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DNA barcoding facilitates discovery and description of two new species of the *Mystacides azureus* Species Group (Trichoptera: Leptoceridae) in Japan

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Abstract

We examined adult specimens of the *Mystacides azureus* Species Group (Trichoptera: Leptoceridae) collected in Japan and confirm three species including *M. azureus* Linnaeus 1761 and two new species, *M. rivularis* and *M. moritai*. Males and females of the new species are described. *Mystacides azureus* in Japan is shown to have a considerable variation in morphology of the male tergum X. We analyzed mitochondrial COI barcodes of the genus *Mystacides* including these three species to confirm their species status. A maximum likelihood phylogeny based on COI barcodes shows monophyly of the new species. It also supports the hypothesis that morphological variation of the male tergum X in Japanese populations is intraspecific in only *M. azureus*.

Key words: caddisfly, mitochondrial COI, cryptic species, DNA barcode, genitalic plasticity

Introduction

The genus *Mystacides* Berthold 1827 is distributed in the Oriental and Holarctic regions and contains 20 described species (Morse 2022). Yamamoto & Ross (1966) classified the genus into four species groups based chiefly on morphology of the male genitalia. Morse & Yang (2002) revised the grouping using cladistic analysis and also recognized four species groups, partially agreeing with the former grouping.

The *Mystacides azureus* Species Group, which is characterized by the morphology of the male phallus, such as lacking parameres, includes 10 species [9 species specified by Morse & Yang (2002) and *M. dabrynos* Malicky 2012] and is distributed mainly in the Oriental and East Palaearctic regions. In Japan, only one species has been recorded in this group, *Mystacides azureus* Linnaeus 1761, which has wide distribution from Europe to Far East Russia.

During an examination of a large number of *Mystacides* specimens collected in various locations in Japan, we found two potentially undescribed species belonging to the *M. azureus* Species Group, with morphologies distinct from that of *M. azureus*. Moreover, *M. azureus* was found to have significant variation in the morphology of male genitalia. We used a mitochondrial COI barcode phylogeny to confirm that the morphological distinctions observed among the provisional *Mystacides* species correspond with phylogenetic evidence, supporting our hypotheses, and describe the two new species. The DNA barcodes also revealed paraphyly within the *M. azureus* Species Group distributed across Asia and Europe, confirming morphological variations, thus suggesting that additional cryptic species diversity occurs within the group.

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Materials and methods

Morphological study

Terminology of genitalia follows that of Morse & Yang (2002). Types of the newly described species are deposited in the collections of Systematic Entomology, Hokkaido University. Unless otherwise stated, types are preserved in 70–80% ethanol. Collectors are abbreviated as follows: NK (N. Kuhara), SN (S. Nakamura), TI (T. Ito), and TN (T. Nozaki). In the material list of *M. azureus*, the number of males is counted separately with respect to the shape of tergum X: short type (no indication), long-twist type (abbreviated as "L"), intermediate between these two types ("I") and reversal type ("R") (details provided below and in Table 1).

TABLE 1. Number of specimens for each type of male segment X in *Mystacides azureus*.

Island/Population	Short type	Long-twist type (L)	Intermediate (I)	Reversal type (R)
Hokkaidô I.	146	3	3	0
Honshû I. (excl. Ômachi Pop.)	75	26	11	0
Ômachi Pop.	0	5	4	26
Shikoku I.	16	8	1	0
Kyûshû I.	15	0	0	0

As reported by Morse & Yang (2002), antisymmetry of male tergum X occurs in all known species of the *M. sepulchralis* Species Group and in at least *M. azureus*, *M. elongatus* Yamamoto & Ross 1966, *M. khasicus* Kimmins 1963, and *M. superatus* Yang & Morse 2000 of the *M. azureus* Species Group, with the left and right positions of the long-curved spines and short-sinuous spines commonly exchanged. This antisymmetry occurs not only is those species but also in the two new species described here. In the illustrations of variation, asterisks indicate mirror-reversed images for easy comparison.

To compare eye-proportions, we measured the length of the left eye and the distance between eyes in selected specimens of both sexes for three species following the method of Gullefors and Petersson (1993).

DNA extraction and COI barcode amplification

Genomic DNA were extracted from a single leg from each of the examined specimens. The standard COI barcode region (658 bp) was amplified using primers and PCR programs following Zhou *et al*. (2016). Barcode amplicons were sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems). Sequences generated for this project were deposited in the BOLD project JPMYS (boldsystems.org) and in GenBank (Specimen details and Accession numbers provided in Table 2).

Phylogenetic analyses

Additional barcode sequences for *Mystacides* collected in Asian, American, and European countries were also obtained from published BOLD projects (Table 2). Barcode sequences longer than 500 bp were included in the phylogenetic analyses. A maximum likelihood-based approach was employed to build the phylogenetic tree using IQ-TREE (v2.2.0, Minh *et al*., 2020). ModelFinder (Kalyaanamoorthy *et al*. 2017) and ultrafast bootstrap approximation (Hoang *et al*. 2017), which were both built in this software, calculated that HKY+F+I+I+R3 as the best-fit substitution model according to Bayesian information criterion scores (BIC). And branch supports were accessed with 1000-bootstrap replicates.

TABLE 2. Specimen information used for mitochondrial phylogeny.

Sample ID	Species	Identifier	Lat	Lon	COI-5P Accession	BIN
013265640	*Mystacides azureus*		53.1268	-4.13319		BOLD:AAB1494
08HMCAD-134	*M. azureus*	Hans Malicky	46.0667	12.7667	KX107257	BOLD:AAB1494
08HMCAD-135	*M. azureus*	Hans Malicky	46.0667	12.7667	KX107258	BOLD:AAB1494
08HMCAD-137	*M. azureus*	Hans Malicky	46.0667	12.7667	KX106462	BOLD:AAB1494
08HMCAD-138	*M. azureus*	Hans Malicky	46.0667	12.7667	KX106997	BOLD:AAB1494
08NWCAD-017	*M. azureus*	Trond Andersen	59.85	9.43333	KX105010	BOLD:AAB1494
08NWCAD-018	*M. azureus*	Trond Andersen	59.85	9.43333	KX103290	BOLD:AAB1494
08OFCAD-1507	*M. alafimbriatus*	Oliver S. Flint, Jr.	48.6733	-122.315	JQ935392	BOLD:AAD9796
09CAUTR-032	*M. dentatus*	Xin Zhou	40.6455	117.422	HM405385	BOLD:ACF1811
09CAUTR-033	*M. dentatus*	Xin Zhou	40.6455	117.422	HM405386	BOLD:ACF1811
09CAUTR-034	*M. dentatus*	Xin Zhou	40.6455	117.422	HM405387	BOLD:ACF1811
09CAUTR-035	*M. dentatus*	Xin Zhou	40.6455	117.422	HM405388	BOLD:ACF1811
09CAUTR-071	*M. dentatus*	Xin Zhou	41.0702	125.015	HM405418	BOLD:ACF1811
09JPMYS-001	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395285	BOLD:AAB1495
09JPMYS-002	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395286	BOLD:AAB1496
09JPMYS-003	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395287	BOLD:AAB1496
09JPMYS-004	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395288	BOLD:AAB1496
09JPMYS-005	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395289	BOLD:AAB1495
09JPMYS-006	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395290	BOLD:AAB1495
09JPMYS-007	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395291	BOLD:AAB1495
09JPMYS-008	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395292	BOLD:ACE4254
09JPMYS-009	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395293	BOLD:AAB1496
09JPMYS-010	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395294	BOLD:ACE4254
09JPMYS-011	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395295	BOLD:AAB1496
09JPMYS-012	*M. azureus*	Takao Nozaki	35.2833	139.583	HM395296	BOLD:AAB1496
09JPMYS-013	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395297	BOLD:AAB1495
09JPMYS-014	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395298	BOLD:AAB1495
09JPMYS-015	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395299	BOLD:AAB1495
09JPMYS-016	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395300	BOLD:AAB1495
09JPMYS-017	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395301	BOLD:AAB1495
09JPMYS-018	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395302	BOLD:AAB1495
09JPMYS-019	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395303	BOLD:AAB1495
09JPMYS-020	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395304	BOLD:AAB1495
09JPMYS-021	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395305	BOLD:AAB9485
09JPMYS-022	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395306	BOLD:AAB9485
09JPMYS-023	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395307	BOLD:AAB9485
09JPMYS-024	*M. azureus*	Naotoshi Kuhara	42.7656	141.719	HM395308	BOLD:AAB1495
09JPMYS-025	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395309	BOLD:AAB9485
09JPMYS-026	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395310	BOLD:AAB9485
09JPMYS-027	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395311	BOLD:AAB9485
09JPMYS-029	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395313	BOLD:AAB9485
09JPMYS-030	*M. rivularis*	Naotoshi Kuhara	42.7656	141.719	HM395314	BOLD:AAB9485
09JPMYS-031	*M. rivularis*	Takao Nozaki	35.1028	138.902	HM395315	BOLD:ABZ1489

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TABLE 2 (Continued)

