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ORIGINAL ARTICLE

Revision of Asian species of the *Drosophila melanica* species group (Diptera: Drosophilidae), with a description of a new species from Vietnam

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Abstract

A new species of the *Drosophila* (*Drosophila*) *melanica* species group (Diptera: Drosophilidae), *Drosophila denruoi* Suwito & Watabe, sp. nov., is described from northern Vietnam. It is proved by crossing experiments that reproductive isolation is complete between this species and other morphologically similar species. Larval ganglion cells of *D. denruoi* possess $2n = 12$ chromosomes, comprised of four pairs of acrocentric, one pair of metacentric and one pair of micro-chromosomes. This is the largest number of acrocentric chromosomes in the group and implies that the species has the most primitive chromosomal configuration in the *melanica* group. Based on the results of crossing experiments and close morphological examination, *Drosophila pengi* Okada & Kurokawa, 1957 from central Japan, once regarded as a synonym of *Drosophila tsigana* Burla & Gloor, 1952, is shown to be distinct from *D. tsigana*. *Drosophila pengi* is thus resurrected as a member of the *melanica* group. We synonymize *Drosophila bisetata* Toda, 1988, described from central Myanmar, with *D. pengi*. In addition, a population from Guizhou, southwestern China, once regarded as *D. tsigana*, is also identified as *D. pengi*. These populations from central Myanmar and southwestern China share the diagnostic characters of *D. pengi*. At the same time, it is reconfirmed that the Hokkaido population is conspecific with European *D. tsigana*. Furthermore, another species, *Drosophila longiserrata* Toda, 1988 described from central Myanmar, is synonymized with *Drosophila afer* Tan, Hsu & Sheng, 1949 from China, based on their having identical karyotypes and external morphology.

Key words: geographic distribution, karyotype, morphology, redescription, re-identification, reproductive isolation.

INTRODUCTION

The *Drosophila melanica* species group, established by Sturtevant (1942), has been intensively studied from the evolutionary point of view (Stalker 1966, 1972; Levitan 1982; Wang *et al*. 2006a; Flores *et al*. 2008), and most of its members inhabit temperate forests of Eurasia and North America.

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We have been studying a drosophilid fauna in subtropical Vietnam since 2008. In the course of this study, we found a species apparently of the *melanica* group and succeeded in establishing it in laboratory culture. This species closely resembles *Drosophila tsigana* Burla & Gloor, 1952 in the general features of its external morphology.

The taxonomy of *D. tsigana* and its relatives is somewhat confused, with a history of nomenclatural changes, and is still not fixed. Currently, the Old World *melanica*-group species, except for *D. moriwakii* Okada & Kurokawa, 1957 of which taxonomical position will be revised elsewhere, are known under four valid

names. These four names are *D. tsigana* recorded from Europe to Japan, *D. afer* Tan, Hsu & Sheng, 1949 from central to southwestern China, *D. bisetata* Toda, 1988 and *D. longiserrata* Toda, 1988 from central Myanmar. *Drosophila tsigana* has been recorded most widely, from the furthest west (Portugal) to the furthest east (Japan) of the Palearctic Region. Over this range there is geographic variation in the color pattern on the abdominal tergites (Watabe *et al.* 1990; Wang *et al.* 2006a). The Japanese population of this species has been reported under different species names. Kikkawa and Peng (1938) identified specimens from Kinki District (Kyoto and Kobe) as a North American *melanica*-group species, *D. melanissima* Sturtevant, 1916, but with "no conviction". However, Sturtevant (1942) pointed out that the Japanese population was distinct from *D. melanissima*. Accordingly, Okada and Kurokawa (1957) described it as a new species, *D. pengi*, based on specimens from Tokyo (type locality), Hokkaido and Kyoto. Watabe *et al.* (1990) later synonymized *D. pengi* with *D. tsigana*, based on morphological comparison of the genitalia and crossing tests between *D. tsigana* (from France) and *D. pengi* (from Sapporo, Hokkaido), although they noticed some differences in the abdominal color pattern and mate preference between them. *Drosophila tsigana* has also been recorded from Sichuan and Guizhou, southwestern China. However, Wang *et al.* (2006a) suggested, based on the results of crossing experiments and comparison of morphology and karyotype with the Hokkaido (Sapporo) population, that the Guizhou population is of a biologically good species distinct from *D. tsigana*, probably being conspecific with *D. bisetata*. In addition, Wang *et al.* (2006b) has revealed, by molecular phylogenetic analyses based on DNA sequence data of two mitochondrial (*ND2* and *COI*) and one nuclear (*Adh*) genes, that the Guizhou population is closer to *D. longiserrata*, to which it was placed as the sister group, than to the Hokkaido population. Complete reproductive isolation between *D. longiserrata* and both Guizhou and Hokkaido populations of *D. tsigana* was proved by Wang *et al.* (2006a). Thus, the species status has not yet been determined for some Asian populations currently called "*D. tsigana*". An important problem impeding resolution of this issue is that the type-locality (Tokyo) population of *D. pengi* has never been included in previous studies.

Therefore, to help clarify the species status of the three populations of *D. tsigana* or related species from Vietnam, Tokyo and Sapporo, we conducted crossing tests and morphological comparisons between populations. We examined specimens from a wider range of localities than previously used including North Africa, Europe, Russian Far East, northeastern and southwestern China, Myanmar and Kyoto (central Japan).

