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Confirmation of a species status and redescription with DNA barcoding of *Pagastia* (s. str.) *angarensis* (Linevich, 1973), stat. resurr. (Diptera: Chironomidae: Diamesinae) from the Russian Far East and East Siberia

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The species *Pagastia* (*Pagastia*) *angarensis* (Linevich) was described as *Syndiamesa angarensis* Linevich based on a larva from the Angara River of the Baikal Lake basin (Linevich 1953). Later, the adult female, pupal, and larval stages were described from the same locality (Linevich 1984; Linevich *et al.* 1991); however, the adult male remained unknown. The male was first reared from mature pupae collected in the Yenisey River in 1988 by L.V. Bazhina and the Volchanka River (Southern Primorye) in 1991 by E.A. Makarchenko. The structure of the hypopygium of the obtained males, characteristics of pupae and larvae, turned out to be very similar to *P.* (*P.*) *lanceolata* (Tokunaga, 1936) from Japan. As a result *P.* (*P.*) *angarensis* was identified as *P.* (*P.*) *lanceolata* and consequently synonymized with this species (Makarchenko & Makarchenko 1999, 2000a,b; Linevich *et al*. 2002; Makarchenko 2006; Ashe & O'Connor 2009).

Previously, Makarchenko *et al.* (2021) used DNA barcodes of specimens identified as *P.* (*P.*) *lanceolata* from the Russian Far East, as well as *Pagastia* aff. *lanceolata* from India and Tajikistan (Makarchenko *et al.* 2021), the taxonomic status of which has not changed to date. After the deposition of DNA barcodes of the adult male of *P.* (*P.*) *lanceolata* from Yunnan Province, China (GenBank accession number OM302510) and the adult female from Kunashir Island (Greater Kuril Chain, BOLD Process ID KUNA081-17), the species status of the Far Eastern samples was questioned due to the significant distances between each of them. Moreover, the type habitat of *P.* (*P.*) *lanceolata* is located in Japan (Tokunaga, 1936), which is geographically closer to Kunashir Island than to the mainland of the Russian Far East.

In 2025, Dr. Kazuo Endo provided us with imaginal material of *P.* (*P.*) *lanceolata* that he collected in Japan, including specimens from an area that is not far from the type locality of the species (Kibune Stream of Kyoto botanical garden). This was to conduct a morphological and genetic comparison of populations of *P.* (*P.*) *lanceolata* from Japan and Eastern Siberia, and the Russian Far East. High interspecific distances between these populations demonstrated that they are independent species and justify the restoration of *P.* (*P.*) *angarensis*.

Below, we present a redescription of the adult male of *P.* (*P.*) *angarensis* with a justification for the restoration of its species status based on morphological and molecular evidence. We also found it appropriate to make a brief redescription of the male *P.* (*P.*) *lanceolata* based on our material.

Materials and methods

Adults were preserved in 96% ethanol for the study of morphology and DNA barcoding and were slide-mounted in Euparal. The morphological terminology and abbreviations used below generally follow Sæther (1980). The photographs were taken using an Axio Lab.A1 (Karl Zeiss) microscope with an AxioCam ERc5s digital camera, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® software.

Total genomic DNA was isolated from seven specimens of *P.* (*P.*) *angarensis* and two specimens of *P.* (*P.*) *lanceolata* using a Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany) or ExtractDNA Blood & Cells (Evrogen, Moscow, Russia). Fragments of the cytochrome c oxidase subunit I (COI-5P) were amplified using 5X ScreenMix-HS DNA polymerase (Evrogen, Moscow, Russia) and primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Amplification

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products were purified by exonuclease I (ExoI) and alkaline phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA) and bidirectionally sequenced by ABI 3500 sequencer (Applied Biosystems) using reagents BigDye terminator v3.1 cycle kit. More details about the PCR regime and sequence can be found in Makarchenko *et al.* (2024). Intra- and interspecific genetic divergence values were calculated using K2P distances implemented in MEGA7 software (Kumar *et al.* 2016). The obtained sequences have been deposited in GenBank under accession numbers PX260992–PX261000.

For the calculation of intra- and interspecific genetic divergence, we used all available sequences of from the *Pagastia* (*P.*) *lanceolata* group in the Barcode of Life Data System and GenBank (accessed on 04 September 2025). From several available sequences of *P.* (*P.*) *orthogonia* Oliver we left one and added to it closely related species *Pagastia* sp. (BIN BOLD:AAP6893). Species delimitation for the obtained dataset followed a distance-based approach using Assemble Species by Automatic Partitioning (ASAP, Puillandre *et al.* 2021) with p-distances.

