the anal point; presence of 20-23 setae placed on both median and posterior areas, setae located along the posterior margin are longer. Laterosternite IX with 2 short setae. Anal point 192 µm long (free distal part), long drop-like to pear-shaped distally, apex with 1-2 terminal setae 8-9 µm long and 3-4 µm wide (Fig. 2B). Sternapodeme linearly extended transversally in its oral part; strongly projecting orally to

Tab. 1. Lengths (in µm) and proportions of leg segments of male *Diamesa planistyla* Reiss (n=4).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P1	1443-1960	1509-1960	1017-1360	508-720	312-480	80-82	131-160	0.67-0.70	3.67-3.84	2.81-2.90
P2	1542-2040	1279-1760	656-840	328-460	213-280	80-82	131-160	0.48-0.51	4.61-4.73	4.20-4.52
P3	1680-2160	1560-2100	1200-1440	640-840	320-480	80-120	160	0.67-0.77	3.56-3.85	2.70-3.10

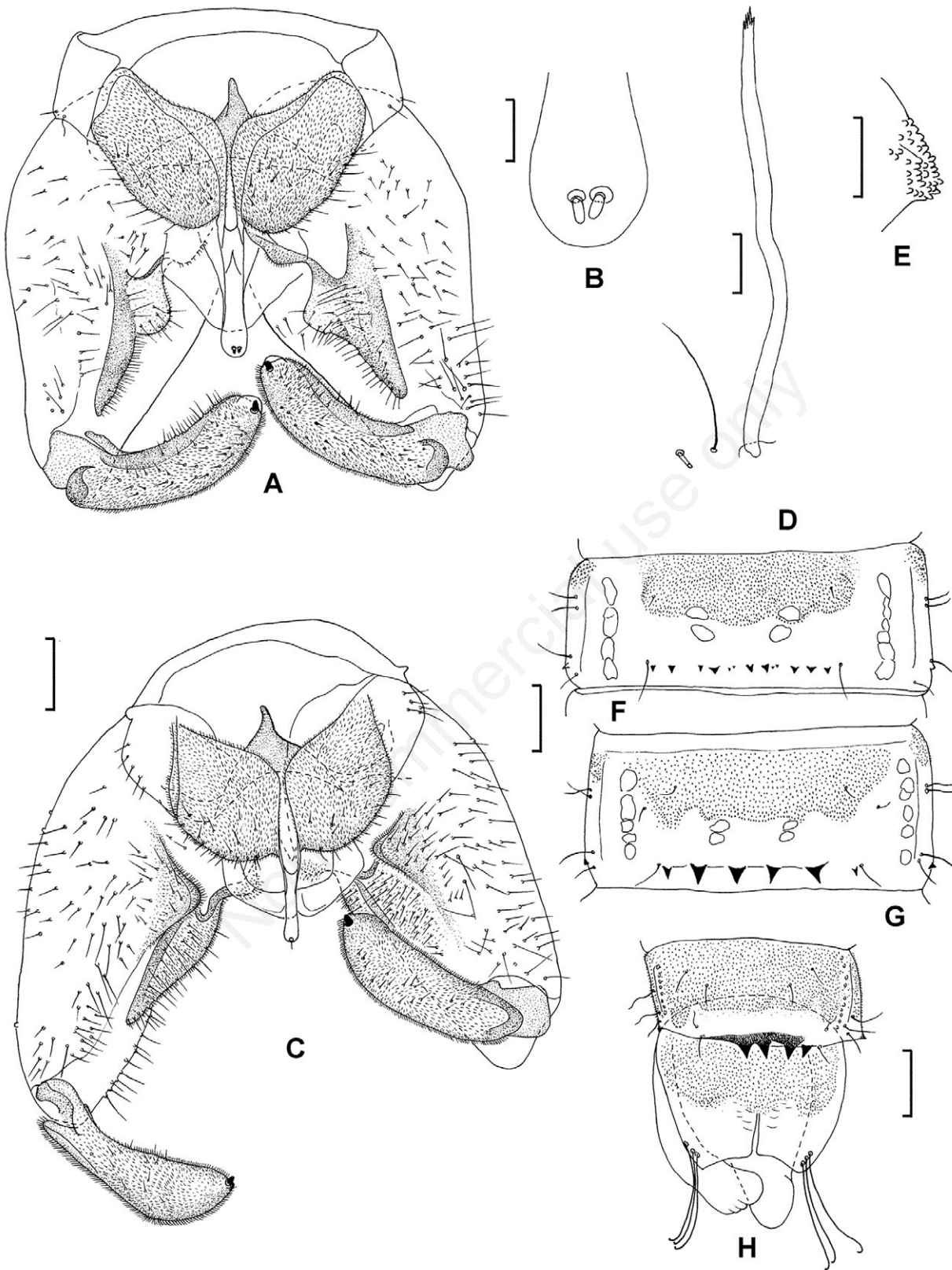


Fig. 2. Adult male (A-C) and pupa (D-H) of *Diamesa akhrovi* sp. nov. (A,B) and *D. alibaevae* sp. nov. (C-H). A,C) hypopygium in dorsal view; B) terminal setae of anal point; D) thoracic horn and precorneals; E) basal part of antennal case; F) tergite II; G) tergite IV; H) tergite VIII and anal segment. Scale bars: A,C) 100 μ m; B) 20 μ m; D,E) 50 μ m; F-H) 200 μ m.

