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A revision of the *Monopis monachella* species complex (Lepidoptera: Tineidae) from China

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The *Monopis monachella* species complex from China is revised, and its relationship to other species complexes of the genus *Monopis* is discussed with reference to morphological, and molecular evidence. Principal component analysis on all available specimens provided supporting evidence for the existence of three species, one of which is described as new: *Monopis iunctio* Huang & Hirowatari **sp. nov.** All species are either diagnosed or described, and illustrated, and information is given on their distribution and host range. Additional information is given on the biology and larval stages of *Monopis longella*. A preliminary phylogenetic study based on mitochondrial cytochrome c oxidase subunit I gene (CO1) sequence data and a key to the species of the *M. monachella* species complex in China is presented. The types of the new species have been deposited in the Institute of Entomology, Hunan Agricultural University, China and Entomological Laboratory, Osaka Prefecture University, Japan.

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ADDITIONAL KEYWORDS: biology - COI mtDNA - new species - taxonomy.

INTRODUCTION

The genus *Monopis* was established by Hübner in 1825 with *Tinea rusticella* Hübner, 1796 as its type species. It is cosmopolitan in distribution but poorly represented in the New World, consisting of some 80 nominal species (Robinson, 2008), and characterized by the forewing with a characteristic subhyaline spot in the discoidal cell and M_3 stalked with CuA₁,

rod-shaped saccus and inner surface of valva with dense patch of setae in the male genitalia. The genus *Monopis* was described in detail by Robinson & Nielsen (1993), and separated into several species complexes, of which the *M. monachella* species complex is one of the most difficult groups to identify (Gaedike, 2000; Gaedike & Karsholt, 2001; Xiao & Li, 2006). The species complex is characterized by the white vertex and thorax, and the forewing with a large white mark at the costa. In this paper, the *M. monachella* species complex from China is revised based on morphological and molecular data. The

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species complex was found to consist of three species, including one new species. The wing venation and genitalia are redescribed and illustrated based on the materials obtained from China.

MATERIAL AND METHODS TAXON SAMPLING

Adult specimens were collected by light trapping and sweeping, or reared in the laboratory from larvae collected in the field. All specimens examined in this study were preserved in the Department of Entomology, South China Agricultural University, China (SCAU), Institute of Entomology, Hunan Agricultural University, China (HUNAU), National Science Museum (Tokyo), Tokyo, Japan (NSMT), and Entomological Laboratory. Osaka Prefecture University. Japan (OPU). Types deposited in BMNH were also examined and the types of the new species were deposited in HUNAU and OPU. DNA and tissues collection have been deposited in OPU, HUNAU, and SCAU. Terminology used in descriptions of morphology follows Robinson & Nielsen (1993). Measurements (in millimetres) were carried out using a binocular microscope. Photographs of adults were taken with a Nikon Coolpix 4500 digital camera. All figures of wing venations and photos of genitalia were carried out using a binocular microscope.

SAMPLES FOR MOLECULAR PHYLOGENETIC STUDY

All three Chinese Monopis monachella speciescomplex species, each represented by one to 11 populations, were sampled for phylogenetic analyses. Three *Monopis congestella* species-complex species, two Monopis flavidorsalis species-complex species, and one Monopis hemicitra species-complex species, each represented by one to two populations, were used for comparison, and Niditinea baryspilas and Phereoeca uterella as outgroups. The collection data for all these samples are shown in Table 1. The adults were killed by ammonia, and the right legs were immediately detached and preserved in tubes containing 99.9% ethyl alcohol, which were temporarily stored at -20 °C for DNA extraction. The adults were spread, dried, and stored in boxes under natural conditions as voucher specimens. Two dried specimens preserved in OPU were also used for DNA extraction.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total genomic DNA was extracted from each individual using the Blood & Tissue Genomic DNA Extraction Miniprep System (VIOGENE). The primers used for the PCR amplification and sequencing to obtain DNA sequences of cytochrome c oxidase subunit I gene (CO1) in this study were as follows: LCO1490 [forward (fwd.); position 5'-3': 1490-1514] 5'-GGTCAACAAATCATAAAGATATTGG-3'; HCO₂ 198 [reverse (rev.); position 5'-3': 806-832] 5'-TAAA CTTCAGGGTGACCAAAAAATCA-3' (Folmer et al., 1994). The PCR products were cleaned up by using the PCR-MTM Clean Up System (VIOGENE). The products of the sequencing amplification protocol (thermal cycling) were purified and melted with Hi-Di formamide for sequence analysis. The sequences were determined using an ABI 3100 genetic analyzer capillary sequencer and an ABI 3730 automatic sequencer, and aligned using the software ClustalX 1.83 (Thompson et al., 1997).

PHYLOGENETIC ANALYSIS

In this study, evolutionary history was inferred using the neighbor-joining (NJ) (Saitou & Nei, 1987) and the minimum evolution (ME) methods (Rzhetsky & Nei, 1992). The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analysed (Felsenstein, 1985). The percentage of replicate trees is shown above the branches (Felsenstein, 1985). The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Takezaki, Rzhetsky & Nei, 2004). The NJ tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The ME tree was searched using the close-neighbourinterchange (CNI) algorithm (Nei & Kumar, 2000). Codon positions were first + second + third + noncoding. There was a total of 658 positions in the final data set with no gaps. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007). The NJ and ME trees with Niditinea baryspilas and Phereoeca uterella as outgroups were reconstructed using equally weighted as implemented.