Bayesian analysis was carried out on the obtained dataset in MrBayes v3.2.7 (Ronquist *et al.* 2012) using Markov Chain Monte Carlo (MCMC) randomization. Four Markov chains (three heated chains, one cold) were run for 5 million generations, with the first 25% of sampled trees discarded as burn-in. Strict clock model (brlenspr=clock: uniform) were used to obtain an ultrametric tree. 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 the COI gene.

Material from this study is deposited in the Bioresource Collection (reg. number 2797657) of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS).

Description

Pagastia (*Pagastia*) *angarensis* (Linevich, 1953), stat. resurr.

(Figs 1–2, 5–8)

Syndiamesa angarensis Linevich, 1953:162.

Potthastia angarensis (Linevich); Linevich 1984:127, Linevich *et al.* 1991: 227.

Pseudodiamesa (*Pagastia*) *oliveri* Makarchenko, 1989: 270.

Pagastia (*Pagastia*) *lanceolata* (Tokunaga, 1936); Makarchenko & Makarchenko 1999: 237, 2000a: 190, 2000b: 173; Erbaeva 2000: 13; Linevich *et al*. 2002: 59; Makarchenko 2006: 271.

Pagastia angarensis (Linevich); Linevich & Makarchenko 1989: 24; Makarchenko 1994: 832.

Material examined. ***Russian Far East:*** 1 adult male, Primorye Territory, Terneiskyi District, Sikhote-Alin' Nature Reserve, Jasnaja River,11.IX.1983, leg. E. Potikha; 3 adult males, 3 pupae, 12 larvae, Partisansk District, Volchanka River, 5.V.1991, leg. E. Makarchenko, males were reared from larvae; 1 adult male the same data except Litovka River, 5.V.1991, leg. E. Makarchenko; 2 larvae, the same data, except Tigrovaya River, 8.IV.2025, N 43.303457,E 133.052454, leg. E. Gorovaya. 2 adult males, Khabarovsk Territory, Nanaisky District, Anyuisky National Park, Pihtsa River (tributary of Gassi Lake, Amur River basin), 23–24.V.2019, N 48.796733, E 136.783783, 26.V.2020, leg. N. Yavorskaya; 1 adult male, the same data, except 26.V.2020, leg. N. Yavorskaya; 1 adult male, the same data, except Mulchi River, N 48.51.418 E 136.47.433, 29.V.2020, leg. N. Yavorskaya. ***East Siberia:*** 3 mature pupae, Krasnoyarsk Territory, Severo-Eniseisk District, Enisey River basin, Bolshoi Pit River, 10.VIII.1988, leg. L. Bazhina; 10 larvae, vicinity of the of Krasnoyarsk City, Sverdlovskij District, Bazaikha Village, Bazaikha River of Enisey River basin, N 55.967589, E 92.820094, 21.VI.2025, leg. N. Kislitsina.

Adult male (n = 4).

Total length 3.4–4.6 mm. Total length/wing legs 1.1–1.26.

Coloration. Head, mesonotum, legs, and abdomen brown to dark brown; scutellum grey; antennae light brown; wings greyish.

Head. Eyes bare and strongly extended dorsomedially. Temporal setae including 4 coronals, 5–6 preoculars, 6–11 verticals, and 14–15 postorbitals. Clypeus with 15–21 setae. Antenna with 13 flagellomeres and developed plume, these setae 754–984 µm long; pedicel with 2–3 setae 60–68 µm long; terminal flagellomere with 1 subapical seta 50–52 µm long. AR 2.03–2.1. Palpomeres lengths (in µm): 40; 84; 140; 180; 172. Palpomere 3 in distal part with sensilla capitata (diameter 12 µm). Palpomeres 1–5 length/head width 1.06.

Thorax. Antepronotum with 3–11 median antepronotals, 40–80 µm long, and 4–11 lateral antepronotals, 64–92 µm long. Acrostichals 20–31 (40–60 µm long), dorsocentrals 18–29 (in 1 row in anterior 2/3 and in 2 rows in posterior 1/3), prealars 10–20, scutellars 37–60 in 3 rows.