form a pointed triangle-like expansion in its median part. Phallapodeme consists of a large plate as in *D. planistyla*. Gonocoxite 590 μm long. Superior volsella small blade-like, covered with short setae. Inferior volsella 312 μm long, consists of 2 distinct dorsal lobes: proximal one rounded to lobe-like, distal one elongated downwards and tapering distally; basal lobe covered with few thick setae, distal lobe densely covered with long thin setae (Fig. 1J; Fig. 2A). Gonostylus (Fig. 1 D,M; Fig. 2A) 288-316 μm long, uniformly elongated and slightly curved medially; apical part with a characteristic sclerotized hyaline and

bare plate of 28 μm long, placed close to the megaseta; preapical megaseta 14 μm long and strong. HR 1.89-2.05.

Pupa and larva unknown.

Diagnostic characters. *D. akhrorovi* sp. nov., is very closely related to *D. planistyla* Reiss and *D. alibaevae* sp. nov. from which it be separated based on the following combination of characters highlighted in the given key below, and additional DNA barcoding results illustrated in Fig. 3.

Tab. 2. Lengths (in μm) and proportions of leg segments of male *Diamesa akhrorovi* sp. nov. (n=1).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P1	1960	1960	1300	640	400	140	200	0.66	3.78	3.02
P2	2000	1740	880	480	300	120	160	0.51	4.39	4.25
P3	2240	2120	1360	800	400	120	160	0.64	3.86	3.21