TAXONOMIC ACCOUNT

MONOPIS MONACHELLA SPECIES COMPLEX

Diagnosis: Medium-sized species. Vertex and frons roughly covered with erect white piliform scales. Antenna filiform, about $0.8 \times$ forewing in length, pecten present, and each flagellomere covered with a row of short scales. Maxillary palpus developed, five-segmented. Proboscis developed. Labial palpus projecting, the second segment densely covered with lateral and apical bristles. Wings elongate, with moderately rounded apex. Forewing pattern simple, ground colour purple black with a large rectangular oblique white marking (including the subhyaline spot at the end of discoidal cell) on the costa from 2/7 to 6/7

DNA number	Таха	Collectors	Collection locality	Collection dates	GenBank accession no. (CO1)
412 437	Niditinea baryspilas-OK, Japan Phereoeca uterella-HAN, China	G.H. Huang <i>et al.</i> G.H. Huang	Sakai, Osaka, JAPAN (100 m asl) Jiangfengling, Hainan, CHINA (970 m asl)	13.vii.2007 20.x.2006	HM016800 HM016801
337 338	Monopis sp.2-HAN, China Monopis monachella-GD, China	G.H. Huang <i>et al.</i> G.H. Huang <i>et al</i> .	Jiangfengling, Hainan, CHINA (970 m asl) Sanvatane, Conchua, Guanedone, CHINA (330 m asl).	20.x.2006 27.iii.2006	HM016803 $HM016804$
339	Monopis iunctio-HAN, China	G.H. Huang	Bawangling, Hainan, CHINA (560 m asl).	25.x.2006	HM016805
340	Monopis longella-HAN, China	G.H. Huang	Bawangling, Hainan, CHINA (1000 m asl).	26.x.2006	HM016806
$341 \\ 342$	<i>M. longella</i> -HAN, China <i>M. longella</i> -GX, China	G.H. Huang <i>et al.</i> G.H. Huang <i>et al.</i>	Jiangtengling, Hainan, CHINA (970 m asl). Mao'ershan, Guangxi, CHINA (320 m asl).	20.x.2006 26.vi.2005	HM016807 HM016808
343	Monopis hemicitra-HAN, China	G.H. Huang et al.	Bawangling, Hainan, CHINA (1000 m asl)	20.v.2005	HM016809
345	M. longella-GD, China	G.H. Huang et al.	Nankunshan, Guangdong, CHINA (510 m asl).	17.iii.2006	HM016810
346 347	<i>M. longella</i> -HAN, China <i>M. hemicitra</i> -HAN, China	L.S. Chen G H Huang <i>et al</i>	Yinggeling, Hainan, CHINA (1000 m asl) Bawanơlino' Hainan' CHINA (1000 m asl)	20.v.2005 20 v 2005	HM0168112 HM016812
402	M. longella-HKD, Japan	T. Hirowatari et al.	Hokkaido, Horoka, Kamishihoro-cho, JAPAN (700 m asl)	17.vii.2000	HM016813
403	M. longella-AT, Japan	T. Sugiyama	Takikyanbuzyo, Takimati, Okazaki, Aichi, JAPAN (100 m asl)	26.vi.2007	HM016814
404	<i>M. longella</i> -AT, Japan	T. Sugiyama	Takimatioku, Takimati, Okazaki, Aichi, JAPAN (100 m asl)	26.vi.2007	HM016815
407	Monopis flavidorsalis-AT, Japan	T. Sugiyama	Takikyanbuzyo, Takimati, Okazaki, Aichi, JAPAN (100 m asl)	10.vii.2007	HM016802
408	M. flavidorsalis-AT, Japan	T. Sugiyama	Takimatioku, Takimati, Okazaki, Aichi, JAPAN (100 m asl)	12.vii.2007	HM016820
409	Monopis congestella-AT, Japan	T. Sugiyama	Takimatioku, Takimati, Okazaki, Aichi, JAPAN (100 m asl)	12.vii.2007	HM016821
501	M. longella-HUN, China	G.H. Huang	Yingzuijie, Hunan, CHINA (300 m asl)	13.vii.2008	HM016816
502	M. longella-GD, China	G.H. Huang	Nanling, Guangdong, CHINA (1000 m asl)	27.vii.2008	HM016817
504 505	<i>M. monachella</i> -HN, China <i>M. lonsella</i> -BKPVietnam	T. Hirowatari B W Lee	Diaoluoshan, Hainan, CHINA (300 m asl) Bahe Bac Kan Prefecture VIRTNAM (600 m asl)	18.iii.2003 2 v 2006	HM016818 HM016819
H009 H028	Monopis zagulajevi-HEN, China Monopis sp.1-HUN, China	G.H. Huang G.H. Huang	Baiyunshan Nature Reserve, Henan, CHINA (900 m asl) Badagongshan Nature Reserve, Hunan, CHINA	17.viii.2008 26.v.2009	HM016822 HM016823
	4	D	(1000 m asl)		
H029	<i>Monopis</i> sp.1-HUN, China	G.H. Huang	Badagongshan Nature Reserve, Hunan, CHINA (1000 m asl)	26.v.2009	HM016824
H068	M. zagulajevi-HEN, China	G.H. Huang	Baiyunshan Nature Reserve, Henan, CHINA (900 m asl)	16.viii.2008	HM016825