MATERIALS AND METHODS

Three culture strains were employed for this study: BV derived from an inseminated female caught in the National Park of Mt. Ba Vi (929 m in altitude), Vietnam, TK from several flies caught in a temple forest of Kisshoin (120 m), Musashi-murayama, Tokyo, and SP from several flies caught at Jozankei (387 m), Sapporo, Hokkaido. They were maintained at 20°C in an incubator on our usual *Drosophila* medium.

Crossing experiments

In no-choice tests, five females were confined together in a vial with either five males from the same or a different strain. The vial was 30 mm in diameter and 100 mm tall. After 48 h, the females were removed and dissected in Ringer solution, and their spermathecae and seminal receptacle were examined for sperm. More than 50 (usually 100) females were checked in each test. The index of premating isolation for each pair was calculated as 1 – (% of different-strain matings, i.e., A♀ × B♂)/(% of same-strain matings, i.e., A♀ × A♂) (Coyne & Orr 1989). This value gives +1 when isolation is perfect between two populations but 0 when there is no isolation.

In choice-by-male tests, five males were confined with five females of the same strain and five of a different strain. The females were again removed after 48 h and then dissected in Ringer solution to detect sperm. The degree of mate-choice preferences was evaluated by isolation index (I.I.): (% of same-strain matings – % of different-strain matings)/(% of same-strain matings + % of different-strain matings) (Stalker 1942). This index gives +1 when same-strain females are completely preferred and –1 when different-strain mates are completely preferred.

To examine post-mating isolation, another ten vials containing five females and five males of a different strain were maintained for one month. When progeny emerged as adults, their fertility was examined by back crossings between these adults and the parental strains.

Morphological observations

In addition to specimens reared from the three strains, some wild-caught specimens including types of *D. tsigana*, *D. pengi*, *D. bisetata* and *D. longiserrata* and specimens reared from the strain E derived from several flies caught at Ariège (42°27′N, 1°30′E), St. Girons, France (Watabe *et al.* 1990) and the strain GZ

from two females caught at Anshun (ca. 700 m in alt.), Guizhou, China (Wang *et al*. 2006a) were examined. External morphology was examined under a stereoscopic microscope, and metric characters were measured with an ocular micrometer. Male and female terminalia were detached from the body, treated with 10% KOH solution at 80°C for several minutes, mounted in a droplet of glycerin on a cavity slide and examined under a compound microscope. The genitalia and other parts were microphotographed using a DinoLite Camera, and drawn from these microphotographs using a Wacom pen tablet, Driver Version 6.05 on Adobe Illustrator CS4. We followed McAlpine (1981) for the morphological terminology, and Zhang and Toda (1992) for the definitions of measurements and indices. New type specimens are deposited in the Vietnam National Museum of Nature (VNMN) and Systematic Entomology, Hokkaido University Museum, Hokkaido University, Sapporo, Japan (SEHU).

Karyotype preparation

Neuroblasts of 3rd instar larvae were treated with 0.1 mg/ml of colchicines solution for 30 min and with 1% of sodium citrate for 20 min, fixed with a solution (carbinol:acetic acid = 3:1) for 2 h, stained with 4% Giemsa solution for at least 30 min, and then air-dried (Imai *et al*. 1977). About 100 nuclear plates were examined. Metaphase chromosomes were photographed using an analog camera (Olympus PM-6) and then saved as digital figures using a film-scanner (Nikon APS IX240).

RESULTS AND DISCUSSION

Crossing experiments

In no-choice tests, more than 90% of females were inseminated in same-strain crosses of the three strains (Table 1). In different-strain crosses between BV and the other two strains, however, insemination percentages were very low (0.0–4.5%), except for the cross between SP♀ and BV♂ with 21.2%. No embryonic development was observed in these crosses. There is therefore complete reproductive isolation between BV and the other two strains. In the crosses between TK and SP, the reciprocal crosses differed in insemination percentage: rather high (47.6%) for TK♀ × SP♂, but very low (7.8%) for SP♀ × TK♂. No larvae were obtained in these crosses. In TK and SP choice-by-male crosses the isolation index was 1.00 in the cross (TK♀ + SP♀)/TK♂, while it was 0.39 in the cross (TK♀ + SP♀)/SP♂ (Table 2).

These three strains are thus each a biologically good species. The Japanese populations currently assigned to *D. tsigana* include two different species. It is not clear, however, whether SP or TK should carry the name *D. tsigana*. If SP is *D. tsigana* then *D. pengi* Okada & Kurokawa, 1957 (type locality: Suginami, Tokyo) should be resurrected for TK. If, however, TK is *D. tsigana*, *D. septentriata* (Takada & Maekawa, 1984) should be resurrected for SP. This name was originally applied to material described from Toyotomi Hot Spring (type locality), Hokkaido under the genus *Nesiodrosophila* Wheeler & Takada but later synonymized with

Table 1 Percentages of inseminated females in crosses among three culture strains (BV from Mt. Ba Vi, Vietnam; TK from Tokyo, Japan; SP from Sapporo, Japan) of the *Drosophila melanica* species group by the no-choice method

♂	BV			TK			SP		
♀	%	(*N*)	Index†	%	(*N*)	Index	%	(*N*)	Index
BV	91.2	(114)		4.5	(89)	0.951	1.6	(127)	0.983
TK	0.0	(102)	1.000	90.6	(64)		47.6	(63)	0.475
SP	21.2	(128)	0.781	7.8	(64)	0.920	96.8	(94)	

†Premating isolation index by Coyne and Orr (1989) (see text).