Sample ID	Species	Identifier	Lat	Lon	COI-5P Accession	BIN
09JPMYS-032	*M. rivularis*	Takao Nozaki	35.1028	138.902	HM395316	BOLD:ABZ1030
09JPMYS-033	*M. rivularis*	Takao Nozaki	35.1028	138.902	HM395317	BOLD:ABZ1030
09JPMYS-034	*M. rivularis*	Takao Nozaki	35.1028	138.902	HM395318	BOLD:ABZ1030
09JPMYS-035	*M. rivularis*	Takao Nozaki	35.1028	138.902	HM395319	BOLD:ABZ1030
09JPMYS-036	*M. rivularis*	Takao Nozaki	35.1028	138.902	HM395320	BOLD:ABZ1030
09JPMYS-037	*M. azureus*	Naotoshi Kuhara	36.6	137.85	HM395321	BOLD:ACE4254
09JPMYS-038	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395322	BOLD:ACE4254
09JPMYS-039	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395323	BOLD:ACE4254
09JPMYS-040	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395324	BOLD:ACE4254
09JPMYS-041	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395325	BOLD:ACE4254
09JPMYS-042	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395326	BOLD:ACE4254
09JPMYS-043	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395327	BOLD:ACE4254
09JPMYS-044	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395328	BOLD:ACE4254
09JPMYS-045	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395329	BOLD:ACE4254
09JPMYS-046	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395330	BOLD:ACE4254
09JPMYS-047	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395331	BOLD:ACE4254
09JPMYS-048	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395332	BOLD:ACE4254
09JPMYS-049	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395333	BOLD:ACE4254
09JPMYS-050	*M. azureus*	Naotoshi Kuhara	36.6	137.844	HM395334	BOLD:ACE4254
09JPTRI-017	*M. moritai*	Naotoshi Kuhara	34.4	136.8	HM395339	BOLD:ABZ1029
09JPTRI-018	*M. moritai*	Naotoshi Kuhara	34.4	136.8	HM395340	BOLD:ABZ1029
09JPTRI-019	*M. moritai*	Naotoshi Kuhara	35.3538	134.899	HM395341	BOLD:ABZ7905
09JPTRI-020	*M. moritai*	Naotoshi Kuhara	35.3538	134.899	HM395342	BOLD:ABZ7905
09JPTRI-021	*M. moritai*	Naotoshi Kuhara	35.3538	134.899	HM395343	BOLD:ABZ7905
10OFSI-0207	*M. pacificus*	Oliver S. Flint, Jr.	42.58	143.49	HQ967433	BOLD:AAN4154
10PROBE-19177	*M. interjectus*	Jonathan Witt	58.6631	-94.1662	HQ986594	BOLD:ACJ9907
11TVCAD-026	*M. dentatus*	Oliver S. Flint, Jr.	42.9169	132.767	KX295370	BOLD:ACF1811
12HMCAD-74	*M. azureus*	Hans Malicky	48.05	15.0333	KX296204	
12HMCAD-189	*M. elongatus*	Hans Malicky	18.6	100.967	KX293450	BOLD:ACH0651
12IQTRA-0020	*M. azureus*	John Morse	35.479	45.195	KX294075	BOLD:ACE0060
12IQTRA-0044	*M. azureus*	John Morse	35.465	45.195	KX292561	BOLD:ACE0060
ARin-2012F281	*M. azureus*	Juha Salokannel	60.5096	26.8798	KX293677	BOLD:AAB1494
ARin-2012F282	*M. longicornis*	Juha Salokannel	60.3633	23.5674	KX295573	BOLD:ACB2178
ARin-2014F096	*M. azureus*	Juha Salokannel	56.79	9.94	KX295349	BOLD:AAB1494
BC ZSM AQU 00624	*M. azureus*	Monika Hess	49.028	11.588	HQ948142	BOLD:AAB1494
BIOUG22331-C09	*M. sepulchralis*	Kate Perez	49.034	-125.553	KX141327	BOLD:AAA8765
BIOUG43950-C04	*M. azureus*	Kate Perez	63.5783	23.736		BOLD:AAB1494
GBOL12182	*M. azureus*	Monika Hess	50.584	6.376	KX291284	BOLD:AAB1494
HM09Ma8	*M. azureus*	Hans Malicky	44.4	8.26667	KX143779	BOLD:AAB1494
HMCAD0111-132	*M. azureus*	Hans Malicky	47.9167	13.9	KX142253	BOLD:AAB1494
HMCAD0810-2	*M. azureus*	Hans Malicky	40.3	-7.88333	KX291447	BOLD:AAB1494
ID-04401	*M. bifidus*	Suvdtsetseg Chuluunbat	48.144	99.783	KX104225	BOLD:AAG3713
JSlk-2011F066	*M. azureus*	Juha Salokannel	60.205	23.702	KX141266	BOLD:AAB1494

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TABLE 2 (Continued**)**

Sample ID	Species	Identifier	Lat	Lon	COI-5P Accession	BIN
MG587011	*M. dentatus*				MG587011	BOLD:ACF1811
NIVA_TER_27	*M. azureus*	Tor ERIK Eriksen	59.9242	11.1588	KX292831	BOLD:AAB1494
NIVA_TER_63	*M. azureus*	Tor ERIK Eriksen	60.1873	11.4673	KX292832	BOLD:AAB1494
NZCAD909-87	*M. azureus*	Stanislaw Czachorowski	54.1783	17.565	HM395764	BOLD:AAB1494
NZCAD909-90	*M. azureus*	Stanislaw Czachorowski	54.1903	17.5567	HM862430	BOLD:AAB1494
NZCAD909-94	*M. azureus*	Stanislaw Czachorowski	54.1903	17.5567	HM862434	BOLD:AAB1494
RBCAD-1099	*M. azureus*	Roger J. Blahnik			KX104248	BOLD:AAB1494
TMAZU_1	*M. azureus*	Mladen Kucinic	45.2527	15.5461		BOLD:AAB1494
TMAZU_2	*M. azureus*	Mladen Kucinic	43.4138	17.0591		BOLD:AAB1494
TMAZU_3	*M. azureus*	Mladen Kucinic	43.533	16.7446		BOLD:AAB1494
TMNIG_1	*M. azureus*	Andela Cukusic	45.3566	16.2812		BOLD:AAB1494
TRD-TRI133	*M. azureus*	Jon K. Skei	63.274	10.561	KX295690	BOLD:AAB1494
TRD-TRI213	*M. azureus*	Jon K. Skei	63.274	10.561	KX292906	BOLD:AAB1494
TRD-TRI214	*M. azureus*	Jon K. Skei	63.274	10.561	KX296479	BOLD:AAB1494
TVTRI0164	*M. dentatus*	Oliver S. Flint, Jr.	43.3363	132.19	KX294760	BOLD:ACF1811
TVTRI0165	*M. dentatus*	T.S. Vshivkova	43.3363	132.19	KX291770	BOLD:ACF1811
UA-SG-TRICH-A79	*M. azureus*	Sophie C. Gombeer	51.0939	5.74344	KX144600	BOLD:AAB1494
UA-SG-TRICH-A80	*M. azureus*	Sophie C. Gombeer	51.0939	5.74344	KX144351	BOLD:AAB1494
UA-SG-TRICH-A83	*M. azureus*	Sophie C. Gombeer	51.2608	5.09272	KX142840	BOLD:AAB1494
UA-SG-TRICH-A90	*M. niger*	Sophie C. Gombeer	51.2241	4.59861	KX141833	BOLD:AAK8719
UA-SG-TRICH-E05	*M. azureus*	Sophie C. Gombeer	51.1584	3.17103	KX140856	BOLD:AAB1494
UA-SG-TRICH-X20	*M. azureus*	Koen Lock			KX143644	BOLD:AAB1494
ZFMK-TIS-2532519	*M. azureus*	Guido Haas	50.8	7.17	MW459693	BOLD:AAB1494
ZFMK-TIS-2532657	*M. azureus*	Guido Haas	50.77	7.36	MW459555	BOLD:AAB1494
ZFMK-TIS-2532659	*M. azureus*	Guido Haas	50.77	7.36	MW459655	BOLD:AAB1494
ZFMK-TIS-2532664	*M. azureus*	Guido Haas	50.77	7.36	MW459909	BOLD:AAB1494
ZFMK-TIS-2534217	*M. azureus*		50.77	7.11	MW459795	BOLD:AAB1494
ZFMK-TIS-2534224	*M. azureus*		50.77	7.11	MW459432	BOLD:AAB1494

***Mystacides rivularis* n. sp.**

Figs 1–3

Mystacides sp.: Nozaki & Tanida 2007: 252.

Diagnosis. This species can be distinguished from *M. azureus*, which is occasionally collected sympatrically with *M. rivularis* **n. sp.**, by shape of the male segment IX that is strongly extended posteroventrad. Among species of the *M. azureus* Species Group, this character state is similar to that of *M. schmidi* Morse & Yang 2002 from Sri Lanka, but distinguishable from the latter by the long sigmoid spine of segment X. The female of this species is characterized by the manta-ray-shaped plate of the spermathecal sclerite.

Adult. Forewing length: male 6.4–7.6 mm (mean = 7.2 mm, n = 10); female 6.8–7.6 mm (mean = 7.3 mm, n = 9). Vertex, thorax, and wings in ethanol brownish black. General morphology typical for the genus. Relative eye size smaller than that in *M. azureus* for both male and female (Fig. 1).