Table 1. Names and collection localities for taxa used in total evidence analysis

	Key to species of <i>Monopis monachella</i> species complex in China
1.	Brownish macula in white marking on upper side of forewing absent
_	Brownish macula in white marking on upper side of forewing present
2.	Brownish macula included in white marking completely
_	Brownish macula connected with outer black margin

dilated in middle where it reaches half-way across wing; all veins present, discoidal cell without chorda, R_5 and M_1 stalked, M_3 and CuA_1 stalked. Hindwing broad with complete venation. Eighth abdominal segment in male without coremata. Male genitalia with uncus bilobed, hook-like, tapered to a pair of sharply pointed apical processes; gnathos arms well developed basal 3/4 triangular and apical 1/4 stout needle-shaped; subscaphium poorly developed, indistinct; tegumen and vinculum fused into a ring; tegumen with anterior margin strongly emarginated; saccus long, rod-shaped; valva simple, rectangular, with apodeme weakly developed, transtilla absent; juxta weakly sclerotized, fused with vinculum; aedeagus almost nearly straight. Female genitalia without corethrogyne, eighth tergum developed, antrum present, ovipositor short, corpus bursae with some modified signa.

Distribution: India, nearly whole Palaearctic from Europe to Far East, Indonesia, Central African Republic, Zaire.

Remarks: Amongst the family Tineidae, it is very difficult to identify members of the *M. monachella* species complex because of individual variations and the similarity of the genitalia. The complex consists of six species [*M. longella* (Walker, 1863); *M. pavlovskii* Zagulajev, 1955; *M. pseudagyrta* Meyrick, 1919; *M. monachella* (Hübner, 1796); *M. monacha* Zagulajev, 1972; *M. mediella* (Fabricius, 1794)] based on the black spot in the forewing and brightness of the hind wing in Asia. Xiao & Li (2006) considered all the differences in appearance amongst the species of *M. monachella* species complex to be individual variation of one species.

Most of the larvae of the subfamily Tineidae feed on a variety of foods, usually consisting of either keratin or chitin including wool, fur, feather, horn, and insect debris, etc. Hinton (1956) described the larvae of 20 species. Petersen (1963) reported some species of several genera (*Tinea*, *Niditinea*, *Tineola*, *Monopis*, *Trichophaga* etc.) collected from nests of Hymenoptera. Robinson (1980) reported some cavedwelling species feeding on bat guano. The larvae of the genus *Crypsithyris* are usually lichenophagous (Fletcher, 1933; Sakai & Saigusa, 2002). Sakai & Saigusa (2002) reported the species *Crypsithyris* crococoma Meyrick, 1934 feeding on fallen leaves. Many tineine larvae are case-bearing, but Pelham-Clinton (1985) reported that *Tinea pallescentella* only builds a case as a temporary shelter for habitation during moulting. The larvae of *Monopis* species are keratophagous and chitinophagous (Robinson & Nielsen, 1993). They are widely found in birds' nests living in a flattened slightly ovate portable case or in a silken tubular tunnel, and perhaps feed on feathers, fur, wool, hides, guano etc. In addition, 12 species have been recorded to be larviparous (Diakonoff, 1955; Căpuşe, 1966; Gozmány & Vári, 1973; Robinson & Nielsen, 1993; Xiao & Li, 2006).

MONOPIS MONACHELLA (HÜBNER, 1796) (FIGS 1A, 2A, 3A, B)

Monopis monachella (Hübner, 1796), Sammlung europäischer Schmetterlinge, 8: 65. Type locality: Europe.

Alucita mediella Fabricius, 1794, Entomologia Systematica, 3 (2): 337. Type locality: Germany.

Monopis monacha Zagulajev, 1972, Trudy Zoologicheskogo Instituta, Leningrad, 52: 352. Type locality: Indonesia (Java). **New synonym**.

Diagnosis: Medium-sized species, very similar to *M. longella* and *M. iunctio* in forewing colour pattern, but distinguished easily from them by the following characters: a brownish macula in the white marking on upper side of forewing absent in *M. monachella*, present in *M. longella* and *M. iunctio*; the former with the brownish macula entirely included in the white marking, but the latter with the brownish macula connected with the outer margin black areas of the whitish marker at the apical margin.

Description: Wingspan 13.5–14.8 mm in male, 14.3–15.6 mm in female; forewing length 6.2–6.9 mm in male, 6.5–7.2 mm in female; length of antenna 5.0–5.4 mm in male, 5.2–5.8 mm in female (Fig. 1A).

Male. Head: vertex and frons white. Antenna, scape yellowish white with pecten consisting of more ten bristles. Maxillary palpus brownish yellow. Labial palpus yellowish white mesally and dark grey laterally with apex yellow, and the bristles on the second segments laterally and apically present.

REVISION OF M. MONACHELLA SPECIES COMPLEX 5



Figure 1. A-C, adults of *Monopis monachella* species complex spp. A, *Monopis monachella* (Hübner, 1796); B, *Monopis longella* (Walker, 1863); C, *Monopis iunctio* Huang & Hirowatari sp. nov., male, holotype; D-F, ecological photos of *Monopis longella* (Walker, 1863) in Japan. D, immature stages and habitats; E, larval case; F, adult with the habitat.