Wing. Length 3.6–4.0 mm; width 0.9 mm. Membrane without macrotrichiae, only with microtrichiae. R and R_1 with 40–48 setae; R_{4+5} with 19–26 setae. Costa extension 66–82 µm long. RM length/MCu length 3.2. Anal lobe developed, outline rounded. Squama with 35–50 setae, 88–136 µm long.

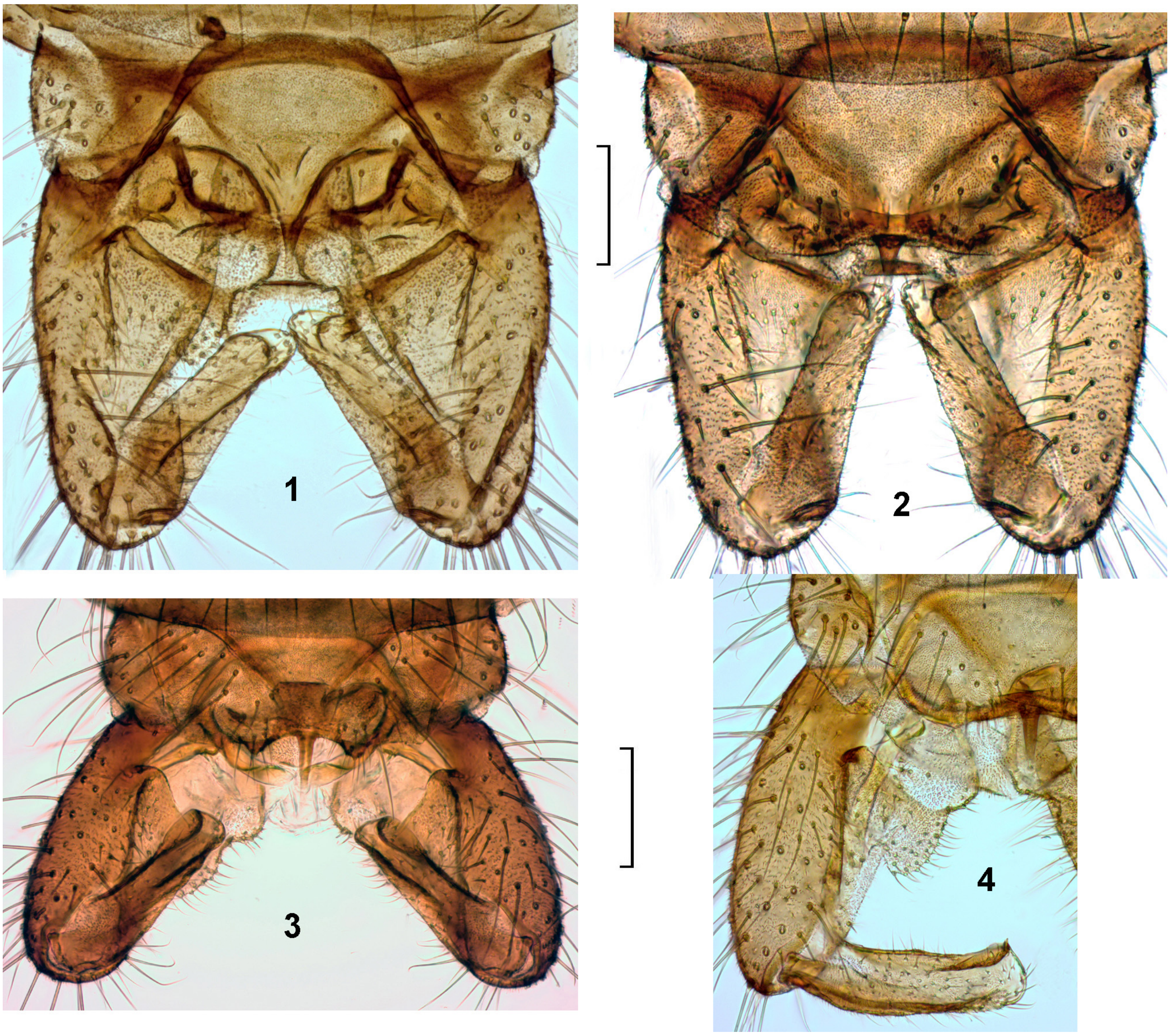
Legs. Spur of fore tibia 88–92 µm long; spurs of mid tibia 60–64 µm and 64–68 µm; of hind tibia 104–108 µm and 64–72 µm long. Hind tibial comb with 12–15 setae. Lengths and proportions of leg segments as in Table 1.

