A taxonomic study of the *Drosophila auraria* species complex (Diptera; Drosophilidae), with description of a new species

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Abstract

The *Drosophila auraria* species complex, especially from the Ryukyu archipelago and Taiwan, was reviewed. A new species, *D. neoasahinai* Watada and Kondo, sp. nov., was described from Okinawa-jima and surrounding islands. Two synonymies were proposed on the basis of the present and previous morphological comparisons and cross experiments; *D. yuwanensis* Kim and Okada, 1988, as a junior synonym of *D. asahinai* Okada, 1964, and *D. quadraria* Bock and Wheeler, 1972, as a junior synonym of *D. triauraria* Bock and Wheeler, 1972. A laboratory stock (No. 14020-0011.01) maintained in the Drosophila Species Stock Center at the University of California, San Diego and so far designated as *D. rufa* Kikkawa and Peng, 1938, was identified as *D. tani* Cheng and Okada, 1985, based on morphology.

Key words: Ryukyus, synonymy, Taiwan, taxonomic status.

INTRODUCTION

The *Drosophila auraria* species complex belongs to the *montium* species subgroup of the *melanogaster* species group, although some taxonomists consider that the *montium* species subgroup should be raised to the rank of species group (Da Lage *et al*. 2007; van der Linde *et al*. 2010). This complex consists of the *D. auraria* and *D. rufa* lineages (Lemeunier *et al*. 1983; Goto & Kimura 2001; Da Lage *et al*. 2007; Miyake & Watada 2007). Five species have been reported in each lineage; *D. rufa* Kikkawa and Peng, 1938, *D. tani* Cheng and Okada, 1985, *D. lacteicornis* Okada, 1965, *D. asahinai* Okada, 1964, and *D. yuwanensis* Kim and Okada, 1988, in the *rufa* lineage, and *D. auraria* Peng, 1937, *D. biauraria* Bock and Wheeler, 1972, *D. triauraria* Bock and Wheeler, 1972, *D. quadraria* Bock and Wheeler, 1972, and *D. subauraria* Kimura, 1983, in the *auraria* lineage. In the *rufa* lineage, *D. lacteicornis*, *D. asahinai* and *D. yuwanensis* have been recorded only from the Ryukyu archipelago, whereas *D. rufa* from the main islands of Japan (including Tanegashima and Yakushima), China and Korea and *D. tani* from the continental regions of China (Fig. 1); in the *auraria* lineage, *D. quadraria* has been recorded only from Taiwan, whereas the other four species from Japan, China and/or Korea (Okada 1964, 1968; Bock & Wheeler 1972; Kim & Okada 1988; Hirai *et al*. 2000; Kondo & Kimura 2008). In our recent study, however, some questions arose on the species status of these species. First, Kim and Okada (1988) differentiated *D. yuwanensis* from *D. asahinai* by some morphological characteristics, but some specimens collected in our surveys in Amami-oshima and Tokunoshima showed

intermediate morphology. Second, Okada (1965) reported *D. lacteicornis* from Okinawa, but specimens collected in our survey in Okinawa and surrounding islands differed from *D. lacteicornis* in morphology (Kondo & Kimura 2008). This paper aims to solve these problems mainly on the basis of morphological and genetic studies on specimens and laboratory strains collected from the Ryukyu archipelago. In addition, we revise the taxonomic status of *D. triauraria* and *D. quadraria* of the *D. auraria* lineage on the basis of previous morphological, phylogenetic and genetic studies (Kimura 1987; Kim *et al*. 1989; Schawaroch 2002; Miyake & Watada 2007) and re-examine the laboratory stocks identified as *D. rufa* by Bock and Wheeler (1972).

MATERIALS AND METHODS

Collections of specimens belonging to the *rufa* lineage were carried out using traps baited with banana and by net sweeping on undergrowth plants in Amami-oshima, Tokunoshima, Iheya-jima, Okinawa-jima, Kume-jima, Ishigaki-jima and Iriomote-jima in the Ryukyu archipelago (Fig. 1) from 1989 to 2009. Collected specimens were dried and pined, preserved in 70% ethanol, or used to establish iso-female lines.