Table 2 Female insemination status in crosses between TK and SP by the choice-by-male method

Mating		Same-strain				Different-strain				
Female	Male	+	(%)	–	(%)	+	(%)	–	(%)	I.I.†
TK+SP	TK	25	(78.1)	7	(21.9)	0	(0.0)	32	(100.0)	1.00
TK+SP	SP	31	(96.9)	1	(3.1)	14	(42.4)	19	(57.6)	0.39

†Stalker's (1942) isolation index (see text).

D. tsigana by Watabe *et al.* (1990). To determine which is the case it is indispensible to compare the candidate strains with European populations of *D. tsigana* (type locality: Oloron, 43°12'N, 0°35'W, France). Strain E from Ariège, approximately 170 km east of the type locality, was once available and used by Watabe *et al.* (1990), but has unfortunately since been lost. However, there is no reproductive isolation between strain E and strain J (from Sapporo) (Watabe *et al.* 1990). These strains crossed freely and produced fertile F_1 and F_2 progeny. It is thus probable that the Hokkaido population (SP and J strains) is *D. tsigana*.

In addition, reproductive isolation between the Guizhou and the Hokkaido populations of *D. tsigana* is very strong, if not complete (Wang *et al.* 2006a). Few fertile F_1 progeny were produced only by crosses between Guizhou females and Hokkaido males. Based on this evidence and morphological comparisons, they suggested that the Guizhou population is conspecific with *D. bisetata*.

Taxonomy

To confirm the hypotheses mentioned above, we examined all available specimens of relevant populations to identify morphological characters by which *D. tsigana* and *D. pengi* can be distinguished from each other. We found some characters that could be used in diagnoses of these species.

Based on crosses and morphological examination, BV is described as a new species, and the resurrected *D. pengi*, *D. tsigana* and *D. afer* are redescribed below.

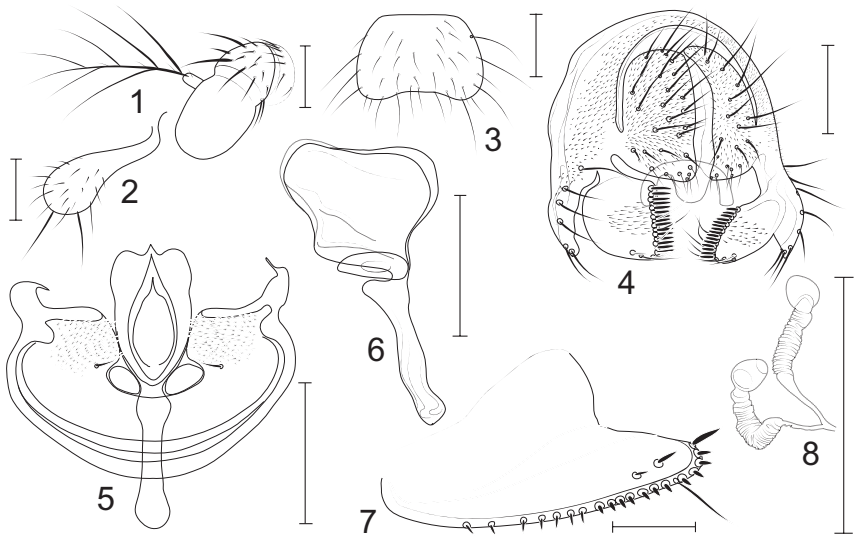
Drosophila (*Drosophila*) *denruoi* Suwito & Watabe, sp. nov. (Figs 1–8)

Diagnosis

Recurved setae on antero-inner margin of male foreleg tarsomere I shorter than width of tarsomere, sparse. Aedeagus roundly expanded dorsad, with neither serrations nor edge on margin (Fig. 6). Male sternite V wider than long (Fig. 3). Surstylus pubescent only mediodistally (Fig. 4). Oviscapt with two lateral ovisensilla (Fig. 7). Spermathecal capsule very small, less sclerotized, membranous, longer than wide, but somewhat irregular in shape, without spinules on surface; basal introvert of duct absent (Fig. 8).

Description (♂ and ♀)

Head: Dorsomedial, tentorial apodeme shorter than half of distance from its base to level of postocellar setae. Dorsolateral, tentorial apodeme gently curved outward around 3/4 from base, reaching around inner, vertical seta. Frons blackish, anteriorly lighter. Posterior, reclinate, orbital seta situated nearer to outer, vertical seta than to anterior margin of frons; anterior, reclinate, orbital seta situated just outside of proclinate, orbital seta. Anterior portion of fronto-orbital plate narrower than frontal vitta. Facial carina as wide as 1st flagellomere. Antennal pedicel and 1st flagellomere blackish brown. Pedicel with thick setae between two prominent setae and inside of lower, prominent seta and fringe of setae longer than upper to medial setulae on anterior to inner, distal margin (Fig. 1). Longest, dorsal branch of arista longer than upper, prominent seta on pedicel (Fig. 1). Prominent, subvibrissal seta present. Palpus



Figures 1–8 *Drosophila* (*Drosophila*) *denruoi* Suwito & Watabe, sp. nov. **1** Antenna; **2** palpus; **3** male sternite V; **4** periphallic organs (caudolateral view); **5** phallic organs (ventral view); **6** aedeagus and paramere (lateral view); **7** oviscapt; **8** spermathecae. Scale line = 0.1 mm.

distally broader than thick, with two to three prominent setae laterally (Fig. 2). Clypeus black. Cibarium: anterior margin ridged; projections at anterolateral corners shorter than half width of anterior margin; anterior portion of hypopharynx broader than posterior portion; roundish flap on subapical portion absent; cross distance between lateralmost anterior sensilla wider than medial, narrowest portion of hypopharynx; medial sensilla approximately six per side, including short, apically somewhat blunt peg-like ones, arranged in anteriorly divergent rows. Prementum with a pair of small setae between longer setae on lower, medial surface. Dorsal arm of lacinia as broad as ventral arm. Labellum with seven pseudotracheae per side.

