Zoosymposia 10: 234–242 (2016)
http://www.mapress.com/j/zs/

ISSN 1178-9905 (print edition)
ZOOSYMPOSIA
ISSN 1178-9913 (online edition)

http://dx.doi.org/10.11646/zoosymposia.10.1.21
http://zoobank.org/urn:lsid:zoobank.org:pub:63D72156-5ACA-4327-BCF3-7B31F201A1C7

Redescription of *Triplectides misakianus* (Matsumura 1931) (Trichoptera, Leptoceridae) in Japan with notes on its habitat

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Abstract

Triplectides misakianus (Matsumura 1931) is redescribed on the basis of adults, larvae, and pupae collected in Japan. This report describes the pupal morphology of this species for the first time. A 658-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene as a potential "DNA barcode" is provided. The habitat and conservation of the species are discussed.

Key words: morphology, taxonomy, adult, pupa, larva, coastal wetland, extinction, CO1

Introduction

The long-horned caddisfly genus *Triplectides* Kolenati 1859 (Trichoptera, Leptoceridae) has been recognized to include 83 valid species worldwide (Morse 2016). In Japan, *Tobikera misakiana* Matsumura 1931 was described on the basis of an adult specimen from Misaki, (Miura-shi) Kanagawa (Matsumura 1931), and no other congeners have been recorded. Tsuda (1942) recognized *Tobikera misakiana* as a junior synonym of *Triplectides magna* (Walker 1852), and recorded the species in Kyoto, Shiga, and Tottori. He subsequently described the adult (Tsuda 1950), larva, and case (Tsuda 1959) of this species.

Uenishi (1993) studied the holotype of *T. misakiana* as well as additional material of this species collected from the 1920s to 1940s in Fukuoka, and concluded that *T. misakiana* is a valid species, clearly distinguishable from *T. magnus*.

Yang and Morse (2000) originally described *Triplectides quadratus* from the People's Republic of China. The male genitalia of *T. quadratus* closely resemble those of *T. misakianus*, but the taxonomical relationship between *T. misakianus* and *T. quadratus* remains unclear. In addition, we do not have enough information on female and pupal morphologies of *T. misakianus*. Therefore, in the present paper we describe male and female adults, pupae and larvae of *T. misakianus* in detail to determine the taxonomic status of the species.

In addition, we provide a 658-bp fragment of mitochondrial cytochrome c oxidase subunit I (COI) gene as a potential "DNA barcode" to supplement general morphological information.

Trichopterologists have suspected that *T. misakianus* is extinct because there have been no new records of this species since 1953 and its habitat has suffered considerable damage due to human activities, including land use changes and use of insecticides in paddy fields. After approximately half a century since the last collection record, Morita (1994) found *T. misakianus* in Mie Prefecture and presented a figure of the male genitalia (Morita 1997). Accordingly, in the present paper, we summarize the collection data and habitat of *T. misakianus*, and discuss its conservation.

 Accepted by T. Vshivkova: 14 Dec. 2015; published: 9 Aug. 2016

Material and methods

Specimens used in this study were collected by the authors and our colleagues whose names are given in the text. Later stages of larvae and pupae of *T. misakianus* were sampled with a D-frame net or by hand. Before using the net, we looked for larval cases attached to emergent shore plants.

The relationship between adult and immature stages was established by rearing larvae to adults. Female specimens of *T. misakianus*, which are preserved in 80% ethanol, were dissected and the number of young larvae inside female abdomens was determined. The genital segments of the male and female were viewed after treatment with hot 85% lactic acid. Photographs were taken with an Olympus DP20 digital microscope camera mounted on an Olympus SZX16 stereoscopic microscope. Partially focused serial images were combined with Helicon Focus software (Helicon Soft Ltd.) to produce completely focused photographs.

Terminology for adults, pupae, and larvae mainly follow Yang and Morse (2000), Wiggins (1996a), and Wiggins (1996b), respectively.