Male genitalia (Figs 2A–2E). Segment IX 1/5 as long dorsally as tall laterally, 1 1/5 as long ventrally as tall laterally; in ventral view apicoventral process of sternum 3/4 as long as rest of sternum, with stout base and slender V-shaped lateral arms. Preanal appendages very long and slender. Tergum X antisymmetrical, produced into two sinuate spines: one spine sigmoid in dorsal aspect, long, extending far beyond apical process of sternum IX; other spine weakly sinuate and shorter than former. Inferior appendages each with dorsomesal lobe oriented vertically, somewhat longer than mid-width in lateral view, its anterior and posterior margins subparallel; posterior margin with three caudal projections: upper caudal projection with acute apex; middle caudal projection narrow and often longest among three projections, with acute apex; lower caudal projection with round apex. Phallus without evident paramere spines or phallicata, with ventromedian spine directed anteroventrad at one third distance from apex and pair of sharp, triangular flanges subapically, apicoventral lip of phallobase constricted subapically in ventral aspect.

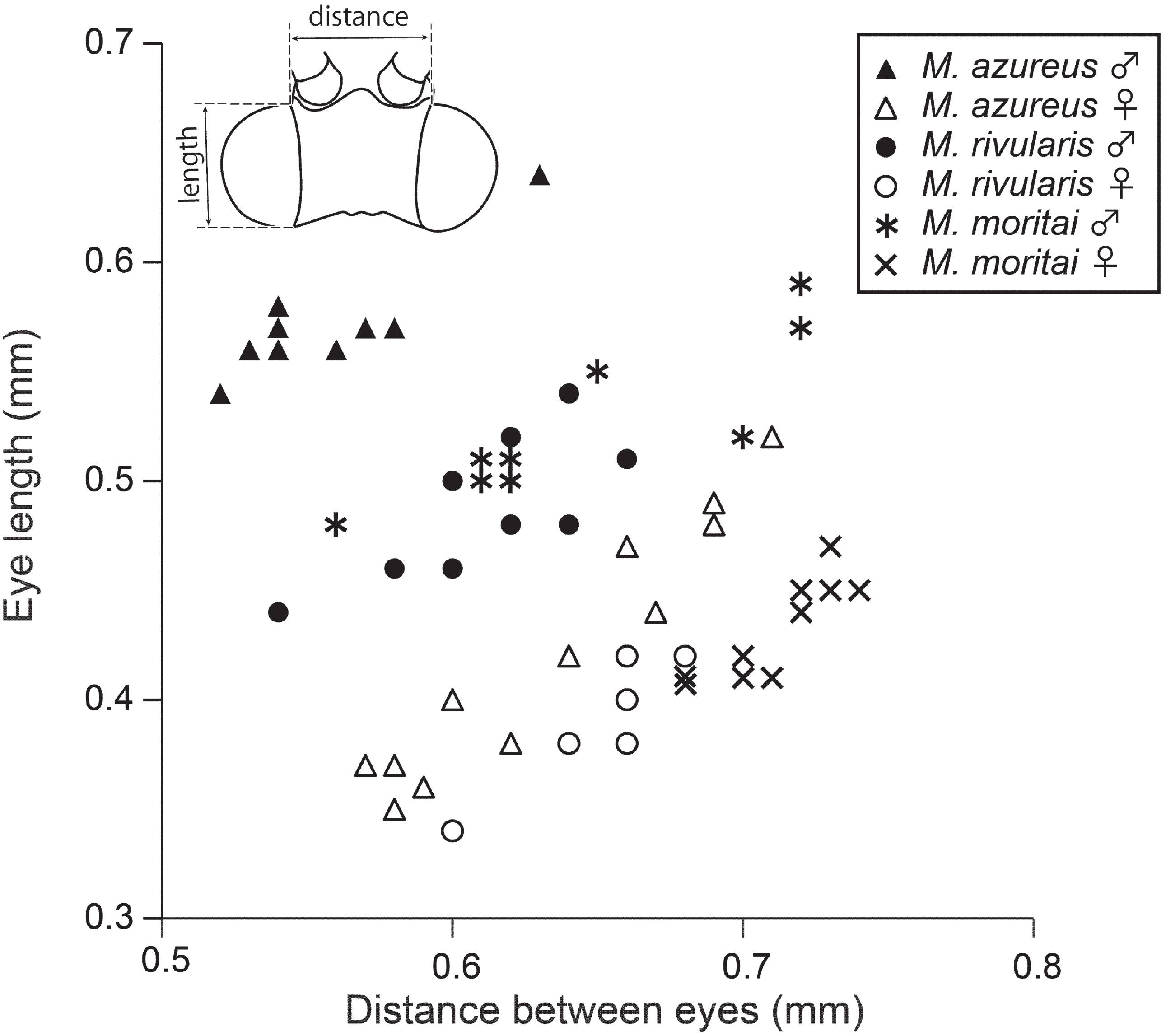

FIGURE 1. Eye proportions in analyzed *Mystacides* spp. An outline drawing of the dorsal view of the head showing the measurement points is inserted in the diagram.

Variation (Figs 3A1–3F3): The shapes of tergum X and inferior appendages are variable geographically. The shorter process of tergum X is about half the length of the longer process in individuals from central Honshû (Figs 3F1–3F3), but the former is prolonged in those from northern Honshû (Figs 3C1–3E1) and almost reaches the tip of the latter in those from Hokkaidô (Figs 3A1–3B2). The middle projection of each inferior appendage tends to be more strongly developed in individuals from Hokkaidô and northern Honshû (Figs 3A1–3E1) than those from central Honshû (Figs 3F1, 3F2) with an exception (Fig. 3F3).

Female genitalia (Figs 2F–2I). Segment IX short in lateral view. Segment X shorter than preanal appendages, narrowly incised ventromedially in dorsal view. Preanal appendages slender, straight, and setose. Lamellae very long, constricted basally, shape in lateral aspect variable geographically; dorsal margin inflated; apicodorsal corner protruded outward; outer surface slightly concave. Gonopod plates triangular in ventral aspect with pair of posterolateral processes short, rounded and apex extending far beyond apex of segment X. Spermathecal sclerite with pigmented manta-ray-shaped plate, anterior and posterior margins extended anterad and posterad mesally and acute.

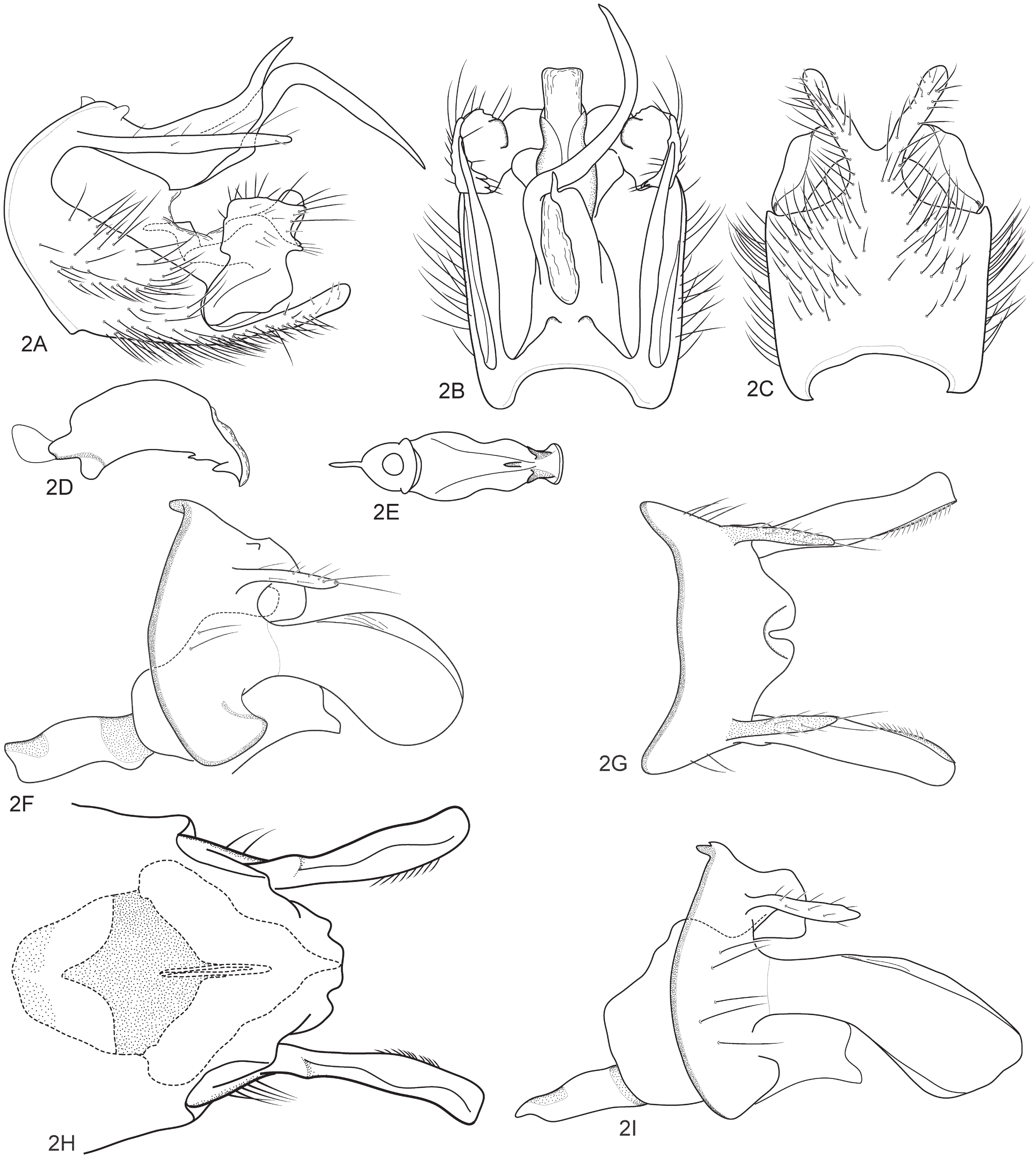

FIGURE 2. *Mystacides rivularis* **n. sp.** 2A–2E, male genitalia: 2A, left lateral; 2B, dorsal; 2C, ventral; 2D, phallus, left lateral; 2E, phallus, ventral. 2F–2I, female genitalia: 2F, 2I, left lateral; 2G, dorsal; 2H, ventral. Specimens from the type locality, Shimizu-chô, Shizuoka, Honshû (2A–2H) and Tomakomai-shi, Hokkaidô (2I).