Thorax: Scutum dark brown, obscure along dorsocentral lines, with two pairs of dorsocentral setae but no long, acrostichal setae. Acrostichal setulae in six regular rows. Postpronotal lobe dark brown, with two prominent setae. Anepisternum and katepisternum dark brown. Katepisternum with ventral setulae, except for several dorsalmost ones, arranged nearly in a single row below katepisternal setae, and setulae anteriorly and laterally beside prominent seta near caudoventral corner. Scutellum dark brown, darker along lateral to posterior margins; anatergite yellowish brown. Halter whitish gray; stalk pale brown.

Wing: Dm-cu crossvein clear.

Legs dark brown, except for paler femora and joints. Foreleg tibia as long as four succeeding tarsomeres together. All tarsomere I as long as two succeeding tarsomeres together.

Abdomen: Tergites nearly entirely dark brown, paler on anterior margin. Sternites pale yellow, darker laterally, wider than long; male V and female VI slightly concave on posterior margin; male V with three to four long setae laterally but without medial tuft of setae on posterior margin (Fig. 3).

Male terminalia: Epandrium with approximately six setae near caudal margin of ventral portion, pubescent except for antero-submedian area, lower half of ventral portion and area around connection to cercus (Fig. 4); ventral portion as broad as dorsal portion, not concave on anterior margin below anteroventral corner, not protruded at caudoventral corner, without tuft of setae on ventromedial margin and transparent patch or plate on caudoventral margin. Surstylus with approximately 15 prensisetae in nearly straight row on distal margin, approximately four trichoid setae around caudoventral corner on outer surface and three to four longer setae on inner, distal margin (Fig. 4). Cercus fused to epandrium at lower portion, pubescent except for narrow area along ventral margin, with approximately 19–23 long setae nearly all over surface and approximately seven setulae at caudoventral corner (Fig. 4). Hypandrium wider than long, not protruded at anterolateral corners, with paramedian seta and large patch of pubescence on lateral lobe (Fig. 5). Parameres dark brown, strongly sclerotized, elongated ellipsoid in lateral view, not pubescent, separated from aedeagus and each other (Figs 5, 6). Aedeagus nearly straight to aedeagal apodeme and ventroproximally expanded in lateral view, without ventral, transparent plate or membrane and a pair of sclerotized, dorsal ridges, bilobed dorsally but fused apically; lateral lobes basally not extended ventrad; apodeme longer than 1/2 length of aedeagus proper, entirely as broad as thick; aedeagal guide short, not reaching to hypandrium (Fig. 6).

Female terminalia: Tergite VIII ventrally with six to nine setae per side, pubescent only on dorsal portion. Oviscapt distally narrowing, with approximately seven trichoid and approximately seven peg-like ovisensilla arranged in single, regular row on proximal and distal, ventral margin, respectively, approximately four apical, peg-like ones, longest apicodorsal one, and ventrosubterminal, trichoid one approximately thrice as long as apicodorsal, marginal one (Fig. 7). Spermathecal, outside duct four times as long as capsule height (Fig. 8).

Measurements: BL = 2.56 mm in holotype (range in 3♂ paratypes: 2.60–2.72 mm, range in 2♀ paratypes: 2.84–3.24 mm), ThL = 0.96 mm (♂: 0.84–0.96 mm, ♀: 1.00–1.04 mm), WL = 2.20 mm (♂: 2.16–2.20 mm, ♀: 2.40–2.52 mm), WW = 0.84 mm (♂: 0.88–0.92 mm, ♀: 0.92–1.04 mm).

Indices: arb = 3/2 (♂: 3/2, ♀: 2–3/2), FW/HW = 0.46 (♂: 0.44–0.47, ♀: 0.47), ch/o = 0.17 (♂: 0.12–0.18, ♀: 0.15–0.17), prorb = 1.00 (♂: 1.00–1.10, ♀: 0.67–1.17), rcorb = 0.56 (♂: 0.40–0.50, ♀: 0.40–0.42), orbito = 0.50 (♂: 0.33–0.57, ♀: 0.43–0.50), vb = 0.50 (♂: 0.38–0.50, ♀: 0.47–0.50), pprnl = 0.56 (♂: 0.58–0.85, ♀: 0.52–0.90), dcl = 0.58 (♂: 0.57–0.67, ♀: 0.64–0.75), dcp = 0.45 (♂: 0.50, ♀: 0.42–0.46), sterno = 0.79 (♂: 0.65–0.85, ♀: 0.58–0.90), m-sterno = 0.16 (♂: 0.20–0.25, ♀: 0.19–0.30), sctl = 0.56 (♂: 0.72–0.79, ♀: 0.71–0.75), sctlp = 0.43 (♂: 0.40–0.48, ♀: 0.44–0.79), C = 2.62 (♂: 2.48–2.68, ♀: 2.63–2.76), 4c = 0.97 (♂: 0.93–1.04, ♀: 0.97), 4v = 1.80 (♂: 1.74–1.83, ♀: 1.76–1.79), 5x = 1.15 (♂ 1.07–1.31, ♀: 1.13–1.58), ac = 2.64 (♂: 2.42–2.55, ♀: 2.36–2.46), M = 0.50 (♂: 0.53–0.55, ♀: 0.52–0.56), C3F = 0.34 (♂: 0.34–0.40, ♀: 0.38–0.67).