Morphological examination was conducted under a stereoscopic dissecting microscope and metric characters were measured with an ocular micrometer. Morphological terminology followed McAlpine (1981) and measurements followed Zhang and Toda (1992). The male and female terminalia were detached from the body,

cleared by warming 10% KOH solution at approximately at 100 °C for a few minutes and observed in a droplet of glycerol under a compound light microscope. Drawings were made with an ocular mesh micrometer and section paper.

Type specimens and other examined specimens were dried using the hexamethyldisilazane (HMDS) critical-point drying method (Brown, 1993), pinned and deposited in the National Museum of Nature and Science, Tokyo (NSMT) and the Biosystematics Laboratory, Graduate School of Social and Cultural Studies, Kyushu University, Fukuoka (BLKU).

To examine reproductive isolation and/or morphological variation among sibs, iso-female lines were collected from Mikyou and Inutabu, Tokunoshima on September 16, 2002 (TKN-M-1 and TKN-I-4, respectively), Materia-no-taki, Amami-oshima on September 25, 2000 (AM-2k-1, AM-2k-3), Motobu-cho, Okinawa-jima on September 22, 2000 (OKI-N2K-2), Kume-jima on May 23, 2001 (KM01-7M), Ishigaki-jima, on November 26, 1998 (ISG-O-10) and Iriomote-jima on November 9, 1996 (IR-96-1). Established iso-female lines were maintained in National BioResource Project at Ehime University (NBRP-EU). In addition to these lines, some iso-female lines of *auraria*- and *rufa*-lineage species were obtained from the Drosophila Species Stock Center at the University of California, San Diego (DSSC-UCSD) and NBRP-EU.

In cross experiments, five virgin females were placed with five males in a vial and examined for the production of hybrids (two replicates were usually used). When F_1 flies were produced, they were examined for the production of F_2 flies by backcross to both parental lines to check fertility. Experiments were conducted at 25 °C under a

long day-length (14 h light: 10 h dark) using cornmeal-malt medium as food.

TAXONOMY

***Drosophila asahinai* Okada** (Figs 2-5)

Drosophila asahinai Okada, 1964: 15.

Drosophila yuwanensis Kim and Okada, 1988: 57. **Syn. nov.**

Okada (1964) described *Drosophila asahinai* with specimens collected from Tokunoshima and Amami-oshima (the holotype was collected from Tokunoshima). Later, Kim and Okada (1988) differentiated specimens collected in Amami-oshima from *D. asahinai* by the difference in the shape of the hypandrium and the aedeagus, the length of the paramedian setae on the hypandrium, the number of rows in the acrostichal setulae and the number of teeth of the sex combs, and described as a new species, *D. yuwanensis*. However, specimens from Amami-oshima and Tokunoshima often showed intermediate morphology. To clarify the species status of these species, morphological analyses were carried out with male specimens collected from Tokunoshima (Kametoku: three males) and Amami-oshima (Naze: nine males) in 1989 and males of iso-female lines from Tokunoshima (TKN-M-1 and TKN-I-4) and Amami-oshima (AM-2K -1 and AM-2K-3).

Table 1 shows morphological characteristics of the specimens examined and

the data from Kim and Okada (1988). In the number of teeth of the sex comb (SC) on the fore leg first tarsomere, the number of rows in the acrostichal setulae (AC) and the shape of the median process of the hypandrium (MH), the variation within an iso-female line was nearly equal to or rather larger than the between-species differences reported by Kim and Okada (1988). The variation in the shape of the median process of the hypandrium in an iso-female line (AM-2K-3) was shown in Figures 2 and 3.

The shape of the proximal margin of the tenth sternite (TS) varied rather continuously; concave or slightly concave in wild-caught males from both islands and also in males of two iso-female lines (TKN-I-4 and AM-2K -1), but nearly straight in males of another two iso-female lines (TKN-M-1 and AM-2K-3) (Table 1).