Thorax: mesonotum and tegula white; tegula with basal 1/4 black. Fore and midleg covered with brownish grey scales; hindleg yellowish white. Forewing elongate about $2.8-3.0 \times$ as long as wide including fringes (about $3.1-3.3 \times$ as long as wide excluding fringe), glossy brownish black except a large rectangular oblique white marking (including the subhyaline spot) present at costa from basal 3/8 to 7/8; fringe short. Hindwing $2.1-2.4 \times$ as long as wide including fringe, $3.1-3.4 \times$ as long as wide excluding fringe; ground colour glossy pale grey to golden yellow.

Abdomen: dorsum pale yellow irrorated with brownish black scales, the end with short yellow scales covering the genitalia, greyish pale ventrally.

Genitalia (Fig. 3A): uncus about $1/2 \times$ length of valva. Saccus long, about $2.0 \times$ length of valva. Valva about $3 \times$ width; inner surface of valva membranous on central portion, and setose on costa and distal 1/4.

Aedeagus moderately thick and cylindrical, about $1.2 \times$ length of valva in length, bearing granular microtrichia on apical portion; vesica with a cornutus consisting of great number of minute to medium-sized spinules.

Female. Very similar to male except a little larger and frenulum consisting of two or three bristles.

Genitalia (Fig. 3B): eighth abdominal segment broad with posterior half setose. Ovipositor short with papillae anales setose. Bursa copulatrix $2.8 \times$ length of apophyses anteriores; ostium surrounded by stout bristles; antrum reaching to basal 1/3 of VII abdominal segment; corpus bursae with three to five thorn-shaped signa, transversely encircling subapical portions of corpus bursae.

Material examined: CHINA: 1 male, Sanyatang, Conghua, Guangdong, 300 m altitude, 18.iii.2006,



Figure 2. Wing venation of *Monopis monachella* species complex spp. A, *Monopis monachella* (Hübner, 1796), male; B, *Monopis longella* (Walker, 1863), male; C, *Monopis iunctio* Huang & Hirowatari sp. nov., paratype, male.

light trapping, G.H. Huang & T. Hirowatari leg. (legulus); 1 female, Diaoluoshan, Forest Bureau, Hainan, 300 m, 18.iii.2003, T. Hirowatari leg. All specimens preserved in SCAU and HUNAU.

JAPAN: 1 male, Hokkaido, Akan-gun, Kushiroshitsugen, 21–26.vii.1991, Y. Arita leg.; 1 male, Midorigaoka, Obihiro, 25.vii.1993, T. Hirowatari leg.; 1 male, Horoka, Kamishihoro-cho, 17.vii.2000, light trapping, T. Hirowatari, N.H. Ahn, Y. Miyamoto, H. Okamoto & K. Yamada leg.; 1 female, Iriomote, Komi, Okinawa Prefecture, 16.xii.1977, T. Saito leg.; 1 male, Yonakuni Island, Tabaru-gawa, Ryukyu, 15.v.1963, Y. Arita leg. All specimens preserved in OPU and NSMT. NEPAL: 1 male, Gandaki, Pokhara city, 850 m altitude, 16–19.vi.1994, light trapping, T. Ueda & Y.S. Bae leg. The specimen preserved in OPU.

THAILAND: 1 male, Nakhon Nayok, Khao Yai, 800 m, 7.viii.1987, S. Moriuti, T. Saito, Y. Arita & Y. Yoshiyasu leg.; 1 male, Chaiyaphum, Chulabhorn Dam, 700 m, 14.viii.1987, S. Moriuti, T. Saito, Y. Arita & Y. Yoshiyasu leg. The specimens preserved in OPU.

Distribution: China (Heilongjiang, Xinjiang, Tianjin, Hubei, Shandong, Henan, Shaanxi, Hebei, Sichuan, Guizhou, Yunnan, Tibet, Zhejiang, Hunan, Taiwan, Guangdong, Guangxi, Hainan); Japan; Philippines; New Guinea; Indonesia; Burma; Thailand; Nepal; India; Sri Lanka; Central Asia; Europe; Africa; USA; Samoa. *Remarks:* This species is characterized by the glossy brownish black forewings, with one large white trapezoid spot along costal margin from 3/8 to 7/8 and the ductus bursae in the female bearing one segment of transversal folds close to antrum. Xiao & Li (2006) reported this species from China but they overlooked species differences and treated some of the *M. monachella* species complex as only one species: *M. monachella*.

MONOPIS LONGELLA (WALKER, 1863) (FIGS 1B, 1D–F, 2B, 3C, D)

Tinea longella Walker, 1863, List of the Specimens of Lepidopterous insects in . . . British Museum, 28: 479. Type locality: India.

Monopis pseudagyrta Meyrick, 1919, Exotic Microlepidoptera, 2: 240. Type locality: India.

Monopis pavlovskii Zagulajev, 1955, Trudy Zoologicheskogo Instituta, Leningrad, 21: 282. Type locality: fUSSR. New synonym.

Diagnosis: See the diagnosis of M. monachella.