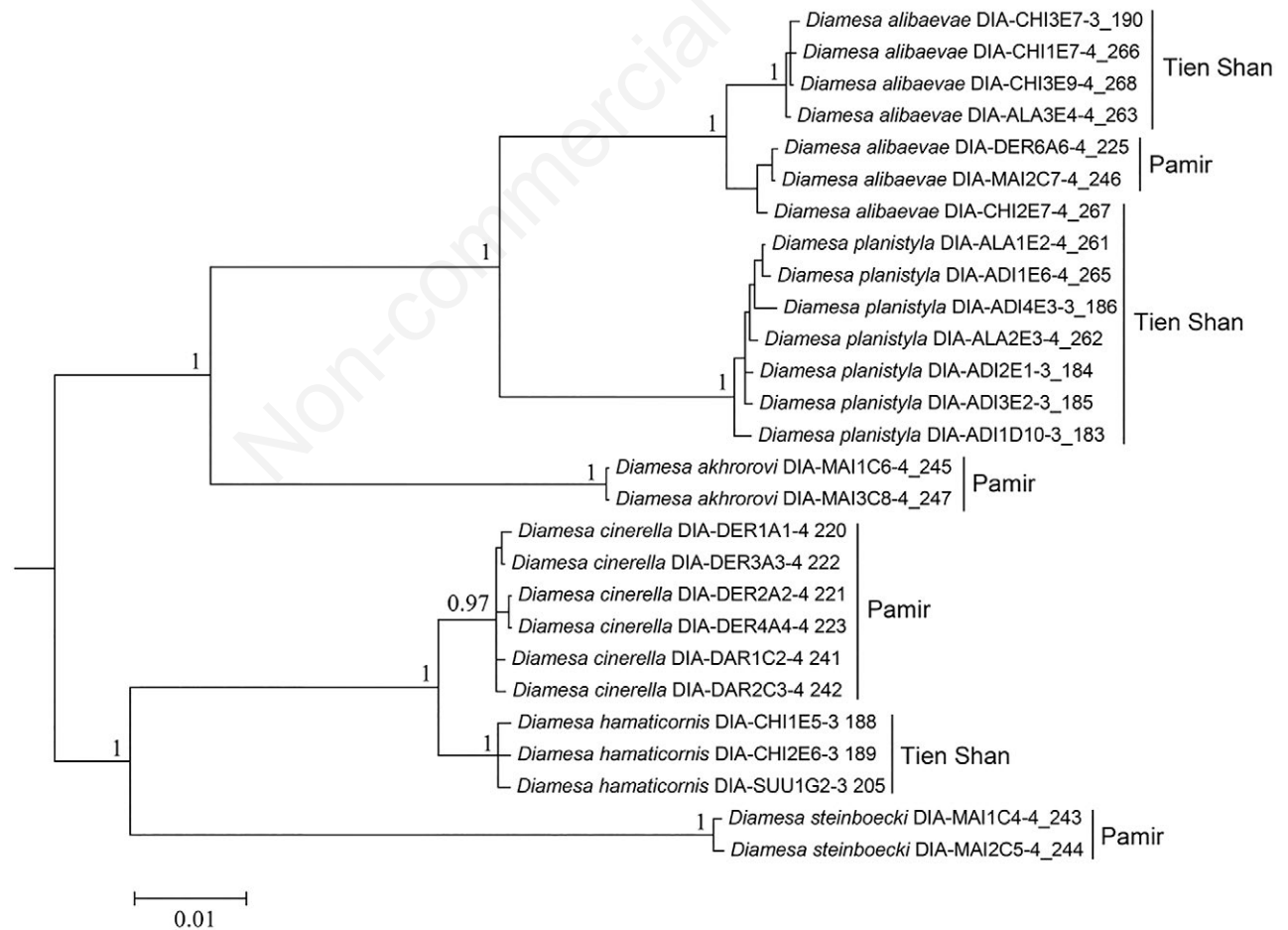


Fig. 3. Bayesian tree of the genus *Diamesa* Meigen from Tien Shan and Pamir on the mitochondrial COI, COII, 16S rRNA and nuclear 18S rRNA, EF-1a sequences (3550 bp). Bayesian posterior probabilities (PP) given in tree nodes.

Distribution. Known only from the type locality in Pamir Mountains.

***Diamesa alibaevae* Makarchenko et Semenchko, sp. nov.**

(Fig. 1 H,I,K,L; Fig. 2 C,H).

Material. Holotype: adult male, Middle Asia, Kirgizstan, Talas Region, Talas district, Tien Shan Mountains, Chychkan River, about 10-15 km below the pass of the Suusamyrl Valley, 2.VI.2016, N 42°11.937', E 72°59.380', leg. D. Palatov. Paratypes: 3 adult males, the same data as for holotype; 1 mature pupa, Kirgizstan, Chuy Region, Alamudun district, Tien Shan Mountains, Kirghiz Range, Adygene River (left tributary of the Ala-Archa River), 28.V.2016, N 42°34.247', E 74°28.649', leg. D. Palatov; 1 adult male, Tajikistan, Gorno-Badakhshan Autonomous Region, Shughnon District, Pamir Mountains, Maisara River near of Maisara Pass, 01.VII.2016, N 37°28.981', E 72°38.348', leg. D. Palatov.

Etymology. The new species is named in honour of Dr. Munovar Alibaeva who was wife of Dr. F. Akhrorov and studied benthos of Pamir rivers and lakes.

Adult male (n=4). Total length: 3.5-3.7 mm. Total length/wing length 0.97-1.1. Colouration: body largely brown to dark brown; wings grey to dark grey; legs light brown to brown.