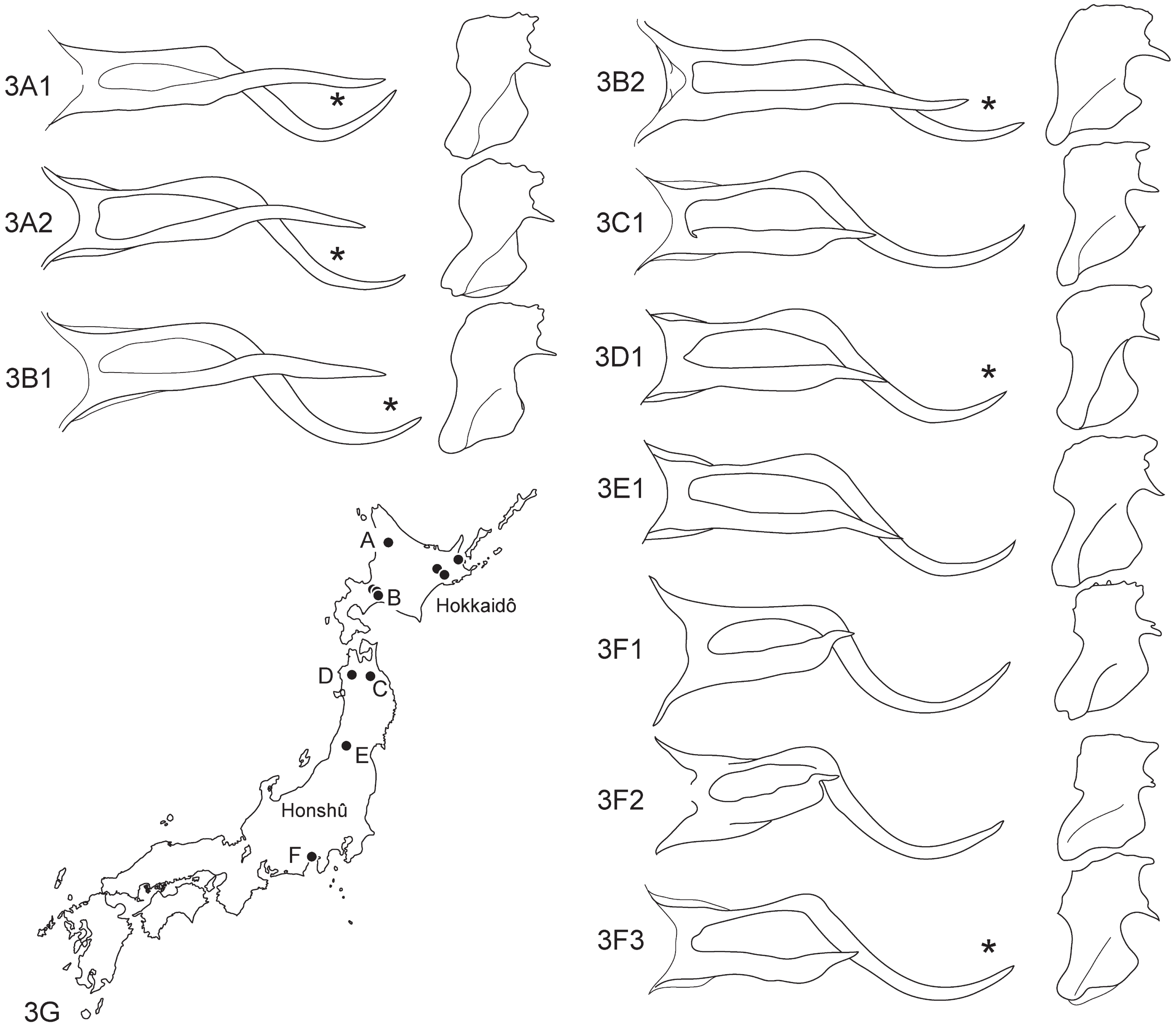

FIGURE 3. Variation of male genitalia and map of collection sites of *Mystacides rivularis* **n. sp.**; the dorsal aspect of tergum X (left) and left lateral aspect of left inferior appendage (right) for each individual are shown. 3A1, 3A2, Horokanai-chô, Hokkaidô; 3B1, 3B2, Tomakomai-shi, Hokkaidô; 3C1, Tatsuko-machi, Aomori, Honshû; 3D1, Nishimeya-mura, Aomor, Honshû; 3E1, Nishikawa-chô, Yamagata, Honshû; 3F1–3F3, Shimizu-chô, Shizuoka, Honshû. 3G, map of collection sites. Asterisks indicate mirror-reversed images for easy comparison.

Variation: Lamellae in lateral aspect are each subelliptical and rounded apically in individuals from the type locality Kakida-gawa, central Honshû (Fig. 2F), but more nearly parallel-sided, gradually heightened caudally, and obliquely truncate apically in those from Hokkaidô (Fig. 2I).

Holotype: ♂, Kakida-gawa, Shimizu-chô, Shizuoka, Honshû, Japan, 35.1031°N, 138.9028°E, alt. 13 m, 27.iv.2002, TN.

Paratypes: 3♂, same data as holotype; 5♂, type locality, 22–23.xi.2002, NK, 2♂ (pinned), type locality, 5.iv.2006, TN.

Other specimens examined. **HOKKAIDÔ: Nemuro**: 1♀, Shibetsu-chô, Shibetsu-shitsugen, 12.viii.1996, K. Kuribayashi, 1♂ 1♀, same location, 21.viii.2013, M. Nakatani; 1♀, same location, 2–3.viii.2013, M. Nakatani; 1♂ 1♀, same location, 3.ix.2013, M. Nakatani. **Kushiro**: 1♂, Kushiro-shi, Akan-chô, Ibeshibetsu-gawa R., 6.viii.1990, NK; 1♂, same location, 6.viii.1990, NK; 3♂ 1♀, Shibecha-chô, Gojikkoku, Shirarutoroetoro-gawa R., 16.vii.2009, TI; 2♂ 7♀, same location, 28.vii.2012, TI; 1♂ 2♀, Shibecha-chô, Kayanuma, Shirarutoroetoro-gawa R., 16.vii.2009, TI; **Kamikawa**: 1♂, Horokanai-chô, small tributary of Shumarinai-gawa R., 10.viii.1999, TI & A.

Ohkawa; 1♂, Horokanai-chô, small tributary of Shumarinai-gawa R., 7.vii.2007, NK. **Ishikari**: 1♂, Chitose-shi, Bibi, Bibi-gawa R., 5–26.ix.1993, TI; 1♂ 1♀, same location, 4.viii.2007, TI; 1♂ 3♀, Eniwa-shi, Izari-gawa R., Eniwa-ôhashi, 11.vii.1999, TI; 1♂, same location, 22.vii.1999, TI; 1♂, same location, 17.vii.2015, TI; 1♀, Sapporo-shi, Hitsujigaoka, 24–31.vii.2009, K. Konishi; 1♂, Sapporo-shi, Nopporo-shinrin-kôen, Osawa-guchi, 17.vi.2002, M. Sakurai. **Iburi**: 3♂ 5♀, Tomakomai-shi, Misawa, Bibi-gawa R., 29.vii.1989, NK; 5♂ 1♀, same location, 17.vii.1990, NK; 1♀, same location, 16.viii.1990, NK; 4♀, same location, 18.viii.1991, TI; 2♂, same location, 12.ix.1993, TI; 1♂, same location, 2.viii.1998, NK; 1♂, same location, 20.viii.2007, TI & A. Ohkawa; 5♂ 8♀, same location, 31.vii.2009, NK; 2♂, Tomakomai-shi, Misawa, small stream, 1.viii.1992, NK; 1♂, same location, 15.viii.1992, NK; 5♂ 2♀, Tomakomai-shi, Uenae, Bibi-gawa R., 22.vii.2001, TI & A. Ohkawa; 1♂, same location, 25.vii.2001, TI; 1♂, same location, 29.vii.2001, TI; 1♂, same location, 30.vii.2001, TI; 3♂ 2♀, same location, 23.viii.2007, TI; 2♂ 3♀, same location, 16.vii.2008, TI. **HONSHÛ: Aomori**: 1♂, Nishimeya-mura, Anmon-gawa R., alt. 240 m, 16.ix.2010, TI; 1♂, Takko-machi, Natsuzaka, Kumahara-gawa R., 18.viii.1996, Suzuki. **Yamagata**: 3♂, Nishikawa-chô, Shizu, Buna-no-izumi, 11.ix.2003, TI. **Shizuoka**: 1♀, type locality, 11.iii.1984, reared and emerged on 7.iv.1984, TN; 3♂ 1♀, same data as holotype; 1♂ 1♀, type locality, 27.iv.2002, TN; 1♀, type locality, 31.viii.2002, TN; 2♂ 1♀, type locality, 14.xi.2002, TN; 6♂ 1♀, type locality, 22–23.xi.2002, NK.