TABLE 1. Lengths (in µm) and proportions of leg segments of *Pagastia* (*P.*) *angarensis* (Linevich), male (n = 4).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1012–1263	1181–1607	918–1099	426–558	295–344	164–180	148–164
P_2	1099–1394	1132–1443	590–640	344–361	213–246	131–148	131–148
P_3	1345–1574	1689–1755	918–951	476–492	295–328	148–164	148–164

TABLE 1. (Continued)

	LR	BV	SV	BR
P_1	0.68–0.72	2.50–3.27	2.27–2.66	3.2–3.4
P_2	0.44–0.51	3.62–3.98	3.74–4.43	3.2–3.4
P_3	0.52–0.56	3.70–3.73	3.19–3.63	3.4–3.6



FIGURES 1–4. Total view of adult male hypopygium of *Pagastia* (*P.*) *angarensis* (Linevich) from the Amur River basin (Russian Far East) **(1–2)** and *P.* (*P.*) *lanceolata* (Tokunaga) from Honshu (Japan) **(3–4),** in dorsal view. Scale bars: 50 µm.

Hypopygium (Figs 1–2, 5–8,). Tergite IX with 13–18 setae on each side, 52–108 µm long and with triangular anal point, 56–88 µm long, without apical peg. Laterosternite IX with 7–14 setae, 92–120 µm long. Transverse sternapodeme narrow, anterior margin nearly straight, 168–220 µm long µm long (Fig. 5). Phallapodeme, 84–88 µm long, with lateral aedeagal lobe only, which 88–96 µm long, lanceolate (Fig. 5). Gonocoxite 256–300 µm long, with basal plate and lobe-like median field. Gonostylus 162–204 µm long, expanded basally and along the outer edge with angular protrusion; megaseta 8–12 µm long (Figs 1–2, 6–8). HR 1.43–1.52.

Remarks. The values of most morphological features of *P.* (*P.*) *angarensis* and *P.* (*P.*) *lanceolata* overlap and the main difference between these two species is the shape of the gonostylus, which in males of *P.* (*P.*) *angarensis* is expanded basally and has an angular protrusion along the inner edge (Figs 1–2, 6–8), while in *P.* (*P.*) *lanceolata* gonostylus is approximately the same thickness along its entire length, not expanded basally and without a protrusion (Figs 3–4, 9–12). The pupae and larvae of these species are indistinguishable.

Distribution. Known from the Russian Far East, East Siberia, and Altai Mountains (Koveshnikov 2016).

Pagastia lanceolata (Tokunaga)

(Figs 3–4, 9–12)

Syndiamesa (*Syndiamesa*) *lanceolata* Tokunaga, 1936: 530.

Pagastia lanceolata (Tokunaga); Hashimoto 1985: 347; Endo 2004: 284; Ashe & O'Connor 2009: 294.

Syndiamesa (*Lasiodiamesa*) *crassipilosa* Tokunaga 1937: 57.

Pseudodiamesa crassipilosa (Tokunaga); Sasa 1989: 64.

Material examined. ***Japan:*** 1 adult male, Honshu, Niigata Prefecture, Kanose-machi, Sanegawa River basin, 18.V.2004, leg. K. Endo; 3 adult male, Honshu, Gunma Prefecture, Kasukawa-mura, Akagi Mount, Fudo-otaki, 8.IV.2001, leg. K. Endo; 2 adult females, Honshu, Yamagata Prefecture, Asahi-machi, Asahi River basin, 19.V.2004, leg. K. Endo; 7 adult males, 3 females, Honshu, Ikawa, Shizuoka City, 15. IX.1996–5. I.1997, leg. H. Niitsuma. All adults were raised from larvae and pupae by H. Niitsuma.

Adult male (n = 2).

Total length 4.0–4.4 mm. Total length/wing legs 1.12–1.13.

Coloration. Head, thorax, legs, and abdomen brown to dark brown; antennae light brown; wings greyish.

Head. Eyes bare and strongly extended dorsomedially. Temporal setae including 4 coronals, 8 preoculars, 10 verticals. Clypeus with 20–24 setae. Antenna with 13 flagellomeres and developed plume, these setae *ca* 900 µm long; pedicel with 3 setae 52–60 µm long; terminal flagellomere with 1 subapical seta 48–52 µm long. AR 1.79.–1.93. Palpomeres lengths (in µm): 44–48; 96–104; 204–216; 260; 268–280. Palpomere 3 in distal part with sensilla capitata (diameter 12 µm). Palpomeres 1–5 length/head width 0.81.