Chromosomes

Male metaphase configuration comprises $2n = 12$ chromosomes, with one pair of medium-sized, metacentric chromosomes (shown with thick arrows in Figure 9; V-shaped), four pairs of acrocentric chromosomes

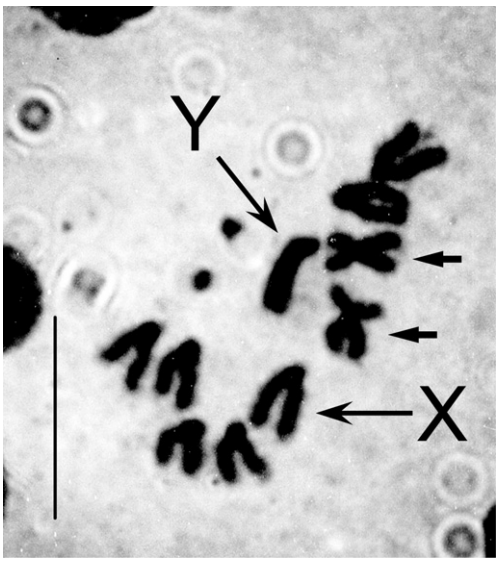


Figure 9 Male metaphase chromosomes of *Drosophila denruoi* Suwito & Watabe, sp. nov. Thick arrows indicate medium-sized, metacentric autosomes. Scale line = 10 μm.

(rod-like) and one pair of micro-chromosomes (dot-like). X chromosome is acrocentric, and Y is heterochromatic and sub-metacentric.

Holotype

♂ (VNMN), "from strain, Mt. Ba Vi (21°04′05.9″N; 105°21′56.9″E), Ba Vi National Park, Vietnam, 16.II.2008, H. Takamori leg.".

Paratypes

Vietnam: 10♂10♀, same data as the holotype (VNMN, SEHU).

Distribution

Vietnam (Fig. 29).

Remarks

Drosophila denruoi is similar to *D. afer* and *D. pengi* in the external morphology, but can be distinguished from them by the diagnostic characters.

Etymology

Referring to the black (den) fly (ruoi) in Vietnamese.

***Drosophila* (*Drosophila*) *pengi* Okada & Kurokawa** (Figs 10–12, 15–17, 20–22, 25, 26; Figs S1, S2 in Supporting information)

Drosophila (*Drosophila*) *pengi* Okada and Kurokawa (1957): 11.

Drosophila melanissima: Kikkawa and Peng (1938): 538; Okada (1955): 99; Okada (1956): 153 (nec Sturtevant, 1916).

Drosophila (*Drosophila*) *bisetata* Toda (1988): 629. **Syn. nov.**

Diagnosis

Epandrium narrower than cercus (Figs 10–12). Hypandrium wider than long (Figs 15–17). Aedeagus proper narrower than long in lateral view (Figs 20–22). Spermathecal capsule wrinkled nearly horizontally on basal half but more or less irregularly on distal half (Figs 25, 26). Foreleg tibia as long as three succeeding tarsomeres together. All tarsomere I as long as three succeeding tarsomeres together.

Supplementary and revised description (♂ and ♀, referring only to differences from D. denruoi*)*

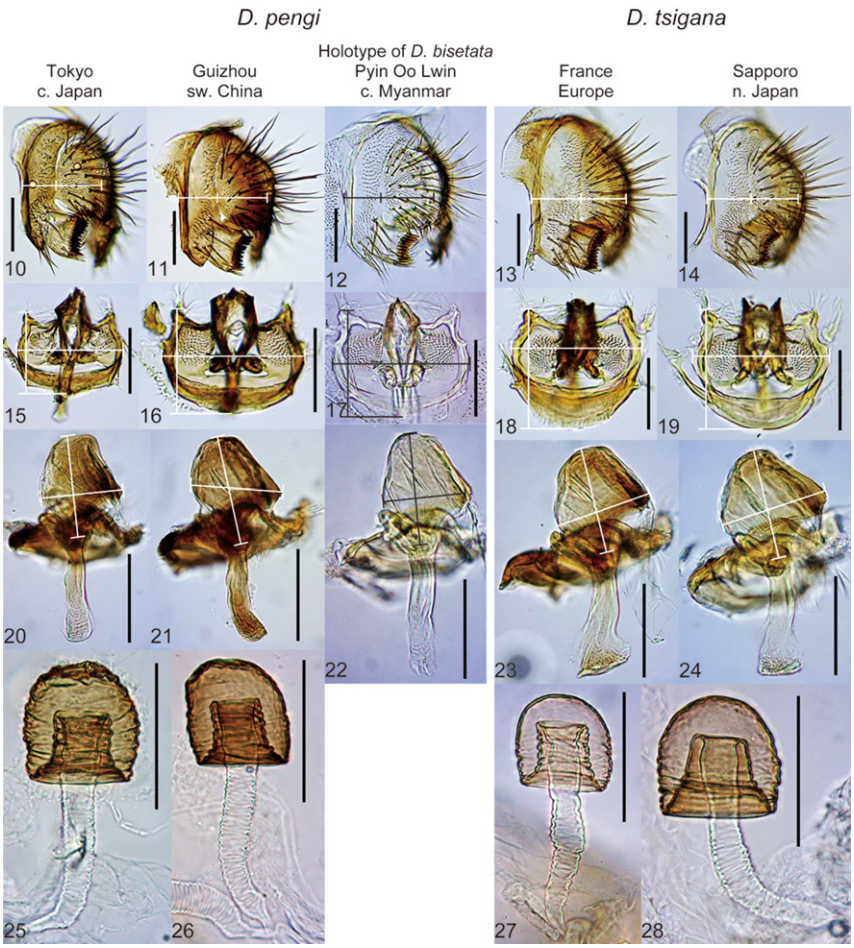
Head: Dorsolateral, tentorial apodeme gently curved outward around 2/3. Pedicel lacking thick seta inside of lower, prominent seta. Palpus with approximately three prominent setae laterally. Cibarium: medial sensilla approximately seven per side. Labellum with six to eight pseudotracheae per side.