Etymology. The specific epithet (Latin adjective, *rivularis* = of a brook or small stream) refers to the habitat of this species.

Distribution. Japan (Hokkaidô, eastern Honshû).

Habitat. Adults are often found beside slowly flowing streams. They are sometimes collected with *M. azureus* sympatrically, but the habitat preference of the new species is narrower than that of the latter.

Mystacides moritai n. sp.

Figs 1, 4A–4I

Mystacides sp. (af. *superatus*): Morita 2011, 217.

Diagnosis. Among species of the *M. azureus* Species Group, the male of this species shares an unusually large caudal projection of the each inferior appendage with that of *M. superatus* from China, but can be distinguished from it by lack of the lower projection of the appendage. Female of this species is similar to that of *M. azureus* but can be distinguished from it by the shape of the lamellae; in lateral aspect, each is parallel sided or slightly tapered in the apical half in the present species but broadest with a dorsal expansion at midpoint in *M. azureus*. It also somewhat resembles that of *M. rivularis* **n. sp.** but can be distinguished from it by the subtriangular plate of the spermathecal sclerite.

Adult. Forewing length: male 6.1–8.1 mm (mean = 7.5 mm, n = 10); female 6.8–8.5 mm (mean = 7.5 mm, n = 8). Vertex, thorax, and wings in ethanol brownish black. General morphology typical for the genus. Relative eye size smaller than that in *M. azureus* for both male and female (Fig. 1).

Male genitalia (Figs 4A–4E). Segment IX 1/8 as long dorsally as tall laterally, 1 1/4 times as long ventrally as tall laterally; in ventral view apicoventral process of sternum 2/3 as long as rest of sternum, with stout base and slender V-shaped lateral arms. Preanal appendages very long and slender. Tergum X antisymmetrical, produced into two spines; longer spine bent inward at one-third from base in dorsal aspect, extending beyond apical process of sternum IX; other spine half as long as longer one, nearly straight in dorsal aspect with acute apex. Inferior appendages each clavate in lateral view with slender base 1/3 as thick as broad apex; upper caudal projection directed dorsad, as long as wide; middle caudal projection unusually large, curved dorsomesad, tapering to acute apex; lower caudal projection lacking. Phallus without evident paramere spines or phallicata, with ventromedian spine directed anteroventrad at one third distance from apex and pair of sharp, triangular flanges subapically, apicoventral lip of phallobase constricted subapically in ventral aspect.

Female genitalia (Figs 4F–4H). Segment IX short. Segment X shorter than preanal appendages, narrowly incised ventromedially in dorsal view. Preanal appendages slender, straight, and setose. Lamellae very long; in lateral aspect each with apical half twice as broad as basal half, somewhat curved downward, parallel sided or slightly tapered in apical half, with round apex; dorsal margin inflated; outer surface slightly concave. Gonopod plates triangular in ventral aspect with short, rounded pair of posterolateral processes; apex extending far beyond apex of segment X. Spermathecal sclerite rhomboid, rounded anteriorly.

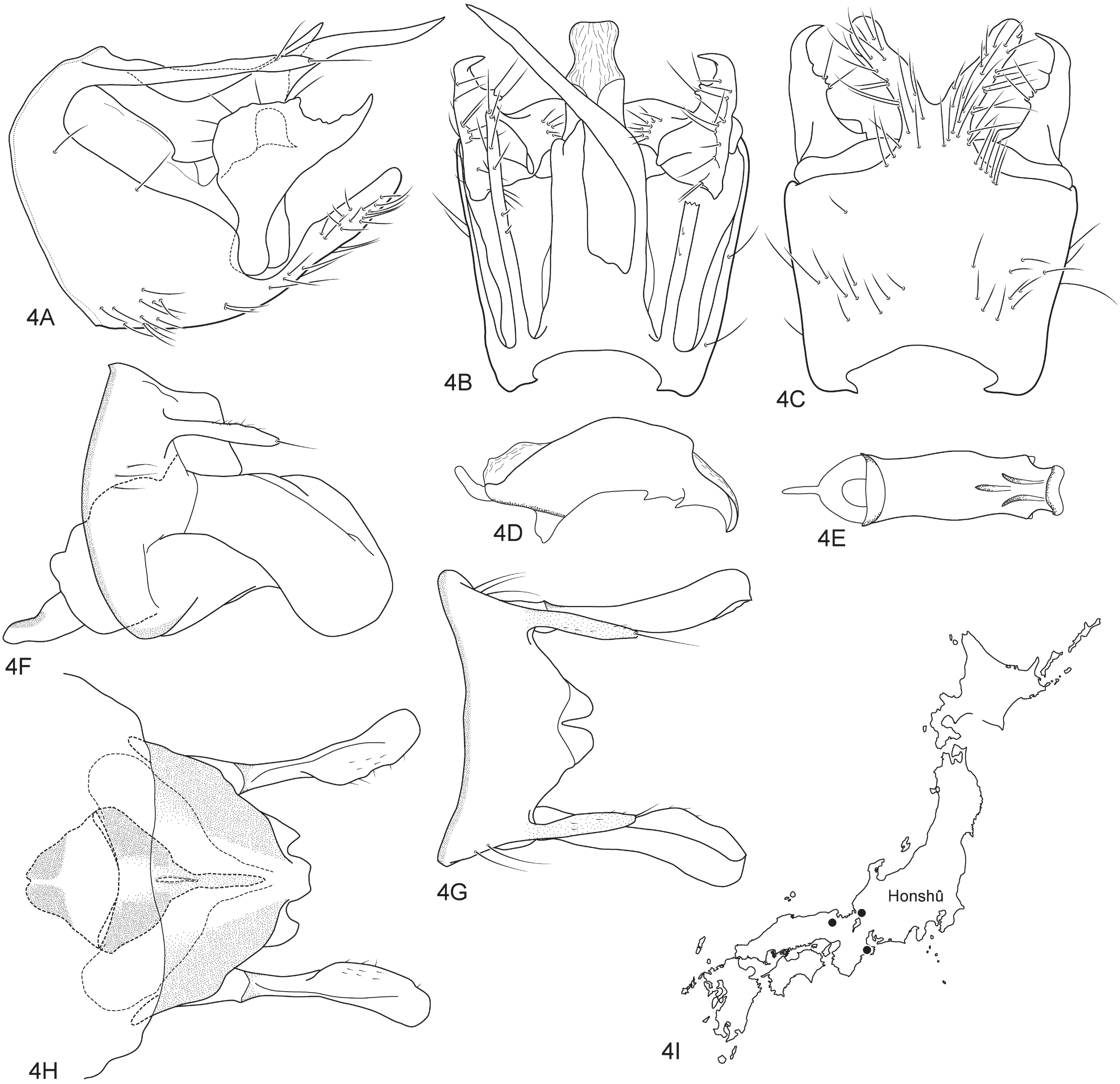

FIGURE 4. *Mystacides moritai* **n. sp.** 4A–4E, male genitalia from the type locality, Shima-shi, Mie, Honshû: 4A, left lateral; 4B, dorsal; 4C, ventral; 4D, phallus, left lateral; 4E, phallus, ventral. 4F–4H, female genitalia from Tsuruga-shi, Fukui, Honshû: 4F, left lateral; 4G, dorsal; 4H, ventral. 4I, map of collection sites.

Holotype: ♂, Honshû, Mie, Shima-shi, Isobe-chô, Natsukusa, 34.359°N, 136.766°E, alt. 20 m, 5.vii.2008, H. Morita.

Paratypes: 1♂, same data as holotype; 7♂, Honshû, Hyôgo, Asago-shi, Wadayama, Kudawa, Ishibe-jinjya, 3.xi.2006, K. Inazu & TI.

Other specimens examined. **HONSHÛ: Fukui**: 5♂ 9♀, Tsuruga-shi, Ikenokôchi-shitsugen, 1.x.2013, TI; 3♂ 11♀, same location, 30.v.2015, TI; 8♂ 7♀, same location, 24.vi.2015, TI; 5♂ 4♀, same location, 21.vii.2015, TI; 4♀, same location, 11.vi.2016, TI. **Hyôgo**: 3♂, Asago-shi, Wadayama, Kudawa, Ishibe-jinjya, 19.v.2009, R.B. Kuranishi.

Etymology. Named *moritai* after Mr. H. Morita, who first provided us the examined specimens of this species, including the holotype.

Distribution. Japan (western Honshû).

Habitat. Adults of this species have been collected at three sites: beside a small stream with low gradient, from a stream flowing through a small marsh, and from a pond recharged by spring water. Adults of *M. azureus* also have been collected at all these sites sympatrically.

Mystacides azureus (Linnaeus 1761)

Figs. 1, 5A–5M, 6A1–6E6

Phryganea azurea Linnaeus 1761: 380.

This species was originally described from Sweden and has been recorded in a broad geographical range from Western and Northern Europe to the Far East, including Japan. Although several authors described or illustrated the male and female of this species (*e.g.*, Mosely 1939; Vshivkova *et al.* 1997), we provide illustrations of male and female genitalia of individuals from Japan for comparison (Figs 5A–5M, 6A1–6E6). We detected intraspecific variation in the shape of the male genitalia among Japanese individuals, although conspicuous morphological variation was not observed in the female.