Thorax. Antepronotum with 8–10 median antepronotals, 40–44 µm long, and 7–8 lateral antepronotals, 50–60 µm long. Acrostichals 25–29 (68–80 µm long), dorsocentrals 19–28 (in 1 row in anterior 2/3 and in 2 rows in posterior 1/3), prealars 14–17, scutellars 56–60 in 3–4 rows.

Wing. Length 3.56–3.88 mm; width 0.96–1.04 mm. Membrane without macrotrichiae, only with microtrichiae. R and R_1 with 56–77 setae; R_{4+5} with 25–26 setae. Costa extension *ca* 60 µm long. RM length/MCu length 2.5. Anal lobe developed, outline rounded. Squama with 50–51 setae, 104–144 µm long.

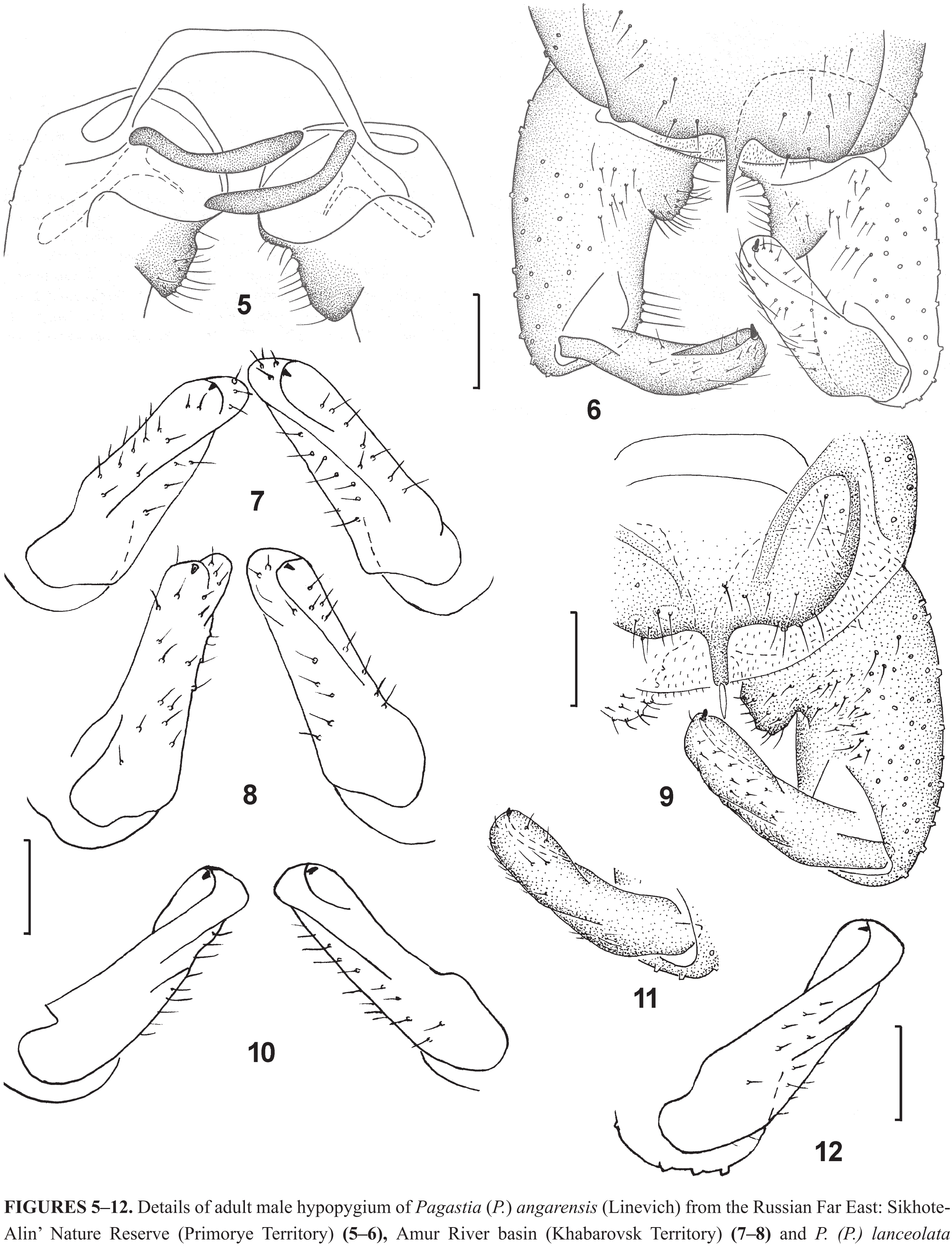
Legs. Spur of fore tibia 96 µm long; spurs of mid tibia 64–68 µm and 64–68 µm; of hind tibia 88–100 µm and 64–80 µm long. Hind tibial comb with 10 setae. Lengths and proportions of leg segments as in Table 2.