According to Okada (1964), two paramedian setae on the median process of the hypandrium (SM) of *D. asahinai* were rather long in his illustration, but were stated "strong and short" in the text. To solve this confusion, we examined the holotype of *D. asahinai* (deposited in the National Museum of Nature and Science), but its phallic and periphallic organs were removed from the body and we could not found them. In the present samples, the length of setae varied rather continuously. For comparison, setae were divided into two types; i.e., "long" when the seta was longer than the distance between the sockets of the setae, and "short" when the seta was shorter than the distance. In all wild-caught males, the setae were short, but thinner than those illustrated by Kim and Okada (1988). On the other hand, the setae were short in TKN-M-1 but long in TKN-I-4, and varied in length in the two lines from Amami-oshima.

The difference in the length of the apical portion of the aedeagus (AE)

between the original descriptions of *D. asahinai* and *D. yuwanensis* was only slight (Okada 1964; Kim & Okada 1988). In the examined specimens, it was rather short in males of iso-female lines from Tokunoshima but long in males of iso-female lines from Amami-oshima and also in wild-caught males from both islands (Table 1; Figs 4, 5).

Thus, most characters denoted by Kim and Okada (1988) to differentiate *D. yuwanensis* from *D. asahinai* varied among individuals from each island and even in iso-female lines. In cross experiments between lines showing rather *D. asahinai*-like (TKN-M-1) and *D. yuwanensis*-like (AM-2k-3) characteristics, F_1 females and males were fertile (Table 2). On the basis of the above results, it is concluded that *D. yuwanensis* is a junior synonym of *D. asahinai*.

Specimens examined. **Tokunoshima:** 3 wild-caught ♂, Kametoku, 20.ix.1989, T. Awasaki: 10 ♂ of the TKN-M-1 line; 10 ♂ of the TKN-I-4 line. **Amami-oshima:** 9 wild-caught ♂, Naze, 23-24.ix.1989, T. Awasaki; 1 wild-caught ♂, Kinsakubaru, 12-19.vii.2006, M. Kondo; 10 ♂ of each the AM-2k-1 and AM-2k-3 lines.

Type specimens. The holotype of *D. asahinai* without phallic and periphallic organs was deposited in the National Museum of Nature and Science, Tokyo (the Okada's collection). The type specimen of *D. yuwanensis* was not found; the type depository was not given in Kim and Okada (1988).

***Drosophila* (*Sophophora*) *neoasahinai* Watada and Kondo, sp. nov.** (Figs. 6–13)

Diagnosis. Hypandrial process apically incised deeply, basally constricted (Fig. 8).

Male and Female. Head: Eye red, with pile. Frontal vitta reddish brown, with a few

interfrontal setulae. Fronto-orbital plate brown. Ocellar triangle dark brown. Facial carina brown, high, narrow. Clypeus gray. Gena brown. Palpus grayish brown, with a terminal prominent seta. Prementam, occiput and postgena yellowish blown. Pedicel dark blown; 1st flagellomere grayish yellow. Arista with 4 (or 5) dorsal and 3 ventral branches; terminal biflucation moderate.

Thorax: Scutum yellowish brown. Acrostichal setulae in 6 rows. Scutellum brown. Humeral pale brown, with 2 setae. Anepisternum and anepimeron yellowish brown, with longitudinal dark brownish stripe along ventral margin. Katepisternum pale brown.

Wing hyaline. Veins brown. C1 setae 2. R_{2+3} straight; R_{4+5} and M_1 nearly parallel. Halter pale brown.

Legs yellowish brown; femora pale yellow. Preapical dorsal setae present on tibiae of all legs, weakly in fore and hind legs; apical ventral setae on fore and mid tibiae. Male fore leg with 2 longitudinal sex combs on first and second tarsomeres. Fore leg first tarsomere twice in length of second tarsomere; second tarsomere half of rest together; sex comb teeth 25 (25–28 in 10♂ paratypes) on first tarsomere, 16 (15–16) on second tarsomere; first tarsomere of mid and hind legs as long as rest together.