Remarks. The males exhibit great variation in the shape of tergum X even among individuals collected at the same time and place. The tergum X is produced into two asymmetrical spines posteriorly: In most populations, one spine is shorter and sinuous in the distal half and the other spine is curved and longer but variable in shape and length. The latter spine is slightly longer than the former and curved mesad in its apical half or bent mesad at mid-length in more than half of the examined specimens (short type; Figs 6A1, 6A2, 6B1–6B4, 6C1, 6C2, 6E1–6E3), which type well agrees with European individuals (Kumanski 1988; Mosely 1939). However, in some specimens, the latter is far longer than the former and curved sigmoidally in dorsal aspect (long twist type; Figs 6A5, 6C5, 6C6, 6E5, 6E6). In addition, a few specimens have the longer spine slightly curved outward subapically, representing an intermediate between the two types (Fig. 6A3, 6A4, 6C3, 6C4, 6E4). In most specimens, the longer spine is crossing beneath the shorter.

Frequencies of the long twist type vary geographically. Only a few or no long-twist type individuals were found in specimens collected in Hokkaidô and Kyûshû, but over 20% of individuals exhibit this type in specimens collected in Honshû and Shikoku (Table 1).

The population in lakes in Ômachi, Nagano, and Honshû, however, exhibit a different type of shape of tergum X from the other populations (Figs 5F–5J): The usually short sinuate spine is usually longer than the curved one (reversal type; Figs 6D1, 6D2), although some specimens have the curved spine extending beyond the sinuate spine (Figs 6D3, 6D4), resembling the intermediate type. Specimens having more extended spines (Fig. 6D5) almost agree with those of the long twist type.

The degree of the posteroventral excisions of the inferior appendages in lateral aspect is also variable (Figs 5A, 5F, 6). The excision of specimens collected in Honshû (Figs 6C–6E) tends to be deeper than that in Hokkaidô (Figs 6A, 6B), but variation of this character within each population is rather small.

The reversal type specimens of the Ômachi population closely resemble illustrations of *Mystacides elongatus* from China by Yang & Morse (2000). In addition, our female specimens of this species show no evident variation in genital morphology and are very similar to illustrations those of *M. elongatus* by them. These facts suggest that *M. elongatus* may be included in the variation of *M. azureus*. Morphological and molecular studies of *M. elongatus* specimens from China would be needed to clarify the relation of *M. elongatus* and *M. azureus*.

HOKKAIDO: **Kushiro**: 57♂ 2♂(L) 3♂ (I) 13♀, Kushiro-shi, Akan-chô, Akan-ko, 14.ix.1999, TI & N. Minakawa; 6♂ 10♀, same location, 7.viii.2007, TI; 6♂ 5♀, Kushiro-shi, Akan-chô, Ibeshibetsu-gawa R., 19.ix.1989, NK; 1♂ 1♂(L) 4♀, Kushiro-shi, Akan-chô, Panketô, 21.ix.1996, TI; 1♂, same location, 14.ix.1999, N. Minakawa; 6♂ 2♀, same location, 3–4.x.2006, TI; 1♂ 2♀, same location, 8.viii.2007, TI; 7♀, Shibecha-chô, L. Shirarutoro-ko, 9.viii.2005, TI; 1♂, same location, 16.viii.2007, TI; 1♂, same location, 25.vii.2008, TI. **Sôya**: 16♂ 8♀, Sarufutsu-mura, Asajino, 31.vii.2007, NK. **Kamikawa**: 1♀, Tôma-chô, Nakadai, 15.vii.2007, NK. **Sorachi**: 1♀, Yuni-chô, Kawabata, small stream, 15–30.viii.2007, NK. **Ishikari**: 1♀, Chitose-shi, Bibi, Bibi-gawa R., 29.viii.1991, TI; 1♀, same location, 5–26.ix.1993, TI; 4♀, Chitose-shi, Neshikoshi, Chitose-gawa R., 4.ix.1997, NK; 1♂, Chitose-shi, Izumisawa, Mamachi-gawa R., 6.viii.2003, NK; 1♀, Chitose-shi, Okotan, Okotanpe-gawa R., 26.ix.1998, NK; 4♂ 1♀, Chitose-shi, L. Shirarutoro-ko, 29.ix.1996, TI & A. Ohkawa; 3♀, same location, 29.ix.1996, A. Ohkawa; 1♂ 5♀, same location, 28.viii.1997, TI & A. Ohkawa; 5♂ 1♀, same location, 25.ix.1997, NK; 3♂ 13♀, same location, 5.viii-7.x.2005, NK; 6♂ 5♀, same location, 4–10.viii.2006, NK; 2♂ 1♀, Chitose-shi, small stream beside L. Shikotsu-ko, 1.x.1993, NK; 4♀, same location, 21.VII-6.x.1996, Y. Nagayasu; 1♀, Sapporo-shi, Misumai, Toyohira-gawa R., 27.vii.1992, NK. **Iburi**: 1♀, Atsuma-chô, Naganuma, Koi-numa, 13.vii.2006, NK; 1♀, Tomakomai-shi, Misawa, Bibi-gawa R., 18.vi.1990, NK; 2♂, same location, 2.viii.1998, NK; 6♀, same location, 5.vii.2009, NK; 2♂ 3♀, Tomakomai-shi, L. Utonai-ko, 1–6.vii.1998, NK; 1♂ 1♀, same location, 21.vi.2003, NK; 9♂, same location, 22.vii.2004, TI. **Oshima**: 4♂ 6♀, Nanae-chô, Konuma, 23.vi.2003, NK; 10♂ 12♀, Nanae-chô, Ônuma, 23.vi.2003, NK. **HONSHU: Aomori**: 1♂(I), Fujisaki-machi, Shirako, Iwaki-gawa R., 2.viii.1996, Suzuki; 1♂ 1♂(L) 2♀, Hirosaki-shi, Akudo, Iwaki-gawa R., 20.viii.1996, Suzuki. **Akita**: 5♂, Kosaka-machi, Towadako-namariyama, L. Towada-ko, 8.viii.1999, H. Kato. **Fukushima**: 1♂ 1♂(L) 3♀, Shôwa-mura, Yanohara-kôgen, 19.viii.2008, N. Katsuma. **Ibaraki**: 4♂ 2♂(L) 3♀, Kasama-shi, Minamikoizumi, 24.vi.2007, N. Katsuma. **Gumma**: 1♂(L) 1♂(I) 2♀, Fujioka-shi, Samba-gawa R., 29.v.1991, S. Ishiwata. **Tôkyô**: 1♂(L), Fussa-shi, Nagatabashi, 9.vi.1985, S. Sasaki. **Kanagawa**: 1♂, Hadano-shi, L. Shinsei-ko, 16.xii.1979, TN; 2♂(I) 15♀, Sagamihara-shi, Fujino-chô, Magino, 9.vi.1988, TN; 2♂ 1♂(L) 5♀, Yamakita-machi, Shiraishi-zawa, 5–6.vii.1984, TN; 1♂(L), same location, 19.vii.1982, TN; 10♂ 4♂(L) 7♀, Zushi-shi, Sakurayama, Morito-gawa R., 20.vii.2009, TN. **Fukui**: 1♂, Tsuruga-shi, Ikenokôchi-shitsugen, 21.vii.2015, TI; 1♂ 1♂(L) 13♀, same location, 11.vi.2016. **Nagano**: 1♂ 1♀, Koumi-chô, Matsubara, L. Chô-ko, 30.v.1997, TI; 3♀, Maruko-machi, Uchimura-kawa, 21.vi.1997, K. Tojo; 1♂(R), Ômachi-shi, L. Aoki-ko, 28.viii.2008, N. Katsuma; 1♂(L) 2♂(R), same location, 23.ix.2009, NK; 1♂(L) 2♂(I) 9♂(R) 17♀, Ômachi-shi, L. Nakatsuna-ko, 27.ix.1990, NK; 3♂(L) 2♂(I) 14♂(R) 19♀, same location, 23.ix.2009, NK. **Gifu**: 1♂(L), Higashishirakawa-mura, Oppara, 27.v.1988, TN. **Shizuoka**: 2♂ 3♂(L) 1♂(I) 1♀, Shimoda-shi, Tateno, 12.iv.2009, S. Inaba. **Mie**: 4♂ 2♂(L) 2♂(I) 2♀, Shima-shi, Isobe-chô, Natsukusa, 5.vii.2008, H. Morita. **Hyôgo**: 2♂ 1♂(I), Asago-shi, Wadayama, Kudawa, Ishibe-jinjya, 3.xi.2006, K. Inazu.& TI. **Shimane**: 1♂ 3♀, Gôtsu-shi, Sakuraechô, Kawagoe, 2.v.1999, SN; 2♂, Oochi-chô, Shiki, 10.viii.1999, SN; 1♂ 1♀, Kawamoto-machi, Inbara, 12.v.1999, SN; 4♂ 1♀, Masuda-shi, Mukaiyokotachô, 7-8.v.2000, SN; 1♂ 4♀, same location, 29.vii.2000, SN; 3♂, Masuda-shi, Yasudomichô, 18.vii.2000, SN; 2♂ 8♀, Masuda-shi, Musochô, 6.v.2001, SN; 2♂ 1♂(L), Masuda-shi, Musochô, Takatsu-gawa R., 27.vii.2000, SN. **Hiroshima**: 1♂(I), Akitakata-shi, Yachiyo-chô, Haji, Haji-dam, 11.vii.2000, SN; 1♂ 2♀, Akitakata-shi, Yoshida-chô, Yoshida, 21.iv.1999, SN; 1♂(L) 1♂(I), Higashihiroshima-shi, Takaya-chô, Nakashima, 13.vii.1999, SN; 1♂, Hiroshima-shi, Asakita-ku, Kabechô, Imaida, 24.iv.1999, SN; 3♂ 1♂(L) 2♀, Kôzan-chô, Uzuto-gawa R., Hattabara-dam, 5.viii.1998, SN; 6♂ 1♂(L) 1♀, same location, 25.vii.2000, SN; 1♂(I), Miyoshi-shi, Minamihatajikimachi, 1.viii.1999, SN; 2♂, same location, 6.x.1999, SN; 1♂ 2♀, Ôtake-shi, Fukase, Kose-gawa R., 8.v.2001, SN; 1♂, same location, 23.vii.2001, SN; 1♂(L) 4♀, Ôtake-shi, Bouroku, Kose-gawa R., 7.v.2001, SN; 5♂ 4♀, Ôtake-shi, Kuritanicho, Yasaka-dam, 28.vii.2000, SN; 1♂, Sera-chô, Io, Hattabara-dam, 26.vii.2000, SN; 2♂ 1♂(L), Yuki-chô, Shimominauchi, 25.iv.1999, SN; 1♂(L), same location, 15.v.2003, TN. **Yamaguchi**: 1♂ 2♀, Iwakuni-shi, Miwachô, One-gawa, Yasaka-dam, 25.ix.2000, SN. **SHIKOKU: Tokushima**: 1♂ 1♂(L), Kitô-son, Takanose-kyô, 18.vii.1998, I. Yamashita. **Kagawa**: 2♂ 1♂(I) 1♀, Takamatsu-shi, Shionoe, 14.ix.2006, NK. **Kôchi**: 11♂ 2♂(L) 6♀, Kami-shi, Monobechô, Befu, 2.viii.2003, I. Yamashita; 2♂(L), Muroto-shi, Sakihama, Sakihama-gawa R., 29.iv.2004, M. Takai; 1♂(L) 1♀, Nankoku-shi, Nakanogawa-rindô, 29.v.2004, M. Takai; 1♂(L), Ochi-chô, Tokoroyama, Ichigaya, 21.vii.2004, K. Nio; 1♂(L), Sukumo-shi, Itchûbara, 28.iv.2001, M. Takai; 1♂, same location, 30.iv.2004, M. Takai; 1♂, Tosashimizu-shi, Mochiishi, Kitayamahigashi, 21.vii.2004, K. Nio. **KYÛSHÛ: Fukuoka**: 1♂ 1♀, Chikushino-shi, Yoshiki, 30.iv.1986, N. Gyotoku; 1♂ 1♀, same location, 9.x.1986, N. Gyotoku; 2♂, Ukiha-shi, Yoshiimachi, Sakurai, Chikuko-gawa R., 8.iv.1997, TN. **Saga**: 4♂ 9♀, Takeo-shi, 22–23.iv.2009, T. Shimizu; 7♂ 1♀, Ureshino-shi, 4.vi.2009, T. Shimizu. **TSUSHIMA**: 5♂ 2♂(L) 2♀, Tsushima-shi, Kamiagata-chô, Sago, upper reaches of Sago-gawa R., 22.vii.2009, R.B. Kuranishi.