Total genomic DNA was extracted from ethanol-preserved tissue and purified using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden). The mitochondrial COI gene was amplified by a polymerase chain reaction (PCR) method using the primer HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') (Folmer *et al*. 1994). PCR products were purified using the ExoSAP-IT (GE Healthcare UK, Buckinghamshire). The purified DNA was sequenced directly by an automated method using the DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare UK, Buckinghamshire) and BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) on an automated sequencer ABI 3130*xl* DNA Analyzer (Perkin Elmer/Applied Biosystems, CA, USA). The sequence chromatographs were assembled using the CLC Workbench software (CLC bio, Aarhus). The COI sequence data of mitochondrial DNA has been submitted to the DNA data bank of JAPAN (DDBJ database).

The holotype of *Tobikera misakiana* deposited in Hokkaido University, Faculty of Agriculture, Systematic Entomology was examined. Newly collected specimens used in the present study are deposited in Natural History Museum and Institute, Chiba (CBM) and partly in the personal collection of the senior author (NK).

Results

Triplectides misakianus (Matsumura 1931)

Figures 1, 2, 3, 4

Tobikera misakiana Matsumura 1931, 1129, female; Tateishi 1934, 76, additional records; Gyotoku 1957, 11, additional records.

Triplectides magna Walker 1842; Tsuda 1942, 287−288, misidentification mentioned by Uenishi (1993); Tsuda 1950, 419, adult.

Notanatolica magna (Walker 1842); Tsuda 1959, 144, larva, case.

Triplectides misakiana (Matsumura 1931); Uenishi 1993, 79, additional records; Uenishi *et al*. 1993, 132, additional records; Morita 1994, 97, additional records; Morita 1997, 96, male; Kuranishi 1999, 43, adult; Tanida 2005, 543, male; Ohtaka *et al*. 2008, 61, additional records.

Ganonema nigripenne Fischer 1972; Kawano *et al*. 2006, misidentification.

Triplectides sp.; Ueda & Miyazaki 2010, 13, larva.

Description. Adult (Fig.1). Body yellowish brown. Body length male 9.6−11.5 mm (n = 13), female 12.5−14.0 mm (n = 15). Head yellowish brown, ocelli absent. Antennae flexible, very long and slender, more than approximately three times as long as folded forewings. Legs yellowish brown, spurs 2, 2, 2. Maximally palpi each with five segments, relative lengths of five segments approximately in male; 1.0 : 2.9 : 3.0 : 1.6 : 2.1, in female; 1.0 : 3.3 : 3.0 : 1.7 : 2.9, in both sexes densely covered with many setae. Length of each forewing of male 14.2 mm−16.0 mm (n = 13), female 15.0−16.0 mm (n = 15); in male, forewing S4 connecting dicoidal and thyridial cells, base of MP separate from S4, but in some specimens connecting to S4; forewing with FI, FII, FV in the male, FI, FII, FIII, FV in the female, hind wing with FI, FII, FIII, FV in both sexes.

Male genitalia. Segment IX rectangular in lateral view, each side with triangular sclerotized strip posterolaterally; tergum IX subtriangular in dorsal view, blunt apically. Segment X thumb-like in lateral view,

rounded apically, with slight constriction in posteroventral margin, suboval in dorsal view, with apicomesal cleft. Preanal appendages long and slender, two-thirds as long as inferior appendages, rounded apically, each with many setae. Subapicodorsal lobe of each inferior appendage oval, rounded apically in lateral and dorsal views, almost as long as main body of appendage, each with many strong setae; harpago arising from ventromesal base of subapicodosal lobe of inferior appendage, hooked apically, directed mesad, tip heavy sclerotized and without sub-apical teeth; mesal ridge of appendage small and triangular in ventral view, rounded apically; basoventral lobe of inferior appendage long and slender, shorter than preanal appendage, rounded apically in lateral and ventral views, each with many strong setae. Phallus tube like, parallel-sided in basal half, slightly constricted in distal one-third in lateral view, rounded apically, with pair of phallotremal sclerites dorsally, ejaculatory duct produced anterodorsally.

Female genitalia. Tergum IX rectangular in lateral view, each with sclerotized concave area posterolaterally, subtriangular in dorsal view, rounded apically, with pair of small papillae posteriorly. Preanal appendages long and oval in dorsal and lateral views, each with many setae. Sensilla-bearing process almost half length as long as preanal appendages, truncated apically, each tip with short 6−7 stout setae. Lamellae semicircular in lateral view, almost as long as sensilla-bearing processes, rounded apically, each with short setae ventrolaterally. Gonopod plate subrectangular in ventral view, depressed at the center posteriorly. Spermathecal sclerite rectangular in ventral view, rounded anteriorly and posteriorly.