Head. Eyes reniform, hairy; macrotrichiae 16-24 μ m long and protruding beyond facets. Temporal setae including (from one side): 2 coronals, 2-4 frontals, 5-14 orbitals, 5-9 verticals and 3-7 postorbitals. Clypeus with 4-6 setae. Antenna with 13 flagellomeres and reduced plume (as in *D. akhrorovi* sp. nov.); flagellomeres 1-12 66-132 μ m long, covered with short setae 40-88 μ m long in basal part; apex of ultimate flagellomere with 5-7 setae 18-40 μ m long. Length of flagellomeres 1-13 (μ m): 104-124, 48-60, 46-60, 44-52, 44-56, 40-50, 40-52, 44-50, 44-68, 44-52, 46-52, 36-48, 244-288; AR 0.36-0.43. Length of palpomeres 1-5 (μ m): 44-60, 92-116, 152-184, 100-124, 124-144; 3rd palpomere with sebsilla capitata diameter of 24-28 μ m. Length of flagellomeres 1-13/length of palpomeres 1-5: 1.58-1.63. Head width/palpomeres 1-5 length: 0.96-1.05.

Thorax. Anteprenotum with 5 lateral setae; dorsocentrals 8; prealars 3; scutellars 16.

Wing. Length 3.2-3.6 mm, width 0.88-1.12 mm. R and R₁ with 24-41 setae, R₄₊₅ with 12-17; Cu₂ occasionally with 1 seta in basal part. Length RM/length MCu: 2.0-2.7. Anal

lobe little reduced, truncated. Squama with 11-20 setae.

Legs. Spur of fore tibia 56-80 μ m, spurs of middle tibia 40-64 μ m both, spurs of hind tibia 62-100 μ m and 96-108 μ m long. Hind tibial comb composed of 14-20 setae. Fore leg with 2 apical pseudospurs on ta₁; mid leg with 4-6 pseudospurs on ta₁, hind leg with 6-10 pseudospurs on ta₁; ta₄ cylindrical. Pulvilli absent. Claws falcated, with pointed apex. Length (in μ m) and proportions of legs as in Tab. 3.

Hypopygium (Fig. 1 H,I,K,L,N; Fig. 2C). Tergite IX broadly semicircular, posterior margin slightly bilobed on each side of the anal point; presence of 16-18 nearly subequal setae placed on both median and posterior areas. Laterosternite IX with 9-12 short setae. Anal point 132 μ m long (free distal part), distal part long drop-like to pear-shaped; apex with 1 single thin terminal seta 12 μ m long (Fig. 1H; Fig. 2C). Sternapodeme and phallapodeme nearly as in *D. akhrorovi* sp. nov. Gonocoxite 582 μ m long, basal inner part with a characteristic angular protuberance (Fig. 1L). Superior volsella reduced. Inferior volsella 196 μ m long, unilobed, consists of a large lobe-like with narrowed distal part, densely covered with thin setae (Fig. 1K; Fig. 2C). Gonostylus 328 μ m long, distal part broadened; inner subapical margin with a small bulge; megasetae 12 μ m long, tooth-like and placed apically (Fig. 1 I,N; Fig. 2C). HR 1.77.

Pupa (n=1). Total length 3.9 mm. Colouration contrasting brown yellowish to dark brown.

Cephalothorax. Frontal apotome with 2 setae 160-172 μ m long. Thorax granulated in both anterior and posterior parts, granulation are smooth and less dense in posterior area. Thoracic horn light yellow, filiform, 348 μ m long, with few small spines apically (Fig. 2D); length (μ m) of precorneal setae: Pc₁ - 104, Pc₂ - 184. Anteprenotum with 3 anteprenotals 17-24 μ m long. Dorsocentrals 2 180 μ m long. Base of antennal sheath distinctly granulated (Fig. 2E).

Abdomen. Tergite I bare. Tergites II-VIII with shagreen and field of spinules in both anterior and lateral parts; posteromedian transverse rows of strong horn-like thick spines present on tergites II-VIII; number of spines by tergite is respectively: 9, 9, 6, 7, 6, 7, 4; size of triangle-like spines becoming gradually bigger from tergites II to VIII (Fig. 2F-H). Posteromedian rows of spines present only on sternites III-VIII; number of spines by sternite is respectively: 10, 10, 16, 14, 7, 9. Spines on tergites almost twice as large as those on