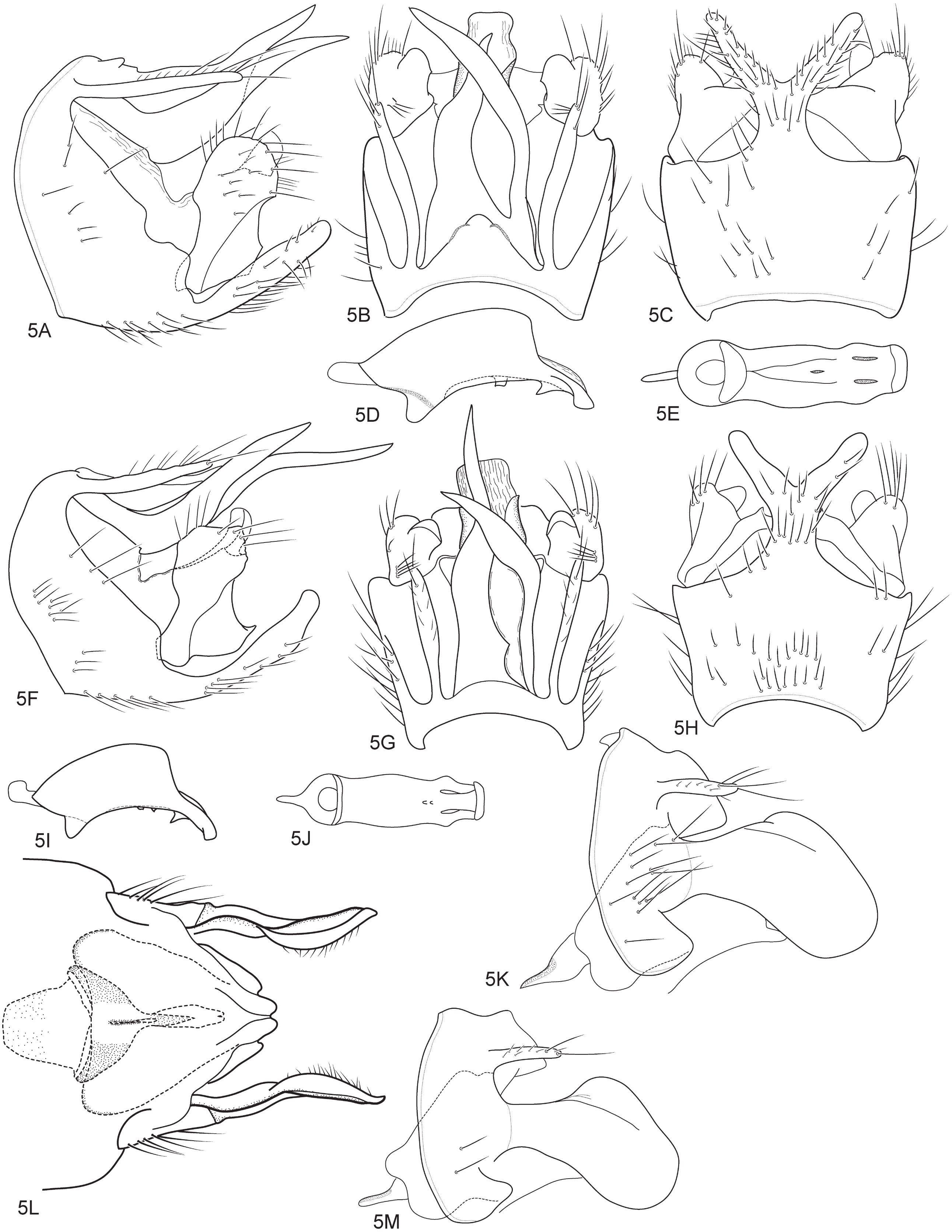

FIGURE 5. *Mystacides azureus* (Linnaeus 1761). 5A–5J, male genitalia: 5A, 5F, left lateral; 5B, 5G, dorsal; 5C, 5H, ventral; 5D, 5I, phallus, left lateral; 5E, 5J, phallus, ventral. 5K–5M, female genitalia: 5K, 5M, left lateral; 5L, ventral. 5A–5E, specimens from Tomakomai-shi, Hokkaidô; 5F–5J, 5M, specimens from Ômachi-shi, Nagano, Honshû; 5K, 5L, specimens from Chitose-shi, Hokkaidô.