Pupa (Fig. 2). Length up to 17.4 mm. Antennae approximately 2.3 times length of body, coiled around posterior end of abdomen. Head: Setal pattern of *T. misakianus* as in Fig. 2C, with one pair of setae on vertex, five pairs on each antennal scape, two pairs of long frontal setae, one pair of setae below and in front of eyes. Labrum rectangular with three long setae at each basolateral corner, three pairs along apicodorsal edge. Mandibles conspicuous, symmetrical, each with acute apical tooth bearing fine serrations along inner edge and with blunt subapical tooth, two long setae on lateral base. Thorax: Forewings reaching abdominal segment VI. Fore and middle tarsi each with hair fringe. Abdomen: Abdominal segment I bearing several fine spines on posterior margin and with weakly sclerotized and wrinkled dorsal plates on posterolateral corners. Paired abdominal hook plates present near anterior margins of segments III−VI and posterior margin of segment V, each with 6−10 small hooks. Lateral fringe on each side of segments III−VIII. Abdominal gills arranged similar to larva, but somewhat shorter and depressed. Dorsum of segment IX with pair of nipple-like, seta-bearing protuberances, ventrolateral portion, near base of each anal process with pair of mound-like processes, each bearing one short and four long setae. Anal processes recurved laterad apically, sharply pointed, each with several minute setae and four long setae on apical portion.

Larva (Fig. 3). Final instar larva. Length 17.1−20.3 mm (n = 5). Head: Width 1.14−1.18 mm (n = 5); dorsally oblong, slightly narrower anteriorly, reddish brown to dark brown with several orange spots; pale patch around each eye. Setae pattern as in Fig. 3B, typical for Leptoceridae. Frontoclypeal apotome as wide behind the constriction as on anterior margin. Ventral apotome widest on anterior margin, gradually narrowing to near middle, distal half rectangular. Labrum with eight setae around the anterior margin, anteroventral edge of labrum with brush of short, secondary setae. Left mandible with three teeth, right mandible with five teeth. Pronotum: Covered by pair of large sclerites; dark brown with light-colored markings; anterior margins and anterolateral corners scalloped, posterior margins with black borders. Fore trochantin horn-shaped. Mesonotum covered by pair of large sclerites. Metanotum with three pairs of sclerites: large, broadly ovate *sa*1 sclerite, each bearing single, very small subapicomesal seta; single long seta at *sa*2 position with extremely small sclerite; and small, tadpole-shaped *sa*3 sclerite, each bearing one long and three short setae. . Metasternum with pair of small, oval sclerites, each with three setae. Legs long, slender, sparsely setose; hind tibiae each divided into two subequal parts. Abdomen cylindrical, segment I with well-developed dorsal and lateral humps; lateral hump sclerites lightly sclerotized, spatulate, each with anterior brush of short, thin setae and single, long, central seta. Abdominal fringe on each side of segments III−VII; segment VIII with lateral tubercles in anterior half, followed by a long fringe. Simple filamentary gills present as follows: Two on each side of segment I (dorsolateral and ventromedial), three on each side of segments II−VIII (dorsolateral, ventrolateral, and ventromedial). Dorsal sclerite of segment IX with five pairs of setae, two long lateral pairs, one mesal pair, and two extremely short pairs between those pairs. Lateral sclerite of each anal proleg well-developed, anal claw with single, large, sharply pointed tooth and one small dorsal accessory hook with small tubercles.

Case (Fig. 4). Case of final instar larva constructed from hollowed-out twig of shrub, stem of cattail (*Typha* spp.), or from several species of emergent plants. Case up to 60 mm, internally lined with silk, and with small lateral hole towards posterior end. Pupal case attached on stems of hydrophytes on pond bottoms.