Tab. 3. Lengths (in μ m) and proportions of leg segments of male *Diamesa alibaevae* sp. nov. (n=4).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV
P1	1560-1920	1440-1920	980-1240	480-640	280-500	120	160	0.65-0.72	3.43-3.85	2.98-3.24
P2	1640-2160	1280-1680	680-920	320-460	220-280	80-120	120-160	0.53-0.56	4.67-4.86	4.10-4.33
P3	1720-2200	1560-1960	1120-1400	560-760	320-400	100-120	120-160	0.70-0.72	3.68-3.93	2.93-3.09

sternites. Tergite IX with field of shagreen and spinules present on anterior half and laterally. Distribution of lateral setation on segments I-VII is as follows: I with 2 pairs of setae 60 µm long, II-VII with 4 pairs; length of LS₁-LS₃ 88-136 µm. Segment VIII with 3 pairs of lateral setae 33-88 µm long, located as in Fig. 8; dorsal setae length 68-128 µm. Anal lobe with 3 needle-like macrosetae 216-260 µm long (Fig. 2H).

Larva unknown.

Diagnostic characters. *D. alibaevae* sp. nov. is keyed near both *D. planistyla* and *D. akhrorovi* sp. nov., from which it can be distinguished based on the combination of characters provided as well in the given key below and results of DNA barcoding illustrated in Fig. 3.

Distribution. Known only from the type locality in Tien Shan and Pamir Mountains.

Key to adult males of related known *Diamesa* species to *D. planistyla* Reiss

1. Antennal plume developed or little reduced (Fig. 1A). Gonostylus with two megasetae (one placed preapically and second located proximally close to the inner edge (Fig. 1 C,G; Reiss, 1968, Fig. 2). Anal point with 1-2 thin terminal setae (Fig. 1F) ... *D. planistyla* Reiss
 - Antennal plume reduced (Fig. 1B). Gonostylus with only one terminal megaseta. Anal point with 1 thin or 1-2 thicker terminal setae ... 2
2. Inferior volsella consists of 2 lobes, from which proximal rounded, distal larger and elongated (Fig. 1J; Fig. 2A). Base of gonocoxite without a distinct protuberance. Gonostylus slightly curved medially, in subapical part with megasetae and chitinized naked plate (Fig. 1 D,M). Anal point with 1-2 wide terminal setae (Fig. 2B) ... *D. akhrorovi* sp. nov.
 - Inferior volsella unilobed, consists of only one large lobe (Fig. 1K; Fig. 2C). Base of gonocoxite with a distinct protuberance (Fig. 1L; Fig. 2C). Gonostylus slightly expanding in its distal part, with only 1 subapical megaseta and tooth, apical area with a slight bulge placed close to the megaseta (Fig. 1 I,N). Anal point with 1 thin terminal seta (Fig. 1H) ... *D. alibaevae* sp. nov.

RESULTS OF DNA BARCODING AND DISCUSSION

The length of aligned sequences given in Tab. S1. Genetic marker COII contains complete COII sequence in front of which 41-42 bp of tRNA-Leu and finally 15-

17 bp of tRNA-Lys. The gene EF-1a in most *Diamesa* was 898 bp, but two specimens of *D. steinboeckii* had an 81 bp intron at the end of EF-1a marker. The tRNA sequences as well as intron in EF-1a were excluded from Bayesian analysis. A final multilocus dataset of 3550 bp, of which 302 were parsimony informative.

We used Bayesian phylogenetic analysis to present *Diamesa* relationships from Tien Shan and Pamir (Fig. 3). In the basis of the constructed tree, two sister groups were formed, *D. cinerella* + *D. hamaticornis* + *D. steinboeckii* and *D. alibaevae* + *D. planistyla* + *D. akhrorovi*. Species *D. cinerella* + *D. hamaticornis* were close however monophyly of this groups was supported (PP=1). Similarly, described species *D. akhrorovi* sp. nov. and *D. alibaevae* sp. nov. united with *D. planistyla* formed a highly supported monophyletic groups (PP=1). Species *D. alibaevae* sp. nov. lives both in the Pamir and in the Tien Shan and has insignificant intraspecific variations.