Description: Wingspan 14.0–16.0 mm in male, 16.0– 18.5 mm in female; forewing length 6.6–7.5 mm in male, 7.5–8.5 mm in female; length of antenna 5.3– 5.9 mm in male, 5.9–6.5 mm in female (Fig. 1B).

Male. Head: vertex and frons roughly covered with white hairs, mixed with a few grey hairs surrounded



Figure 3. Genitalia of *Monopis monachella* species complex spp. A, *Monopis monachella* (Hübner, 1796), male; B, ditto, female; C, *Monopis longella* (Walker, 1863), male; D, ditto, female; E, *Monopis iunctio* Huang & Hirowatari sp. nov., male, holotype.

eye margins. Antenna smooth, scape yellowish white with pecten consisting of some ten grey bristles. Maxillary palpus yellowish white. Labial palpus drooping or forwardly directed with apex yellow, covered with yellowish white scales mesally, dark grey scales laterally; the first and second segments with more than ten black bristles laterally and apically.

Thorax: mesonotum white, anterior and posterior end dark brownish grey; tegula white, basal 1/3 black. Fore and midleg extensively covered with dark grey scales, apical portion of each tarsomere yellowish white; hindleg covered with yellowish white scales, mixed with greyish pale white; hind tibia bearing dense, long, yellowish white hairs dorsally and ventrally. Forewing about $2.7-2.9 \times$ as long as wide including fringe (about $3.0-3.2 \times$ as long as wide excluding fringe), ground colour glossy brownish black, a large rectangular oblique white marking (including the subhyaline spot) present at costa from basal 2/7 to 6/7, which includes a brown macula along costa from basal 3/7 to 5/7; fringe short, consisting of apically six to eight dentated dark grey scales. Hindwing 2.1–2.3 times as long as wide including fringe, 3.1–3.4 times as long as wide excluding fringe; Rs and M_1 independent basally; 1A+2A sinuate (Fig. 2B); ground colour glossy dark grey to pale grey, with basal 3/5 of costa lightly yellowish white; fringe fairly short, consisting of greyish white scales with apex usually 3–4 forked.

Abdomen: dorsum glossy grey with short pale white scales covering the genitalia, pale greyish ventrally.

Genitalia (Fig. 3C): uncus about $2/7 \times$ length of valva. Vinculum narrow. Saccus long, with apex slender, about $2.3 \times$ length of valva. Juxta narrow. Valva about $3.5 \times$ width, broadly rounded distally; inner surface of valva membranous on central portion, and setose on costa and distal 1/3. Aedeagus moderately thick and cylindrical, about $2 \times$ length of valva, bearing granular microtrichia on apical portion; vesica with a cornutus consisting of great number of minute to medium-sized spinules.

Female. Similar to male, but larger and frenulum consisting of three bristles.

Genitalia (Fig. 3D): eighth abdominal sternum broad with posterior half setose. Ovipositor short with papillae anales setose. Bursa copulatrix $2.5 \times$ length of apophyses anteriores; ostium surrounded by a few bristles; antrum reaching to middle of VII abdominal segment; corpus bursae with four to five thorn-shaped signa, transversely encircling subapical portions of corpus bursae.

Material examined: JAPAN: 1 male, Hokkaido, Horoka, Kamishihoro-cho, 17.vii.2000, light trapping, T. Hirowatari et al. leg.; 1 male, Kiba, Komatsu C., Ishikawa Prefecture, 20.v.1983, A. Tomisawa leg.; 1 female, Urayama, Nagoya, Aichi Prefecture, 8.v.1973, K. Tobi leg.; 1 male, Ohsima, Wakayama Prefecture, 30.ix.1971, F. Komai leg.; 198 males 325 females, Honshu, 7.vii.–13.viii.2007, breeding, G.H. Huang leg.; 1 male, Yamatetyo, Osaka, 5.ix.1978, light trapping, S. Moriuti leg.; 1 female, Oyamada, Kagoshima Prefecture, 4.vi.1984, T. Nakahara leg.; 1 male, Yona, Okinawa Prefecture, 18–21.x.1973, M. Owada leg. All the specimens preserved in OPU and NSMT.

CHINA: Guangdong: 1 male, Nankunshan Nature Reserve (N.R.), 500 m above sea level (asl), 17.iii.2006, light trapping, G.H. Huang & T. Hirowatari leg.; 1 male, Nankunshan N.R., 550 m asl, 30.iii.2004, M. Wang & L.S. Chen leg.; 1 female, Nanling N.R., 1300 m asl, 22.vii.2004, G. H. Huang, M. Wang & H. Lin leg.; 1 male, Guangzhou, 60 m asl, 20.xi.2004, M. Wang leg.; 1 male, Nanling N.R., 1000 m asl, 27.vii.2008, G. H. Huang leg. Guangxi: 1 male, Mao'ershan N.R., 320 m asl, 26.vi.2005, G.H. Huang, L.S. Chen & J.X. Liu leg. Hainan: 1 male, Bawangling N.R., 1000 m asl, 26.x.2006, G.H. Huang and Z. Li leg.; 1 male, Qingjie, Wuzhishan city, 1000 m asl, 11-14.v.2005, M. Wang & L.S. Chen leg.; 6 males 1 female, Jianfengling, 970 m asl. 19-21.x.2006, G.H. Huang & T. Hirowatari leg. Hunan: 1 male, Yingzuijie N.R., 300 m asl, 13.vii.2008, G. H. Huang leg.; 1 male, Badagongshan N.R., 1200 m asl, 27.v.2009, G. H. Huang leg. All the specimens preserved in SCAU and HUNAU.