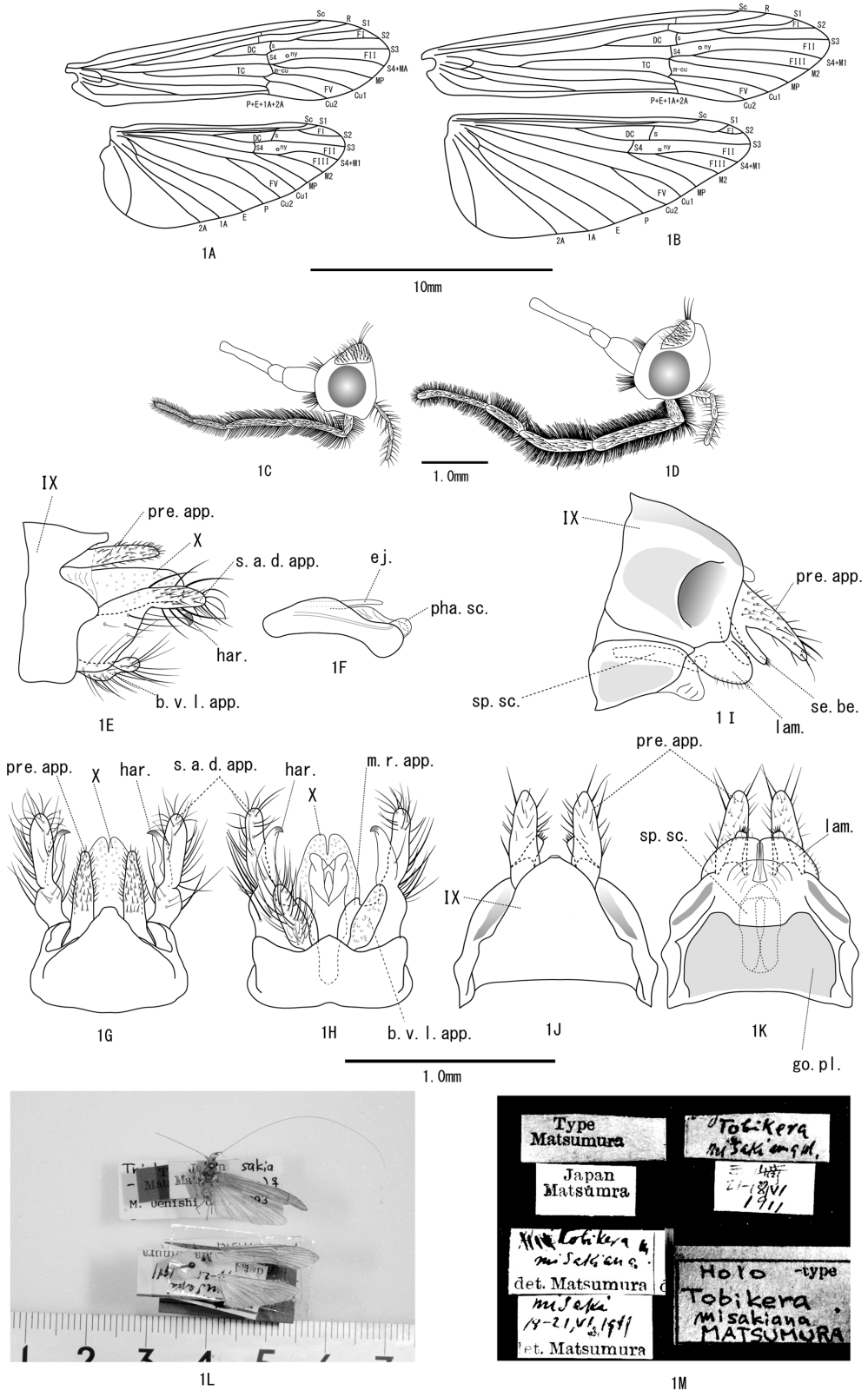