TABLE 2. Lengths (in µm) and proportions of leg segments of *Pagastia* (*P.*) *lanceolata* (Tokunaga) (n = 2).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5
P_1	1328–1361	1558–1689	1263–1296	623–640	426–443	197	164
P_2	1410–1492	1509–1624	738	377–410	279	131–148	131–164
P_3	1574–1640	1837–2000	1115–1160	574	344–361	148–164	148–164

TABLE 2. (Continued)

	LR	BV	SV	BR
P_1	0.76–0.81	2.87–3.08	2.29–2.35	2.4–2.5
P_2	0.45–0.49	3.85–3.98	3.96–4.22	2.8
P_3	0.58–0.61	3.68–3.85	3.06–3.14	3.7–3.9



FIGURES 5–12. Details of adult male hypopygium of *Pagastia* (*P.*) *angarensis* (Linevich) from the Russian Far East: Sikhote-Alin' Nature Reserve (Primorye Territory) **(5–6),** Amur River basin (Khabarovsk Territory) **(7–8)** and *P. (P.) lanceolata* (Tokunaga) from Japan: Shizuoka Prefecture **(9, 11),** Niigata Prefecture **(11),** Gunma Prefecture. **5,** endoskeleton; **6, 9,** total view of hypopygium; **7–8, 10–12,** gonostylus. Scale bars: 50 µm.

Hypopygium (Figs 3–4, 9–12). Tergite IX with 10–17 setae on each side 115–156 μm long and with triangular anal point 72–80 μm long, which sometimes with apical peg. Laterosternite IX with 9–12 setae, 90–108 μm long. Transverse sternapodeme narrow, anterior margin nearly straight, 216 μm long μm long. Phallapodeme, 136–148 μm long, with lateral aedeagal lobe only, which 100–120 μm long, lanceolate. Gonocoxite 288–308 μm long, with basal plate and lobe-like median field. Gonostylus 196–212 μm long, approximately the same thickness along its entire length, not expanded basally, and without a protrusion (Figs 3–4, 9–12); megaseta 8–12 μm long. HR 1.45–1.47.

Distribution. Japan (Honshu, Hokkaido and Shikoku) (Endo 2004), Russian Far East (Kunashir Island of Kurile Archipelago).

Results of COI DNA barcoding

We sequenced fragments of the cytochrome oxidase subunit I (658 bp in length) of seven specimens of *P.* (*P.*) *angarensis* from the Russian Far East and East Siberia, as well as two specimens of *P.* (*P.*) *lanceolata* from Japan (Yamagata Prefecture). Mean intraspecific sequence divergence within these taxa were 0.56% and 0.71% respectively (Table S1). The average intraspecific pairwise K2P distances between *P.* (*P.*) *angarensis* and *P.* (*P.*) *lanceolata* were 7.96% and corresponds to the species level for the genus *Pagastia* (Makarchenko *et al.*, 2021) and is confirmed by the results of ASAP analysis. The intraspecific distances between the remaining species of the *P.* (*P.*) *lanceolata* group were slightly higher – 9.19%–13.15% (Table S1).

The high interspecific distances between *P.* (*P.*) *tianmumontana* Makarchenko *et* Wang (GenBank accession number MZ231025) and the remaining species may be due to the use of the larva (Lin *et al.* 2022) and hence its misidentification.

Bayesian analyses confirm monophily of *P.* (*P.*) *angarensis* with high nodal support (Bayesian posterior probability, BPP = 1) (Fig. 13). Samples of *P.* (*P.*) *lanceolata* formed the sister clade to *P.* (*P.*) *angarensis* with high support (BPP = 0.99). *P.* (*P.*) *lanceolata* from Yamagata Prefecture of Japan and Kunashir Island of Russia were found to be conspecific according to ASAP analysis (Fig. S2).