VIETNAM: 2 males, Sa Pa, Lao Cai, 1600 m asl, 19–22.vi.1999, light trapping, Y.R. Sawada leg.; 1 female, Babe, Bac Kan Prefecture, 600 m, 2.v.2006, B.W. Lee leg. All the specimens preserved in OPU.

THAILAND: 2 males, Chiang Mai, Doi Pui, 1300 m asl, 26–27.x.1985, light trapping, S. Moriuti, T. Saito & Y. Arita leg.; 1 male, 1 female, Doi Inthanon, 1&3.xi.1983, light trapping, S. Moriuti, T. Saito & Y. Arita leg.; 1 male, Doi Inthanon, 8–12.ix.1987, S. Moriuti, T. Saito, Y. Arita & Y. Yoshiyasu leg. All the specimens preserved in OPU. Distribution: China (Shanghai, Jiangsu, Zhejiang, Hunan, Guangdong, Guangxi, Hainan, Yunnan, Shaanxi, Xinjiang); Japan; Russia (Far East); Korea; Vietnam (new record); Thailand; India; Pakistan; Iran.

Remarks: This species was recorded from China as *Monopis pavlovskii* Zagulajev by Zagulajev (1960) and Petersen & Gaedike (1993), and the latter was considered as the junior synonym of M. monachella (Hübner, 1796) by Xiao & Li (2006). This species can be easily distinguished from the other species of the M. monachella species complex by the characters given in the diagnosis. Sakai (2002) reported that one M branch of the forewing in this species is absent, but we could not find the specimen with only two M branches of the forewing.

Monopis iunctio Huang & Hirowatari **sp. nov.** (Figs 1C, 2C, 3E)

Diagnosis: See the diagnosis of M. monachella.

Description: Male. Wingspan 14.2–15.8 mm, forewing length 6.5–7.3 mm, length of antenna 5.4–6.0 mm (Fig. 1C).

Head: vertex and frons densely covered with rough, erect, yellowish white scales except the edges, which have brownish black scales. Antenna smooth, brownish black except apical part white, almost $0.8 \times$ length of forewing; scape smooth scaled, yellowish white with pecten containing more than six black bristles. Compound eyes large, covered with brownish grey scales along the edge. Maxillary palpus sparsely covered with pale yellow scales. Labial palpus yellowish white mesally, and brownish black laterally, the second segment with a bundle of black scales ventrally and more than ten long, strong, black bristles laterally and apically.

Thorax: dorsum including tegula smooth with dense white scales, anterior and posterior end of mesonotum dark brownish grey; tegula with basal 1/4 black. Fore- and midleg brownish black; hindleg pale black with hind tibia bearing long yellowish pale hairs. Forewing about $3.0 \times$ as long as wide including fringes (about $2.9 \times$ as long as wide excluding fringe); ground colour purple black with a large rectangular oblique white marking (including the subhyaline spot) on the costa from 2/7 to 6/7dilated in middle where it reaches halfway across wing, which has a black sheep-foot-shaped spot along the costa from basal 1/2 to 6/7, costa with a very narrow black streak from basal 2/7 to 1/2; fringe short, black; R₁ arising from 1/8 of discoidal cell. Hindwing about $1.8 \times$ as long as wide including fringe (about $2.6 \times$ as long as wide excluding fringe); ground colour brownish grey, semihyaline, with short pale brown fringe except basal 1/2 of costa; all veins present and free, M_1 ending at the termen (Fig. 2C).

Abdomen: dorsum greyish brown, heavily irrorated with pale white scales; venter with grey; the end with dense, slender, short grey scales covering genitalia.

Genitalia (Fig. 3E): uncus about $3/8 \times$ length of valva. Vinculum very narrow. Saccus relatively short, about $1.8 \times$ length of valva. Valva about $2.8 \times$ width, inner surface of valva membranous on central portion, and setose on distal 1/2. Aedeagus straight, cylindrical, entirely stout, about $1.5 \times$ length of valva, weakly sclerotized, cornutus indistinct.

Female. Unknown.

Holotype: Male, China: Hainan, Bawangling National N.R., 560 m asl, 11.x.2006, light trapping, G.H. Huang and Z. Li leg. Deposited in Institute of Entomology, HUNAU.

Paratypes: 1 male, Thailand: Chiang Mai, Doi Pakia, 1500 m asl, 5–7.ix.1987, S. Moriuti, T. Saito, Y. Arita & Y. Yoshiyasu leg.; 1 male, Thailand: Khao Soi Dao, 400 m asl, 7–8.x.1985, H. Kuroko, S. Moriuti, T. Saito & Y. Arita leg. Paratypes deposited in Entomological Laboratory, OPU.

Distribution: South China; Thailand.

Etymology of specific epithet: From the Latin *iunctio* (= connection), referring to the sheep-foot-shaped black spot in the white marking connected with the outer margin of the whitish marker in forewing.

Remarks: The new species can be distinguished from other species based on the characters given in the diagnosis of M. monachella.