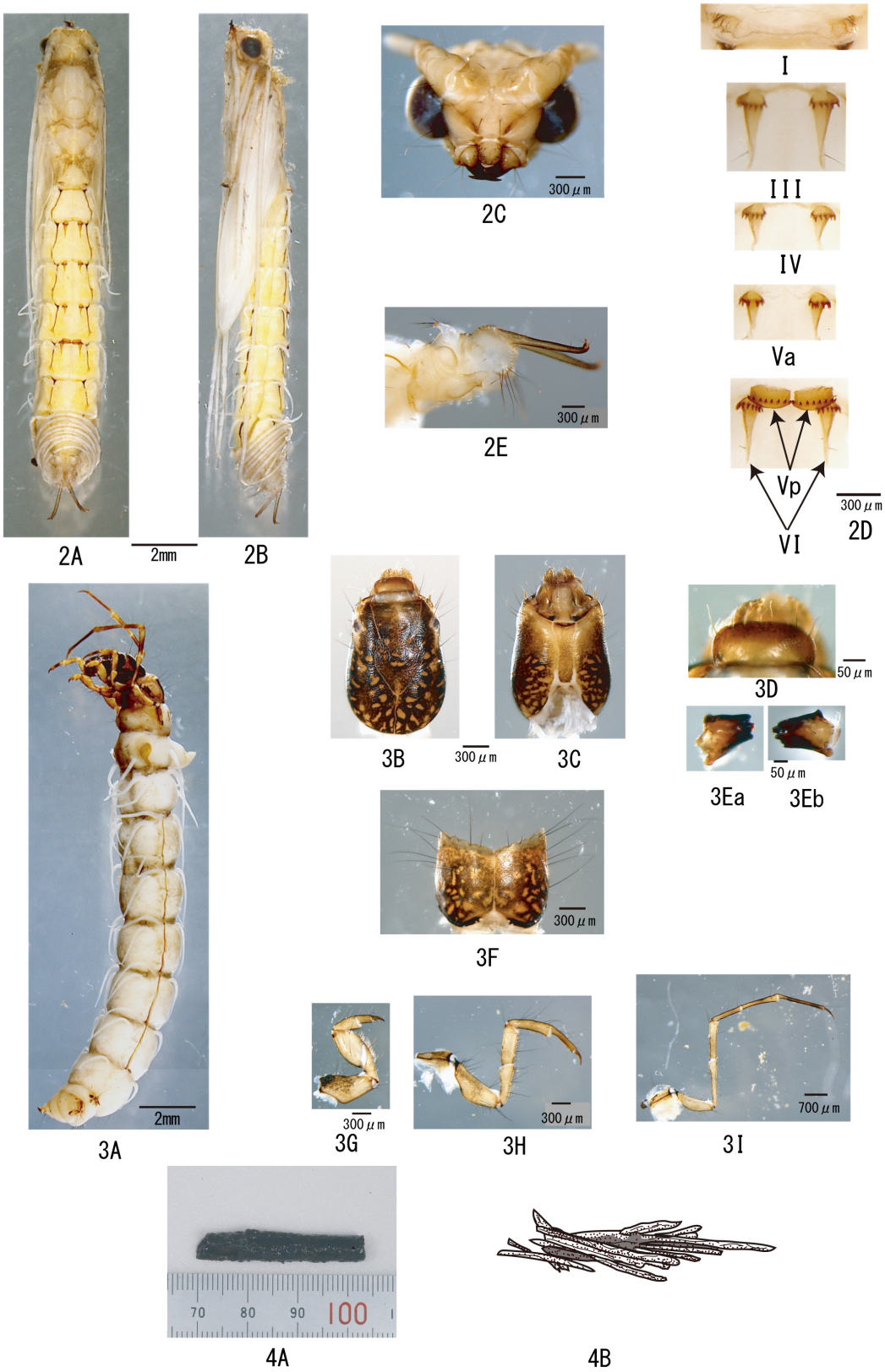