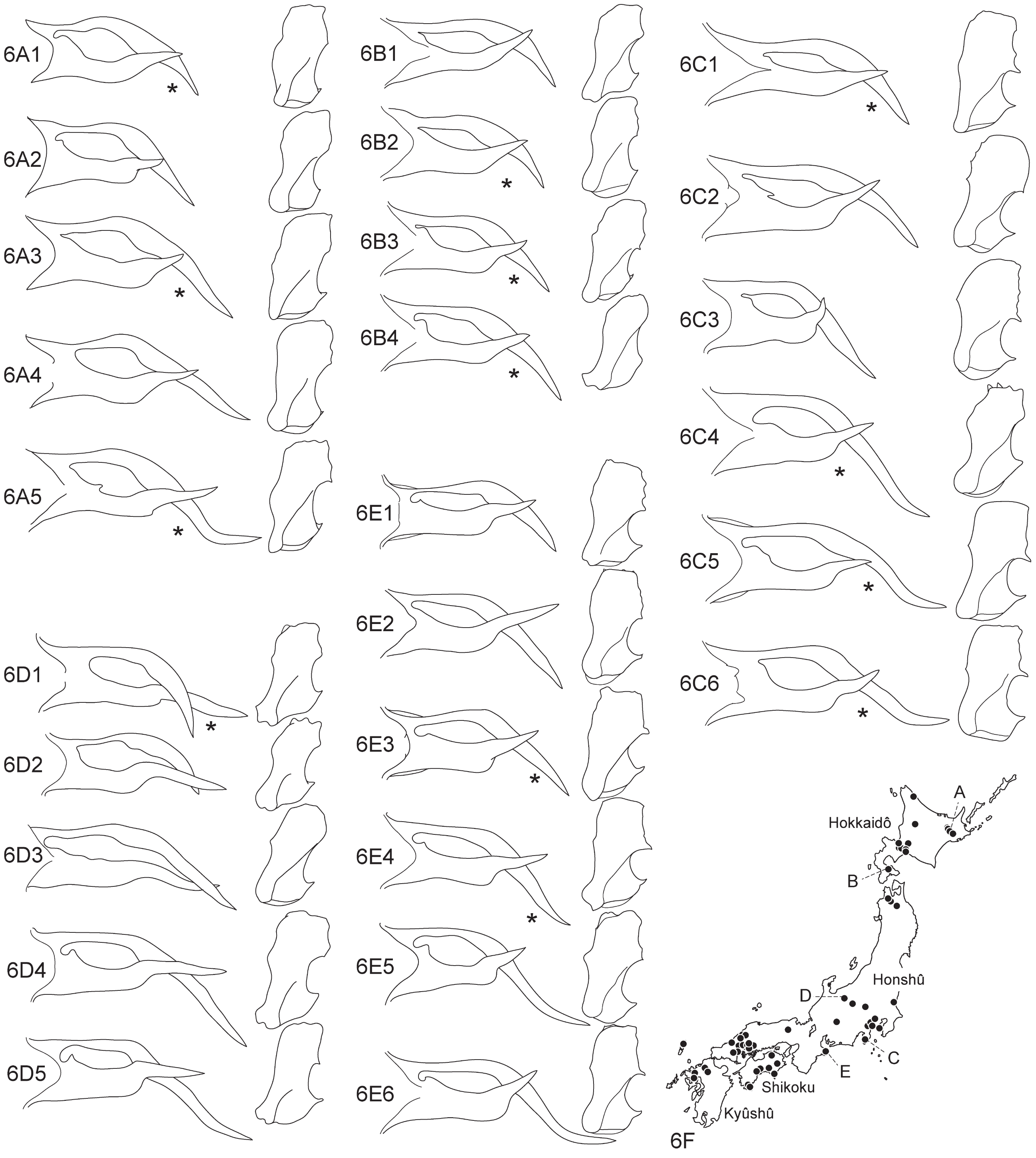

FIGURE 6. Variation of male genitalia in 5 populations and map of collection sites of *Mystacides azureus* (Linnaeus 1761); the dorsal aspect of tergum X (left) and left lateral aspect of left inferior appendage (right) for each individual are shown. 6A1–6A5, Kushiro, Hokkaidô; 6B1–6B4, Nanae-chô, Hokkaidô; 6C1–6C6, Shimoda-shi, Shizuoka, Honshû; 6D1–6D5, Ômachi-shi, Nagano, Honshû; 6E1–6E6, Shima-shi, Mie, Honshû. 6F, map of collection sites. Asterisks indicate mirror-reversed images for easy comparison.

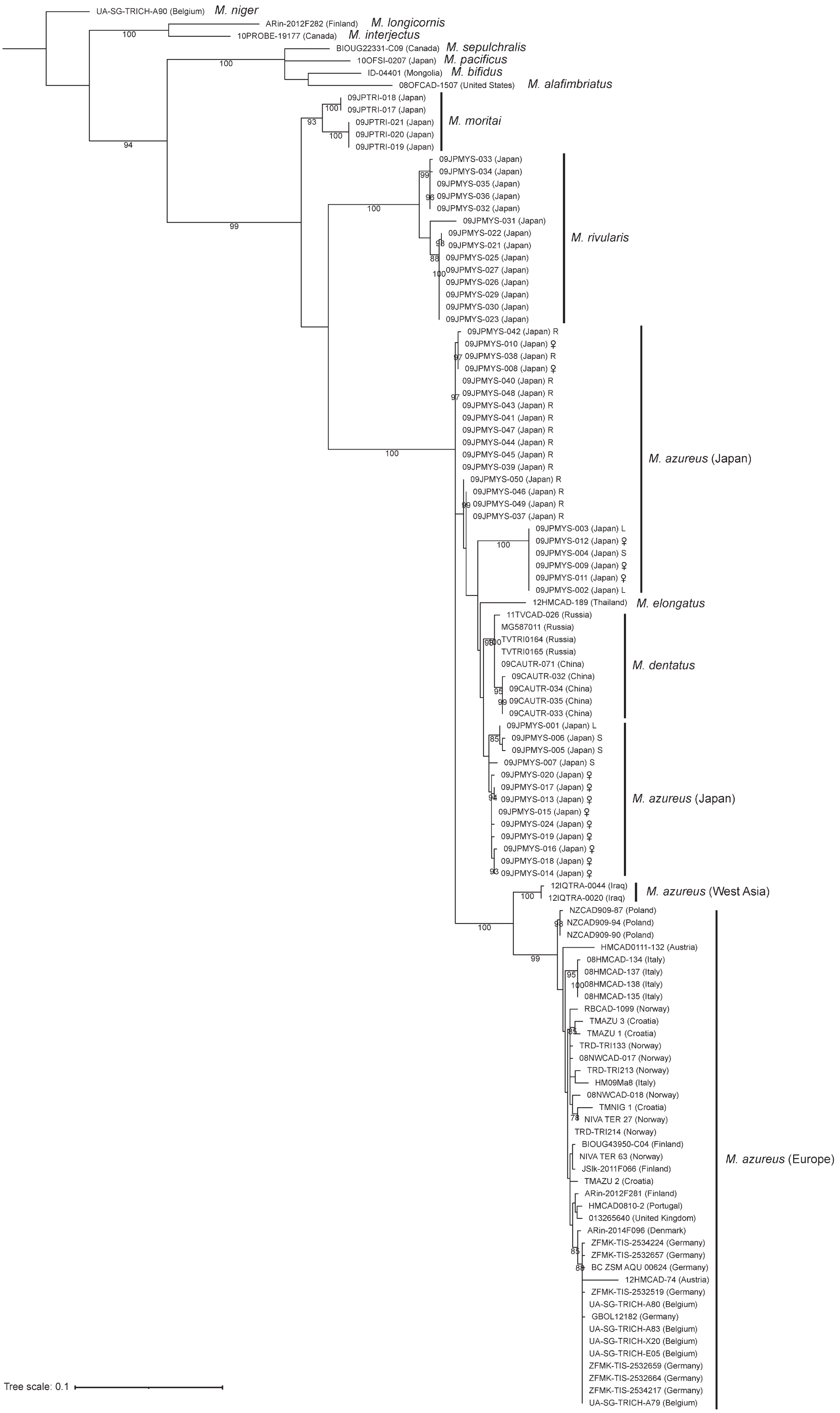

FIGURE 7. The maximum likelihood phylogeny of *Mystacides* based on the COI barcode sequences. For males of *M. azureus* (Linnaeus 1761) from Japan, morphological types of segment X are abbreviated to the right of the country names as follows: S, Short type; L, Long-twist type; R, Reversal type.

Eye measurements

The measurements of the eye length and the distance between eyes revealed that the relative eye size was larger in males than in females in all species and larger in *M. azureus* than in *M. rivularis* **n. sp.** and *M. moritai* **n. sp.** in both sexes (Fig. 1). Moreover, the degree of sexual dimorphism was greater in *M. azureus* than in the other two species. The degree of sexual dimorphism in adult eye size of leptocerid caddisflies has been shown to be related to swarming flight patterns: Species including *M. azureus* that perform vertical zigzagging movements in swarming tend to have larger eyes in males (Gullefors & Petersson 1993). The differences of relative eye size between *M. azureus* and the other two species in this study may also be associated with swarming flight patterns, although the flight patterns of the latter two species are unknown. Furthermore, habitat differences may also be related to their relative eye size. While *M. azureus* mainly inhabits lakes with wide open water surfaces, the latter two species are found in streams and may have a smaller area of mating behavior.

Mitochondrial phylogeny based on DNA barcodes

The maximum likelihood phylogeny based on COI barcode sequences revealed that the *M. azureus* Species Group is monophyletic (Fig. 7) for the five species of this group that were included in the analysis (*i.e*., *M. azureus*, *M. dentatus* Martynov 1924, *M. elongatus*, *M. rivularis* **n. sp.**, and *M. moritai* **n. sp.**). The two new species *M. moritai* **n. sp.** and *M. rivularis* **n. sp.** were successively basal to the remaining *M. azureus* Species Group members, each monophyletic. The remaining species of the *M. azureus* Species Group together form a monophyletic group that was subdivided into two clusters: the East Asian (Japan and China), or East Palearctic-Oriental cluster, and the European plus West Asian (Iraq) or West Palearctic cluster. Members of the European plus West Asian cluster were all *M. azureus* but the East Asian cluster included *M. azureus*, *M. dentatus*, and *M. elongatus*, suggesting a hypothesis of potential cryptic species within this cluster.

In terms of morphological variation in male genitalia of *M. azureus* from Japan, 4 individuals of the short type (09JPMYS004–007) and 3 individuals of the long-twist type (09JPMYS001–003) collected on the same day from a single population, at Morito-gawa, were used for DNA analyses. The sequences of these seven individuals were divided into two mtCOI clusters that did not correspond with their respective morphological types. In addition, two individuals of the long-twist type (09JPMYS002, 003) and an individual of the short type (09JPMYS004) have identical COI sequences. Moreover, individuals of the Ômachi population (09JPMYS037, 042–050), males of which are of the reversal type, are paraphyletic and make a mtCOI cluster with individuals of the Morito-gawa population. As a result, mtCOI sequence evidence supports an alternative hypothesis that each morphological type, based on male tergum X, is merely intraspecific variation in a single species, *M. azureus*. Sequences of other genes should be used to test these competing hypotheses.

In congruence with morphological evidence, our molecular data also suggested that *M. elongatus* and *M. dentatus* from China should be reconsidered whether they are valid species. Because we have not examined the morphology of these two species in detail, a conclusion about their validity is outside the scope of this work and remains left for future work.

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