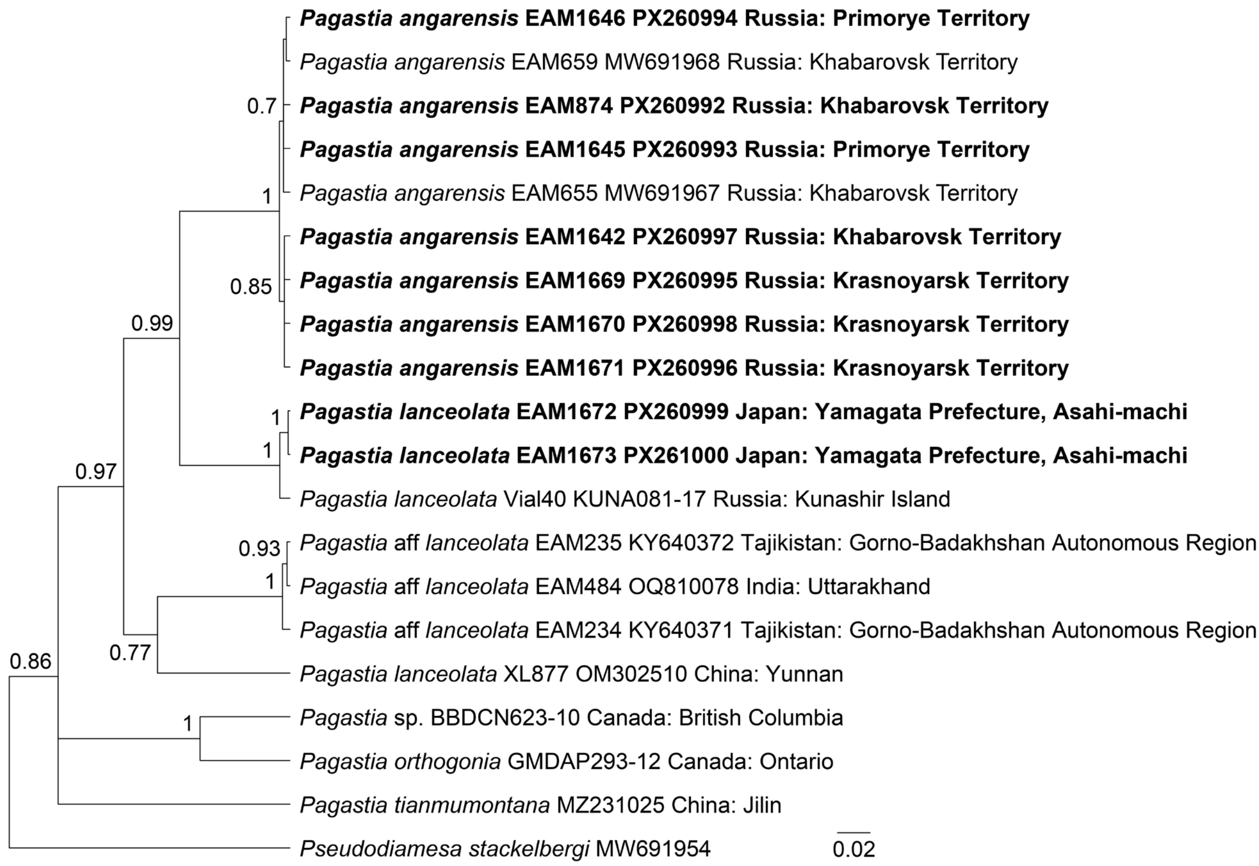


FIGURE 13. Ultrametric Bayesian inference (BI) tree based on the cytochrome c oxidase I (COI) nucleotide sequence data of the *Pagastia* (*P.*) *lanceolata* group and *Pseudodiamesa stackelbergi* (Goetghebuer) as outgroup. Bayesian posterior probabilities (higher than 0.7) are given above the tree nodes. Specimens obtained in this study are bold.

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper.
Supplementary Table S1
(Table S1. Intraspecific and interspecific K2P nucleotide distances of six *Pagastia* species from *Pagastia lanceolata group* estimated using COI sequences)

Supplementary Figure S2
(Figure S2. Results of ASAP analysis with the best ASAP-score of 1.5 using a threshold distance of 0.0684)