FIGURE 1. *Triplectides misakianus* (Matsumura 1931) adult. 1A, right male fore- and hind wings, dorsal. 1B, right female fore- and hind wings, dorsal. 1C, male head, left lateral. 1D, female head, left lateral. 1E–H, male genitalia: E, left lateral; F: phallus, left lateral; G, dorsal; H, ventral. 1I–K, female genitalia: I, left lateral; J, dorsal; K, ventral. 1L, holotype, female. 1M, specimen data labels pinned together with the holotype specimen. IX = segment IX, X = segment X, pre.app. = preanal appendage, s.a.d.app. = subapicodosal lobe of inferior appendage, b.v.l.app. = basoventral lobe of inferior appendage, m.r.app. = mesal ridge of inferior appendage, har. = harpago, pha. = phallus, ej. = ejaculatory duct, pha.sc. = phallotremal sclerite, se.be. = sensilla-bearing process, lam. = lamella, go.pl. = gonopod plate, sp. sc. = spermathecal sclerite.



FIGURES 2–4. *Triplectides misakianus* (Matsumura 1931) pupa, larva, larval case. 2A–D, pupa: 2A, body, dorsal. 2B, body, left lateral. 2C, head, frontal. 2D, abdominal segments and hook plates. 3A–I, larva: 3A, body, left lateral; 3B, head, dorsal; 3C, head, ventral; 3D, labrum, dorsal; 3E, mandibles: 3Ea, left, left lateral, and 3Eb, right, right lateral; 3F, pronotum, dorsal; G, right front leg, right lateral; H, right middle leg, right lateral; I, right hind leg, right lateral. 4A–B, case: 4A, lateral; 4B, same, redrawn from Tsuda (1959). I = abdominal segment I, III–VI = abdominal hook plates III–VI.

DNA barcode A 658-bp fragment of the COI gene is from a specimen (CBM-146883) collected in Higusa-numa, Yokoshibahikari-machi, Chiba Prefecture. Higusa-numa is *ca*.100 km northeast of the type locality of *T. misakianus*. The sequence data has been deposited as a DNA barcode of *T. misakianus* in the DDBJ Nucleotide Sequence Database under accession number AB778888.

Specimens examined. Holotype (Figs. 1L, M): Female of *Tobikera misakiana* Matsumura 1931; type locality Misaki, Miura-shi, (Miura-hantou or peninsula) **Kanagawa** Prefecture. **Aomori**: 1 female, Hirataki-numa, Tsugaru-shi, 4.ix.2012, H. Iketake (N. Katsuma: NK); 7 males, 11 females, Hiyamizu-numa, Tsugaru-shi, 30.vii.2012, Y. Murakami (NK). **Chiba**: 1 male, Higusa-numa, Yokoshiba-hikari-machi, 25.vii.1999, (R.B. Kuranishi :RBK), CBM-ZI 146904; 8 larvae, ibid, 10.iv.2010, RBK; 16 larvae, 1 pupa, ibid, 1.v.2010, RBK, CBM-ZI 146912−146919; 7 larvae, ibid, 6.v.2010, RBK, CBM-ZI 146920−146921; 2 males, ibid, 7.v.2010 (22. v.2010 emerged), NK; 1 female, ibid, 7.v.2010 (28.v.2010 emerged), NK; 1 female, ibid, 11.vi.2010, RBK, CBM-ZI 146911; 4 males, 3 females, ibid, 22.vi.2010, RBK, CBM-ZI 146883, 146907−146910; 6 males, ibid, 28.v.2010, NK. **Mie**:1 male, Funakoshi-ike, Daio-cho, Shima-shi, 2.vi.1996, RBK, CBM-ZI 146905; 1 larva, *ibid*, 7.v.2008, (H. Morita: HM), CBM-ZI 146906, 3 males, *ibid*, 1.vi.2008, HM, (NK). **Shimane**: 7 larvae, Noishidani-cho, Izumo-shi, 8.vii.2011, (M. Hayashi:MH), CBM-ZI 146922−146924; 5 larvae, Koryo-cho, Izumo-shi, 6.x.2011, MH, CBM-ZI 146925−146927; 1 male, Noishidani-cho, Izumo-shi, 25.V.2012 (8.vi.2012 emerged), MH (NK); 2 females, ibid, 25.V.2012 (30.v.2012 and 8.vi.2012 emerged), MH (NK).

Distribution and habitats. Japan: Honshu, Kyushu. Until the 1950s, *T. misakianus* was recorded in lowland lentic habitat, such as lakes, paddy fields, or even in a pond near an urban area (Tateishi 1934; Tsuda 1942; Gyotoku 1957; Uenishi *et al*. 1993). Tateishi (1934) reported that a lot of *Tobikera misakiana* occurred around a pond from June to October in Yukuhashi, Fukuoka, but the last specimen was collected in 1953. Recently, the distribution of this species has been restricted to coastal lakes or ponds, rich in wetland vegetation (Morita 1994; Kuranishi 1999; Kawano *et al*., 2006; Ueda 2010).