Abdomen: Tergum pale brown. In male, II to V tergites each with caudal dark brown band; VI+VII entirely dark brown, anterolateral margin nearly pale brown; VIII brown. In female, II tergite with medially interrupted caudal dark brown band; III to V each with caudal dark band, projected anteriorly at middle. Sternum pale brown.

Male terminalia (Figs. 6–12): Epandrium dark brown, with 5–7 setae on dorsal part; ventral portion, brown, with 6–8 setae laterally and 10–13 setae along posterior margin (Fig. 6). Surstylus dark brown, semicircular, fused to epandrium, with 7–9 short prensisetae in a row on nearly caudal margin, with 16–20 long prensisetae on ventral to inner margin (Fig. 6). Cercus separated from epandrium, not pubescent; upper part dark brown, triangular, with 21–25 setae on lateral surface, ventrally with large, black, stout spine on margin to ventral lobe; ventral lobe pale, conical, differentiated as "secondary clasper" bearing 4 (or 5) large, black, stout, ventrally curved spines on posterior margin and 12–15 setae on anterior margin, of which uppermost one stout (Fig. 6). Tenth sternite dark brown, anteriorly bifurcated (Fig. 7). Hypandrium brown, longer than wide, somewhat pentagonal, pubescent in caudal margin; caudomedial process strongly protruded, subapically with a pair of paramedian setae (Fig. 8). Paramere brown, hemispherical, each with a row of 4 or 5 sensilla (Fig. 8). Aedeagus pubescent, nearly straight, apically tapered and curved dorsad, basally with a pair of ventrolaterally dilated hook-like processes; apodeme fused to aedeagus, nearly straight (Fig. 9). Gonopod fused to each other, forming bridge connecting caudal ends of hypandrium, medially incised triangurally(Fig. 10). Ejaculatory apodeme with oval plate, protruded at each side; stem of apodeme nearly straight, slender (Figs. 11, 12).

Female terminalia (Figs. 13, 14). Oviscapt dark orange brown, subapically broadened, apically triangular, with 17–21 marginal peg-like sensilla, subapically with one trichoid sensilla; anteroventral bridge narrow, *ca*. 1/7 as long as oviscapt (Fig. 13).

Spermathecal capsule broader than long (Fig. 14).

Measurements: Body length=2.64 mm in holotype (paratypes: 2.31–2.69 in 10♂, 2.60–3.07 in 10♀); thorax length=0.96 mm (0.97–1.10 in ♂, 0.95–1.15 in ♀); wing length=2.08 mm (2.00–2.45 in ♂, 2.24–2.47 in ♀); wing width=0.86 mm (0.80–1.02 in ♂, 0.88–1.04 in ♀).

Indices: FW/HW(frontal width/head width)=0.43 (0.43–0.50), ch/o (maximum width of gena/maximum diameter of eye)=0.11 (0.08–0.19), prorb (proclinate orbital/posterior reclinate orbital in length)=1.00 (0.60–1.00), rcorb (anterior reclinate orbital/posterior reclinate orbital in length)=0.44 (0.35–0.50), vb (subvibrissa/vibrissa in length)=0.95 (0.76–0.95), dcl (anterior dorsocentral/posterior dorsocentral in length)=0.63 (0.61–0.95), sctl (basal scutellar/apical scutellar in length)=0.96 (0.71–1.05), sterno (anterior katepisternal/posterior katepisternal in length)=0.64 (0.45–0.68), orbito (distance between proclinate and posterior reclinate orbitals/distance between inner vertical and posterior reclinate orbital)=0.73 (0.56–0.83), dcp (length distance between ipsilateral dorsocentrals/cross distance between anterior dorsocentrals)=0.51 (0.48–0.61), sctlp (distance between ipsilateral scutellars/cross distance between apical scutellars)=1.05 (1.05–1.33), C (a/b; a: 2nd costal section between subcostal break and R_{2+3}, b: 3rd costal section between R_{2+3} and R_{4+5})=2.22 (2.16–2.65), 4c (b/d; d: M_1 between r-m and dm-cu)=1.37 (1.18–1.50), 4v (c/d; c: M_1 between dm-cu and wing margin)=2.50 (2.11–3.13), 5x (e/f; e: CuA_1 between dm-cu and wing margin, f: dm-cu between M_1 and CuA_1)=3.23 (2.08–3.71), ac (b/h; h: distance between distal ends of R_{4+5} and M_1)=2.74 (2.38–3.17), M (e/d)=0.91

(0.74–1.08), C3F (g/b; g: length of heavy setation on 3rd costal section)=0.63 (0.52–0.61).