Legs: Recurved setae on antero-inner margin of male foreleg tarsomere I slightly longer than width of tarsomere, dense.

Abdomen: Tergites nearly entirely dark brown, paler on anterior margin in male, with broad, dark brown bands medially broken on II to IV but not broken on V and VI in female. Male sternites III to V as long as wide.

Male terminalia: Epandrium with 9–11 setae on ventral portion, pubescent except for antero-submedian area, lower 1/3 of ventral portion and area around connection to cercus (Figs 10–12). Surstylus pubescent nearly entirely on outer surface, except for dorsal margin and caudoventral area, with 10–13 prensisetae (Figs 10–12). Hypandrium less protruded at anterolateral corners (Figs 15–17). Aedeagus with fine serrations and edge on dorsal margin (Figs 20–22).

Female terminalia: Tergite VIII ventrally with 15–17 setae per side. Oviscapt with three lateral and 28–32 marginal ovisensilla; ventro-subterminal, trichoid ovisensillum as long as apicodorsal, marginal one. Spermatheca: capsule strongly sclerotized, regular in shape, longer than wide, apically slightly concaved, not constricted distinctly, with spinules over surface and basal introvert distinctly wrinkled; duct not constricted distally in introvert; outside duct twice as long as capsule height, wider than 1/3 of basal diameter of introvert (Figs 25, 26).



Figures 10–28 Diagnostic characters for *Drosophila* (*Drosophila*) *pengi* Okada & Kurokawa, 1957 (**10**, **15**, **20**, **25**: ♂♀ from the strain TK established from flies collected at Kisshoin, Musashi-murayama, Tokyo, Japan; **11**, **16**, **21**, **26**: ♂♀ from the strain GZ established from flies collected at Anshun, Guizhou, China; **12**, **17**, **22**: holotype ♂ of *Drosophila bisetata* Toda, 1988) and *Drosophila* (*Drosophila*) *tsigana* Burla & Gloor, 1952 (**13**, **18**, **23**, **27**: ♂♀ from the strain E established from flies collected at Ariège, St. Girons, France; **14**, **19**, **24**, **28**: ♂♀ from the strain SP established from flies collected at Jozankei, Sapporo, Hokkaido, Japan). **10–14** Periphallic organs (caudolateral view); **15–24** phallic organs (**14–19:** ventral view; **20–24:** lateral view); **25–28** spermatheca. Scale line = 0.1 mm.

Specimens examined

Japan: holotype, ♂ (first deposited in the Department of Biology, Tokyo Metropolitan University, Tokyo, Japan, but later relocated to the National Museum of Nature and Science, NSMT, Tsukuba, Japan; Fig. S1), Suginami, Tokyo, 30.vii.1951; allotype, ♀ (Fig. S1; NSMT), Suginami, Tokyo, 27.vii.1951, Onishi leg.; 2♂2♀, from the culture strain (TK) established from flies collected at a temple forest of Kisshoin (120 m in alt.), Musashi-murayama, Tokyo in x.2008; 10♂16♀, Uryuzan, Kyoto, 7.x.2008, J. Yoshimoto leg. **China**: 1♂1♀, from the culture strain (GZ) established from flies collected at Anshun (ca. 700 m in alt.), Guizhou, 23–24.ix.2002; 1♂1♀, Mt. Emei, Sichuan, 15–20.vii.1992, ex banana trap, M. J. Toda leg. **Myanmar**: 1♂ (holotype of *D. bisetata*: SEHU 31265; Fig. S2), Pyin Oo Lwin, 30.xii.1981- 6.i.1982, M.J. Toda leg.

Distribution

Japan (Kanto, Kinki), China (Sichuan, Guizhou), Myanmar (Fig. 29). Previous records from South Korea and other districts (Tohoku, Chubu, Chugoku, Shikoku and Kyushu) of Japan should be reconsidered in the light of current knowledge.

Remarks

Toda (1988) described *D. bisetata* based on only one male (holotype) specimen collected from Pyin Oo Lwin, central Myanmar, designating the presence of two pairs of paramedian setae on the hypandrium, in contrast to one pair in the other species of the *melanica* group, as the most important, diagnostic character for this species. However, Wang *et al*. (2006a) found a variant male having two paramedian setae on one side and one on the other side of hypandrium among specimens reared from the GZ strain, and considered the holotype of *D. bisetata* to be such a variant. We found a few such variants in specimens reared from the TK strain as well. Close examination of the holotype specimen has revealed that it shares all the newly found diagnostic characters with the type-locality (Tokyo) population of *D. pengi*, and with the Guizhou population of