Japanese name. Misaki-tsuno-tobikera.

Bionomics. The species of the *T. australis* Group are well-known as viviparous species (Morse & Neboiss 1982). We confirm that *T. misakianus* is a viviparous species also, and therefore probably belonging to that species group.

Morita (1997) reported that several small larvae were attached to the tip of the female abdomen. We collected four pregnant females and counted 278, 658, 659, and 698 first instar larvae in their abdomens.

In spring (late March−mid May) at Higusa-numa (Chiba), we found several final instar larvae of *T. misakianus* attached to emergent plants just below the water surface. In late May, final instar larvae had moved to the pond bottom and attached to large plant debris, where they grew into pupae.

Discussion

The *T. australis* Group of the genus *Triplectides* was defined by Morse & Neboiss (1982) based on adult morphologies. Comparing those definitions with the morphological characters of *Triplectides misakianus, T. misakianus* has the following combination of characters of the *T. australis* group; in the wing venation of an adult: (1) the posterior angle of the discoidal cell in the forewing is clearly separated from the thyridial cell by a short base of vein S4; (2) tibial spurs are 2, 2, 2 in both sexes; in the male (3) tergum X lacks sharp dorsal or ventral carinae and (4) segment IX has a weakly sclerotized strip on each side; in the female (5) segment VIII has expansive posterior membranes laterally that are invaginated into the abdomen, (6) preanal appendages each have an enlarged and sclerotized sensilla-bearing process ventrally, and (7) the spermathecal sclerite is rectangular; in the pupa (8) the inner margins of the mandibles are serrated, (9) a pair of anterior hook plates on abdominal tergum III are about the same length as those of segment VI, (10) anal prolegs are long and slender, slightly recurved subapically, and (11) abdominal segment IX has one short and four long setae on each side ventrolaterally the near base of each anal process; and in the larva (14) the frontoclypeal apotome is as wide behind the constriction as on the anterior margin, (15) abdominal gills are composed of single filaments, (16) the mesonotum is paler than the pronotum, and (17) the anterolateral corners of the pronotum are scalloped. Therefore, we conclude that *T. misakianus* is a member of the T. *australis* Group.

Triplectides misakianus is closely similar to *T. quadratus* Yang & Morse 2000 from China in the general appearance of its genitalia. However, the male of *T. misakianus* can be distinguished from *T. quadratus* by

having a longer and more slender harpago on each inferior appendage and the apex of the harpago extends close to the tip of the subapicodorsal lobe of the inferior appendage, approximately three-fourth of the length of this appendage in *T. misakianus* (approximately two-thirds in *T. quadratus*). In addition, the preanal appendages are approximately two-thirds the length of the inferior appendages, being longer in *T. misakianus* than in *T. quadratus*. The female of *T. misakianus* is also distinguishable from *T. quadratus* by having a rectangular spermathecal sclerite in ventral view (nearly square in *T. quadratus*). Consequently, it is concluded that *T. misakianus* and *T. quadratus* are different species.

Tsuda (1959) illustrated a larval case of *T. misakianus,* which was not only smaller in size but also had a quite different shape from our study sample (Fig. 4B). We could not find a larval case of such a shape in this species. According to Holzenthal (1988), some neotropical *Triplectides* species inhabit discarded cases of other caddisflies. It is probable that Tsuda (1959) described a discarded case of a limnephilid caddisfly (*e.g.*, *Nothopsyche* sp.) harboring a *T. misakianus* larva.

Matsumura (1931) proposed *Tobikera* as a new name of the genus in his original description of *Triplectides misakianus*. The word *tobikera* means "caddisfly" in Japanese, but he did not express an intention to use "tobikera" for this species. Two possibilities may explain his use of the name: First, the unusually large body size relative to other leptocerid caddisflies; second, the extremely common species distribution in the area of sample collection.