BIOLOGICAL NOTES

In 2007, the tineid moths living in the birds' nests (*Strix uralensis*) were studied in several localities in Japan. Detritus from the bottom of six nests at different localities that had been abandoned by young birds, and one nest from a natural cavity of a tree were collected. The localities, collectors, and collecting dates of the bird nests and the numbers/species of tineid moths that emerged are shown in Table 2. *Monopis longella* Walker, 1863, and other three tineid moths [*M. flavidorsalis* (Matsumura, 1931); *M. congestella* (Walker, 1864); *Niditinea baryspilas* (Meyrick, 1937)] were reared from the detritus. One to four species were collected from each of the nests. Feathers, pellets, and bone were separated from the detritus, and all identified moth larvae were reared in

			Tineid moths			
Locality	Collector	Date	Monopis longella	Monopis flavidorsalis	Monopis congestella	Niditinea baryspilas (Meyrick, 1937)
-	I. Inamori	16.vi.2007		5		23
7	T. Tanigawa	16.vi.2007	3	1		
3	Y. Nasu	16.vi.2007		1		
4	T. Sugiyama	26.vi.2007	37	80		
5	T. Sugiyama	26.vi.2007	328+	12	59	
9	T. Ishii	7.vii.2007	26	9	18	IJ
7	N. Azuma	9.vii.2007	1			

cases (height 3 cm, diameter 9 cm) under laboratory conditions. The remaining detritus was put into large cases $(30 \times 23 \times 10 \text{ cm})$ under the same conditions. The larvae fed on the feather, fur, pellets, and the skin and flesh attached to bird bone in the detritus of the bird nests, and constructed a tube-like larval case made of fur. Pupation took place in a flat oval cocoon of fur. The pupa protruded from the cocoon before emergence. The larvae of these species have also been found in other bird nests [Ural owl (Nasu *et al.*, 2007a) and great tit (Nasu, Murahama & Matsumuro, 2008b)] and from carnivore faeces (Nasu *et al.*, 2007b, 2008a). The breeding and collecting data indicate that the species has several generations per year.

The larvae of *M. longella* feed on animal hair and feathers, and usually live in bird nests, creating tubular tunnels. The mature larva creates a cocoon just before pupation, which is composed of food material and silk. Adults can be collected at light. All species of the *M. monachella* species complex may have the same biological characters based on the similar biological characters in the *M. falvidorsalis* species complex, *M. congestella* species complex, and *Niditinae baryspilas* species complex.

RESULTS

PHYLOGENETIC ANALYSIS

Data analysis

The alignment of all the CO1 sequences is 658 nucleotide positions in length. The average nucleotide frequencies across species (A = 31.2%, C = 16.7%,G = 13.5%, and T = 38.7%) deviate greatly from the equilibrium of 25% for each nucleotide type, showing an A+T-rich pattern. Analyses of the estimates of evolutionary divergence between sequences were conducted in MEGA4 and the number of base differences per site from analysis between sequences is shown in Table 3. All results are based on the pairwise analysis of 26 sequences, including two outgroups and ten comparative individuals. All of 658 positions were eliminated from the data set. The genetic distances of the M. monachella species complex for CO1 sequences ranged from 0 to 0.100 (M. monachella vs. M. iunctio) (Table 3). Few intraspecific sequence variations were found. Only one sequence of *M. longella*, from an individual collected from Hokkaido, Japan, was included, with the uncorrected genetic distance (p-distance) amongst conspecific forms varying from 0.000 to 0.005.

Phylogenetic relationships

The bootstrap consensus trees based on evolutionary relationships of 26 taxa including outgroups were computed for the *CO1* sequences (658 sites, with 262

variable sites, 25 singleton sites, and 237 parsimonyinformative characters). NJ and ME analysis in MEGA 4.1(Tamura et al., 2007) was carried out for the data set with a total of 658 positions in the final data set aligned by CLUSTALX 1.83 with no gaps. Bootstrap analyses (Felsenstein, 1985) were carried out based on a full heuristic search of 1000 pseudoreplicates using the nucleotide p-distance model. The NJ bootstrap consensus tree (Fig. 4) shows largely similar topology to the ME tree (Fig. 5). In these two trees, the monophyly of the genus Monopis is well supported with respect to the outgroup species with bootstrap values of 94 and 95%, respectively. Each of the species represented by multiple populations proved to be monophyletic with strong confidence. The monophyly of the M. monachella species complex is also well supported in NJ and ME trees with high bootstrap values of 96 and 94%, respectively. The monophyly of the other three species complexes represented by multiple populations for comparison is also strongly supported in all these trees with a bootstrap value of 99%. In the *M*. monachella species complex, all the trees indicate that *M. iunctio* diverged first amongst the species included, with the rest forming a monophyletic clade that subsequently diverged into two subclades, one consisting of M. monachella and the other of M. longella.

DISSUSSION

Phylogenetic relationships amongst species or species clusters

Moore (1995) reported that congeneric species of moths show an average sequence divergence of 6.5% in the mitochondrial gene CO1, whereas divergences amongst conspecific individuals average only 0.25%. Hebert et al. (2004) reported that sequence divergences (CO1 barcoding) for the 45 pairwise NJ comparisons amongst ten taxa representing ten species of the neotropical skipper butterfly average 2.97% and range from 0.32 to 6.58%. In this study, the species M. monachella and M. longella represented by multiple populations show the divergence comparing the conspecific individuals average of M. monachella and M. longella only 0.0 and 0.1% (Table 3), respectively. In the *M. monachella* species complex, the average sequence divergence of the congeneric species is 7.5%. All the phylogenetic trees (Figs 4, 5) suggest with high confidence the sister relationship amongst the three species of *M. monachella* species complex.