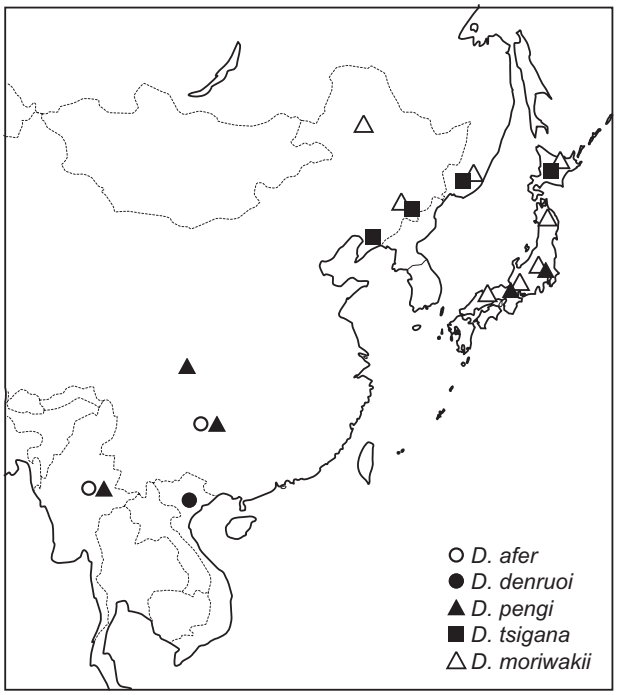


Figure 29 Geographic distributions of the *Drosophila melanica* species group in East and Southeast Asia.

southwestern China (Figs 10–12, 15–17, 20–22). Thus, we have confirmed the hypothesis proposed by Wang *et al.* (2006a), and further synonymized *D. bisetata* Toda, 1988 with the resurrected *D. pengi* Okada & Kurokawa, 1957 here.

Drosophila (*Drosophila*) *tsigana* Burla & Gloor (Figs 13, 14, 18, 19, 23, 24, 27, 28; Fig. S3)

Drosophila tsigana Burla and Gloor (1952): 164.
Drosophila (*Drosophila*) *tsigana*: Watabe *et al.* (1990): 314.
Nesiodrosophila septentriata Takada and Maekawa (1984): 42.

Diagnosis

Epandrium as wide as cercus (Figs 13, 14). Hypandrium as wide as long (Figs 18, 19). Aedeagus proper as wide as long in lateral view (Figs 23, 24). Spermathecal capsule nearly smooth on distal half, apically not concaved (Figs 27, 28).

Supplementary and revised description (♂ and ♀, referring only to differences from D. pengi*)*

Head: Palpus with two to three prominent setae laterally. Cibarium: medial sensilla six to seven per side.

Legs: Foreleg tarsomere I slightly shorter than and mid- and hindleg ones as long as three succeeding tarsomeres together.

Abdomen: Tergites geographically varying in color pattern: in European and Liaoning (northeastern China) populations, tergites nearly entirely dark brown, paler on anterior margin in male, with broad, dark brown bands sometimes medially broken on II and III in female; in Russian Far East and Hokkaido (northern Japan) populations, tergites with broad, dark brown bands medially broken on II to IV (V in pale specimens) but not broken on V and VI in both sexes ("Fig. 1" in Watabe *et al.*, 1990). Male sternites III to V wider than long.

Male terminalia: Epandrium with 13–16 setae on ventral portion, pubescent except for antero-submedian area and lower 1/3 of ventral portion (Figs 13, 14). Surstylus pubescent nearly entirely on outer surface, except for dorsal margin (Figs 13, 14). Hypandrium more or less protruded at anterolateral corners (Figs 18, 19). Aedeagus with slightly coarse serrations on dorsal margin (Figs 23, 24).

Female terminalia: Tergite VIII ventrally with 18–21 setae per side. Oviscapt with 27–32 marginal ovisensilla.

Specimens examined

France: paralectotypes, 2♀ (ZMZ: Zoologisches Museum der Universität Zürich-Irchel, Zürich, Switzerland; Fig. S3), Oloron, B.P./F, 9–11.ix.1950, H. Burla leg.; 1♂1♀, from the culture strain (E) established from flies collected at Ariège (42°27′N, 1°30′E), St. Girons. **Morocco**: 1♂ (ZMZ), Ifrane, viii.1963, W. Greuter leg. **Russia**: 1♀ (ZMZ), Mirny, Caucasus, viii/ix.1981, V. G. Mitrofanov leg.; 21♂31♀, Ussurian Nature Reserve, valley of R. Komarovka, Primorye, 22.vii.1994, M. J. Toda leg. **China**: 4♂7♀, Qianshan, Liaoning, 15–16.viii.1991, Y.-g. Hu leg.; 10♂27♀, Shenyang, Liaoning, 10.viii.1991, Y.-g. Hu leg. **Japan**: 2♂1♀, from the culture strain (SP) established from flies collected at Jozankei (387 m in alt.), Sapporo, Hokkaido.

Distribution

Morocco, Portugal, France, Austria, Hungary, Russia (European parts, Far East), China (Jilin, Liaoning), Japan (Hokkaido) (Fig. 29).

Drosophila (*Drosophila*) afer Tan, Hsu & Sheng

Drosophila (*Drosophila*) *afer* Tan *et al.* (1949): 200.
Drosophila (*Drosophila*) *longiserrata* Toda (1988): 627. **Syn. nov.**

Diagnosis [modified from that defined for D. longiserrata *by Toda (1988)]*

Aedeagus dorsally with sclerotized, broad, long process between lateral lobes; dorsal margin of lateral lobe serrate along nearly entire length ["Figs 7, 8" in Toda (1988)]. Foreleg tibia as long as four succeeding tarsomeres together; tarsomere I as long as two succeeding tarsomeres together.