Combining phylogenetic analysis and DNA barcoding allows to effectively species-level identification approach (Ekrem *et al.*, 2007, 2010b; Montagna *et al.*, 2016). For DNA barcoding, we used only 658 bp fragment of the mitochondrial gene cytochrome c oxidase I (COI) according to Hebert *et al.* (2003). The average interspecific genetic distance based on Kimura 2 parameter (K2P) model found in Tien Shan and Pamir species of the genus *Diamesa* was 0.095 which concordance to Montagna *et al.* (2016).

The K2P sequence divergence between *Diamesa akhrorovi* sp. nov. and other species of the genus *Diamesa* from Pamir and Tien Shan (*D. alibaevae*, *D. cinerella*, *D. hamaticornis*, *D. planistyla*) was 0.102 - 0.115 (Tab. S4). The species independence of *D. alibaevae* sp. nov. on *D. planistyla* determined by 0.083 K2P sequence divergence. Distances between *D. cinerella* + *D. hamaticornis* was 0.023 which is at the lower level of species independence (Montagna *et al.*, 2016). According to Montagna *et al.* (2016), *D. cinerella* and *D. tonsa* are closely related species and *D. hamaticornis* probably also includes to *D. cinerella* / *D. tonsa*.

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New data on taxonomy and systematics of the genus *Diamesa* Meigen (Diptera: Chironomidae: Diamesinae) from Tien Shan and Pamir Mountains, with description of two new species

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Tab. S1. PCR primers and length of aligned sequences for three mitochondrial (COI, COII, 16S rRNA) and two nuclear (18S rRNA, EF-1a) markers.

Locus	Forward primer	Reverse primer	Reference	Length of aligned sequences used in analyses (bp)
COI	COIF-ALT: ACAAATCAYAARGAYATYGG	COIR-ALT TTCAGGRTGNCCRAARAAYCA	Mikkelsen <i>et al.</i> , 2006	658
COII	Mtd13-mod: AATATGGCAGAATAGTGCAA	Mtd20-mod TGGTTAAGAGACCATTACTTG	Ekrem <i>et al.</i> , 2010	754
16S rRNA	LR-J-12887: CCGGTTTGAACCTCAGATCATGT	LR-N-13398 CACCTGTTTATCAAAAACAT	Simon <i>et al.</i> , 1994	510
18S rRNA	18S_Fw1_O: CTTGTCTCAAAGATTAAGCCAT	18S_Rw1_917 CGAGRTCCTATTCCATTATTCC	Own production	798
EF-1a	Efs_149_Cardio: AAGGAAGCACAAAGAAATGG	Efa_1043_Cardio: CCGTTTGAAATTTGTCCTGG	Ekrem <i>et al.</i> , 2010 with modifications	978

Tab. S2. The best models for the partitions used in Bayesian Inference.

Partition names	Best Model	Combined Length, bp	Reference
COI_pos3, COII_pos3	GTR+G	448	Tavaré, 1986
COI_pos1, COII_pos1	GTR+I	447	
EF-1a_pos3	GTR+G	300	
COI_pos2	F81+I	219	Felsenstein, 1981
COII_pos2,	F81	228	
EF-1a_pos1	F81	300	
EF-1a_pos2	F81+I	299	
16S_rRNA	HKY+I	511	Hasegawa <i>et al.</i> , 1985)
18S_rRNA	HKY+I	799	

Tab. S3. List of taxa, samples ID and GenBank accessions.