There are no additional specimens since Matsumura's description of the type locality: Misaki, Miura-shi (Miura-hantou or peninsula), Kanagawa Prefecture. Takakuwa *et al*. (2006) stated that *T. misakianus* is an extinct species in Kanagawa Prefecture. Until the 1950s, there were many paddy fields and small ponds in the Misaki area, but they completely disappeared after the 1970s when the paddies and ponds were converted into residential land (Ohba & Miura Peninsula Insect Study Club 2005).

Prof. Matsumura described one tephritid fruit fly species *Trypeta misakiana* Matsumura (1916) collected in Misaki by him (Matsumura 1916), in the same locality where his *Tobikera misakiana* was collected. Shibuya (1928) described one crambid moth *Sybrida misakiensis* Shibuya 1928, and indicated that the specimen of *Sybrida misakiensis* was collected by Prof. Matsumura on the same day and in the same locality as *T. misakiana* (Fig. 1M). Takakuwa *et al*. (2006) reported that both a fruit fly "*Urophora misakiana* (Matumura, 1961)" and a crambid moth "*Datanoides misakiensis*" (Shibuya 1928) were vulnerable species in Kanagawa Prefecture, because there were no additional specimens from either the type locality or any other area of Kanagawa. The extinction of these three species of different ecological niches may signify that human activity has led to large environmental changes in the Misaki.

We reviewed past records of *T. misakianus*. Tateishi (1934) reported *T. misakianus* as the first record from Kyushu and also a lot of individuals of *T. misakianus* occurred around ponds from June to October in Yukuhashi, Fukuoka. In addition, Gyotoku (1957) recorded *T. misakianus* in Fukuoka and reported that the species was not abundant at that time, but was sometimes attracted to light traps in paddy fields. Gyotoku (1957) reported specimen records of *T. misakianus* collected in 1953 at Yoshii-machi, Kanakawa (Ukiha-shi); after this record, there were no additional records from Kyushu. In Kyoto, Tsuda (1942) collected *T. misakianus* in 1936 at Kitashirakawa (urban area of Kyoto) and in 1939 at the Botanical Garden of Kyoto University, but we could find no any evidence of surviving *T. misakianus* in Kyoto. In Shiga, Tsuda (1942) reported that four male specimens of *T. misakianus* were collected in 1940 at Katada by Mr. Yuki, but there is no record after Tsuda (1942) of a *T. misakianus* collection. Therefore, Uenishi (2011) designated *T. misakianus* as an endangered species. In Tottori, Tsuda (1942) said that one male specimen of *T. misakianus* was collected in 1939 in the Togo-ko by Dr. M. Uéno, but there was no additional record from Tottori. A comprehensive review of the past records suggests that *T. misakianus* was common in lowland areas until the 1940s but became abruptly scarcer thereafter. We have been searching for *T. misakianus* based on the past records, but we could not find any new specimens. Therefore, when, after nearly half a century had passed since the last collection record of *T. misakianus*, Morita (1994) found *T. misakianus* in Funakoshi-ike, Daio-cho, Mie prefecture, we were very surprised!

Since Morita's (1994) discovery, *T. misakianus* has been found in five localities in Japan, being common in (1) coastal lakes or ponds, (2) in areas of rich wetland vegetation, and (3) with low human impact. At present, such an environment is extremely rare in Japan because wetlands are threatened by several human activities including land improvement. These wetlands have also become isolated and have lost continuity with other ponds. We should consider the value of coastal wetlands and their biodiversity. The species merits ecological study and conservation measures.

FIGURE 5. Distribution map. ▲= Recorded before 1940s, ●= Recorded after 1990s.

Acknowledgements

We wish to express our sincere gratitude to Dr. A. Ohtaka, Hirosaki University, for a critical reading of the manuscript, and Dr. T. Nozaki, Kanagawa and Mr. M. Uenishi for their valuable advice and information. Our thanks are also due to Dr. Yang Lian-fang, Nanjing Agricultural University, People's Republic of China, for her kind information about Chinese *Triplectides* species and Dr. M. Hayashi, Hoshizaki Green Foundation, Mrs. H. Morita, Mie; Y. Murakami, Aomori; H. Iketake, Aichi for gifts and loans of specimens. This work was supported in part by JSPS KAKENHI Grant Number 15K06940 (R. B. KURANISHI).

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