MORPHOLOGICAL VS. MOLECULAR AFFINITIES

Xiao & Li (2006) considered all the differences between the species of the *M. monachella* species

Table 3. Uncorrected pair	rwise	p-dis	stance	es am	ongst	the c	ytoch	rome	c oxid	ase sı	tinudu	t I sec	Juence	s of t	he M_0	nopi	s spec	ies wi	th ou	tgroup	SC				
		53	ŝ	4	5	9	7	8	9 1	0 15	l 12	13	14	15	16	17	18	19	20	21	22	5	24 2	5 26	
1 Niditinea baryspilas-OK, Japan																									
2 Phereoeca uterella-HAN, China	0.164																								
3 Monopis hemicitra-HAN, China	0.193	0.210																							
4 M. hemicitra-HAN, China	0.193	0.211	0.002																						
5 Monopis congestella-AT, Japan	0.187	0.166	0.164	0.164																					
6 Monopis sp.1-HUN, China	0.164	i 0.178	0.185	0.185	0.096																				
7 Monopis sp.1-HUN, China	0.164	i 0.178	0.185	0.185	0.096	0.000																			
8 Monopis sp.2-HAN, China	0.161	0.149	0.164	0.164	0.079	0.087	0.087																		
9 Monopis flavidorsalis-AT, Japan	0.187	0.166	0.184	0.184	0.143	0.132	0.132	0.116																	
10 M. flavidorsalis-AT, Japan	0.187	0.166	0.181	0.181	0.141	0.129	0.129	0.116	0.005																
11 Monopis zagulajevi-HEN, China	0.199	0.172	0.191	0.191	0.149	0.141	0.141	0.123	0.027 0	.030															
12 Monopis zagulajevi-HEN, China	0.199	0.172	0.191	0.191	0.149	0.141	0.141	0.123	0.027 0	.030 0.	000														
13 Monopis iunctio-HAN, China	0.181	0.164	0.178	0.178	0.150	0.143	0.143	0.125	0.132 0	.132 0.	138 0.7	138													
14 M. monachella-GD, China	0.184	0.182	0.176	0.176	0.149	0.143	0.143	0.129	0.135 0	.132 0.	138 0.7	138 0.1	00												
15 M. monachella-HN, China	0.184	0.182	0.176	0.176	0.149	0.143	0.143	0.129	0.135 0	.132 0.	138 0.7	138 0.1	00 0.00	0											
16 Monopis longella-HAN, China	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.7	138 0.0	30.0 66	3 0.05	~										
17 M. longella-HAN, China	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.7	138 0.0	30.0 66	3 0.05	0.000	_									
18 M. longella-GX, China	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.7	138 0.0	30.0 66	3 0.05	0.000	0.000	_								
19 M. longella-GD, China	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.	138 0.0	30.0 660	3 0.05	0.000	0.000	0.000								
20 M. longella-HAN, China	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.7	138 0.0	30.0 66	3 0.05	0.000	0.000	0.000	0.000							
21 M. longella-HKD, Japan	0.173	0.178	0.164	0.164	0.149	0.149	0.149	0.131	0.132 0	.132 0.	138 0.7	138 0.0	30.0 66	5 0.05	0.005	300.0	0.005	0.005	0.005						
22 M. longella-AT, Japan	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.	138 0.0	30.0 660	3 0.05	0.000	0.000	0.000	0.000	0.000	0.005					
23 M. longella-AT, Japan	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.	138 0.0	30.0 66	3 0.05	0.000	0.000	0.000	0.000	0.000	0.005	0.000				
24 M. longella-HUN, China	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.	138 0.0	30.0 66	3 0.05	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000			
25 M. longella-GD, China	0.173	0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.	138 0.0	30.0 66	3 0.05	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	000.0		
26 M. longella-BKP, Vietnam	0.173	3 0.176	0.164	0.164	0.146	0.149	0.149	0.131	0.132 0	.132 0.	138 0.	138 0.0	99 0.0£	3 0.05	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	000.0	000	
The numbers in bold are the intrasp	ecific d	listance	s.																						



Figure 4. Bootstrap consensus neighbor-joining tree deduced from the cytochrome c oxidase subunit I gene sequences. Numbers indicate bootstrap proportions (%).

complex in appearance to be the individual variation of one species based on morphological characters. In this study, the two species represented by multiple populations, *M. monachella* and *M. longella*, were strongly supported by the high bootstrap value of 99% based on molecular data (mtDNA *CO1*, 658 bp). Looking at morphological characters, the presence or position of the black spot in the wing markings was considered as the difference amongst species, and the size of the black spot and the brightness of the hind wing were regarded as the individual variation. As a result of these data, we conclude that the *M. monachella* species complex in China consists of three species, including one new species and two new synonyms.

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0.01

Figure 5. Bootstrap consensus minimum evolution tree deduced from the cytochrome c oxidase subunit I gene sequences. Numbers indicate bootstrap proportions (%).

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