Taxa	Sample ID:	COI	COII	16S rRNA	18S rRNA	EF-1a
1. <i>Diamesa planistyla</i>	DIA-ADI1D10-3_183	MG913444	MG913460	MG913390	MG913417	MG913487
2. <i>D. planistyla</i>	DIA-ADI2E1-3_184	MG913445	MG913461	MG913391	MG913418	MG913488
3. <i>D. planistyla</i>	DIA-ADI3E2-3_185	MG913446	MG913462	MG913392	MG913419	MG913489
4. <i>D. planistyla</i>	DIA-ADI4E3-3_186	MG913447	MG913463	MG913393	MG913420	MG913490
5. <i>D. hamaticornis</i>	DIA-CHI1E5-3_188	KY640389	MG913464	MG913394	MG913421	MG913491
6. <i>D. hamaticornis</i>	DIA-CHI2E6-3_189	KY640387	MG913465	MG913395	MG913422	MG913492
7. <i>D. alibaevae</i>	DIA-CHI3E7-3_190	MG913448	MG913466	MG913396	MG913423	MG913493
8. <i>D. hamaticornis</i>	DIA-SUU1G2-3_205	KY640388	MG913467	MG913397	MG913424	MG913494
9. <i>D. cinerella</i>	DIA-DER1A1-4_220	KY640394	MG913468	MG913398	MG913425	MG913495
10. <i>D. cinerella</i>	DIA-DER2A2-4_221	KY640390	MG913469	MG913399	MG913426	MG913496
11. <i>D. cinerella</i>	DIA-DER3A3-4_222	KY640395	MG913470	MG913400	MG913427	MG913497
12. <i>D. cinerella</i>	DIA-DER4A4-4_223	KY640391	MG913471	MG913401	MG913428	MG913498
13. <i>D. alibaevae</i>	DIA-DER6A6-4_225	MG913449	MG913472	MG913402	MG913429	MG913499
14. <i>D. cinerella</i>	DIA-DAR1C2-4_241	KY640392	MG913473	MG913403	MG913430	MG913500
15. <i>D. cinerella</i>	DIA-DAR2C3-4_242	KY640393	MG913474	MG913404	MG913431	MG913501
16. <i>D. steinboecki</i>	DIA-MAI1C4-4_243	KY640397	MG913475	MG913405	MG913432	MG913502
17. <i>D. steinboecki</i>	DIA-MAI2C5-4_244	KY640398	MG913476	MG913406	MG913433	MG913503
18. <i>D. akhrorovi</i>	DIA-MAI1C6-4_245	MG913450	MG913477	MG913407	MG913434	MG913504
19. <i>D. alibaevae</i>	DIA-MAI2C7-4_246	MG913451	MG913478	MG913408	MG913435	MG913505
20. <i>D. akhrorovi</i>	DIA-MAI3C8-4_247	MG913452	MG913479	MG913409	MG913436	MG913506
21. <i>D. planistyla</i>	DIA-ALA1E2-4_261	MG913453	MG913480	MG913410	MG913437	MG913507
22. <i>D. planistyla</i>	DIA-ALA2E3-4_262	MG913454	MG913481	MG913411	MG913438	MG913508
23. <i>D. alibaevae</i>	DIA-ALA3E4-4_263	MG913455	MG913482	MG913412	MG913439	MG913509
24. <i>D. planistyla</i>	DIA-ADI1E6-4_265	MG913456	MG913483	MG913413	MG913440	MG913510
25. <i>D. alibaevae</i>	DIA-CHI1E7-4_266	MG913457	MG913484	MG913414	MG913441	MG913511
26. <i>D. alibaevae</i>	DIA-CHI2E7-4_267	MG913458	MG913485	MG913415	MG913442	MG913512
27. <i>D. alibaevae</i>	DIA-CHI3E9-4_268	MG913459	MG913486	MG913416	MG913443	MG913513

Tab. S4. Between Kimura 2 parameter nucleotides mean distances (under the diagonal) and standard error estimates (above the diagonal) of *Diamesa* obtained in this study (bold) and from GenBank.

<i>Diamesa</i> species	1	2	3	4	5	6	7	8	9	10
1. akhrorovi		0.011	0.011	0.011	0.014	0.012	0.014	0.012	0.014	0.013
2. alibaevae	0.102		0.010	0.010	0.012	0.011	0.011	0.012	0.011	0.011
3. <i>bertrami</i>	0.106	0.084		0.009	0.012	0.010	0.011	0.010	0.011	0.009
4. cinerella	0.100	0.072	0.091		0.011	0.005	0.012	0.013	0.010	0.006
5. <i>latitarsis</i>	0.119	0.087	0.098	0.087		0.011	0.014	0.013	0.011	0.011
6. hamaticornis	0.103	0.079	0.094	0.020	0.088		0.012	0.013	0.011	0.004
7. planistyla	0.115	0.078	0.087	0.097	0.106	0.098		0.014	0.013	0.012
8. <i>simplex</i>	0.108	0.100	0.088	0.101	0.118	0.106	0.116		0.012	0.012
9. <i>steinboeckii</i>	0.103	0.099	0.091	0.084	0.092	0.085	0.104	0.107		0.011
10. <i>tonsa</i>	0.105	0.083	0.093	0.027	0.092	0.013	0.098	0.106	0.089	

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