Supplementary and revised description (♂ and ♀, referring only to differences from D. pengi*)*

Head: Cibarial, medial sensilla approximately six per side. Labellum with six pseudotracheae per side.

Abdomen: Sternites wider than long, except for male V as wide as long.

Male terminalia: Surstylus with 9–11 prensisetae.

Female terminalia: Tergite VIII ventrally with 14–16 setae per side. Oviscapt with 25–26 marginal ovisensilla. Spermathecal capsule not concave apically.

Specimens examined

China: 1♂1♀, from the culture strain established from flies collected at Anshun (ca. 700 m in alt.), Guizhou, 23–24.ix.2002. **Myanmar**: 1♂ (holotype of *D. longiserrata*: SEHU 31221; Fig. S4), Pyin Oo Lwin, 30.xii.1981-6.i.1982, M.J. Toda leg.; 1♀ (paratype of *D. longiserrata*: SEHU 31222; Fig. S4), same data as holotype of *D. longiserrata*.

Distribution

China (Guizhou), Myanmar (Fig. 29). The record from "Hangchow", Zhejiang, central China by Tan *et al.* (1949) should be reconsidered in the light of current knowledge.

Remarks

The culture strain used by Wang *et al.* (2006a) originated from Anshun, approximately 200 km southwest of Meitan (one of the collection localities reported in the original description of *D. afer*), Guizhou, China. This strain and some concurrently collected specimens used in the molecular phylogenetic study of Wang *et al.* (2006b) were tentatively identified as *D. longiserrata* by M. J. Toda (Wang *et al.* 2006a). Wang *et al.* (2006a) compared the karyotype and external morphology of Guizhou *D. longiserrata* with the original descriptions of these characters for *D. afer*, of which syntypes have been lost (Brake & Bächli 2008). They strongly suggested, based on these characters being identical in the two forms, that they are conspecific. According to this suggestion and re-examination of the holotype of *D. longiserrata*, we have here synonymized *D. longiserrata* Toda, 1988 with *D. afer* Tan, Hsu & Sheng, 1949. *Drosophila afer* resembles *D. pengi* in having the epandrium narrower than the cercus, the hypandrium wider than long and the spermathecal capsule entirely wrinkled. Their close relationships are indicated in the molecular phylogenetic trees by Wang *et al.* (2006b), where *D. afer* (there referred to as *D. longiserrata*) and *D. pengi* (referred to as *D. tsigana* from Anshun) are placed as sisters to each other. However, they can be distinguished from each other by the diagnostic characters.

Geographic distribution and evolution

Species of the *melanica* group, according to our revisions and collections, occur in subtropical and temperate areas (Fig. 29). As subtropical, Oriental members of the *melanica* group, two species, *D. afer* and *D. pengi*, are recorded from southwestern China and Myanmar. To this fauna, we have added one more species, *D. denruoi*, from northern Vietnam. All Old World members, *D. afer, D. pengi*, *D. tsigana*, *D. moriwakii* and *D. denruoi*, possess acrocentric X chromosomes. Of the New World members only one species, *D. micromelanica* Patterson in Sturtevant & Novitski, 1941, has acrocentric X, but the remaining five species have a large metacentric X. Based on comparative analyses of banding patterns of salivary gland chromosomes, Stalker (1972, 1976) considered that the metacentric X would have resulted from centric fusion of the acrocentric X and an acrocentric autosome in an ancestral species. Thus, the acrocentric X is considered to be "ancestral" and the metacentric X to be "derived" in the chromosomal evolution of the *melanica* group (Stalker 1966, 1972; Levitan 1982; Clayton & Guest 1986; Flores *et al.* 2008). Furthermore, *D. denruoi* lacks large metacentric autosomes, which are seen in the metaphase plates of its related species, *D. afer, D. pengi* and *D. tsigana*, with $2n = 10$ chromosomes (Tan *et al.* 1949; Stalker 1966; Wang *et al.* 2006a). The three pairs of the acrocentric autosomes of *D. denruoi* are all approximately half as long as the pair of large metacentric ones of these three species. Differences in the chromosome number between *D. denruoi* and its relatives might be explained by centric fusion of two pairs of acrocentric autosomes found in *D. denruoi* or by centric fission of one pair of metacentric autosomes found in *D. afer, D. pengi* and *D. tsigana*. The reduction in chromosome number is likely to be a frequent trend in the genus *Drosophila* Fallén, since the formation of two acrocentric chromosomes from a metacentric one demands an additional centromere (Clayton & Guest 1986). Thus, the hypothesis of centric fusion appears much more probable than the alternative. This implies that *D. denruoi* is closest to the ancestral form as far as karyotype evolution is

concerned. The discovery of such a species, *D. denruoi*, from northern Vietnam suggests that the *melanica* group originated in the subtropical Oriental Region. Phylogeographic analyses, based on molecular data, for all Old and New World *melanica*-group species would provide more crucial evidence for this hypothesis. In addition, since our knowledge of the *melanica* group in Southeast Asia, especially from tropical mountainous areas with cooler climate, is still scanty, further faunal studies are needed to clarify the origin and early diversification of the *melanica* group.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Holotype and allotype of *Drosophila* (*Drosophila*) *pengi*.

Figure S2 Holotype of *Drosophila* (*Drosophila*) *bisetata*.

Figure S3 Female paralectotypes of *Drosophila* (*Drosophila*) *tsigana*.

Figure S4 Holotype and paratype (♀) of *Drosophila* (*Drosophila*) *longiserrata*.