Holotype: ♂ (deposited in NSMT: No. NSMT-I-Dip 6772) from iso-female line (OKNH2K), Hiji-otaki, Kunigami-son, Okinawa-jima, 22.ix.2000, M. Watada. The line is maintained in NBRP-EU.

Paratypes: 10♂10♀ (NSMT: 5♂5♀ (Nos. NSMT-I-Dip 6773-6782) and BLKU: 5♂5♀), from the same strain as the holotype.

Other specimens examined. **Okinawa-jima:** 2♂2♀, Yona, Kunigami-son, 24-31.iii.2004, M. Kondo; 8♂6♀, Yonaha-dake, Kunigami-son, 16-23.iv.2005, M. Kondo; 1♂, Nishihara-cho, 8.v.2005, M. Kondo; 1♀, Nishihara-cho, 28.iv.2008, M. Kondo; 1♂, Sueyoshi-koen, Naha, 2.vii.2009, M. Kondo; 1♂, Sueyoshi-koen, Naha, 5.vii.2009, M. Kondo. **Kume-jima:** 5♂5♀, 4.iv.2004, M. Kondo; 1♂1♀, 5.vii.2004, M. Kondo; 2♂2♀, 29.x.2004, M. Kondo; 5♂2♀, 9.ii.2005, M. Kondo; 3♀, 8-15.ii.2005, M. Kondo. **Iheya-jima:** 4 iso-female lines, 30.iv.2004, M. Watada.

Etymology. Morphologically close to *Drosophila asahinai*.

Distribution. Central Ryukyus (Okinawa-jima, Kume-jima, Iheya-jima: Fig. 1).

Remarks. This species resembles *D. asahinai* and *D. lacteicornis* in the external morphology, but can be clearly distinguished from them by the shape of the hypandrial process: in *D. asahinai* and *D. lacteicornis,* the process is basally not constricted and apically less concaved (Figs. 15, 17). In addition, the hook-liked process of the aedeagus is smaller in *D. neoasahinai* than in *D. asahinai* (Fig. 16), and the aedeagus is more pubescent in *D. neoasahinai* than in *D. lacteicornis* (Fig. 18). This species is

reproductively isolated from *D. asahinai* or *D. lacteicornis* (Table 2).

Drosophila tani **Cheng and Okada**

Drosophila tani Cheng and Okada, 1985: 202.

Drosophila tani was described from China by Cheng and Okada (1985) as a closely related species of *D. rufa*. These species are easily differentiated by the phallic and periphallic organs; those of *D. tani* and *D. rufa* were illustrated by Cheng and Okada (1985) and Okada (1954), respectively. In the University of Texas Drosophila collection, there were two stocks designated as *D. rufa*; No. 1736.3 (newly numbered as 14020-0011.01) collected from Hangchow, China and No. 3040.16 (newly numbered as 14028-0661.1) from Kirishima, Japan (the stocks were moved from the University of Texas to the National Drosophila Species Resource Center at Bowling Green State University in 1982, and now maintained in DSSC-UCSD: new numbers were given at the Bowling Green Resource Center and modified at DSSC-UCSD). In our examination of their male phallic and periphallic organs, however, the No. 14020-0011.01 stock was determined as *D. tani*, and the No. 14028-0661.1 stock as *D. rufa*. In addition, a specimen of which the phallic and periphallic organs were illustrated by Bock and Wheeler (1972: Figs. 103, 104) was considered to be of *D. tani*, probably from the No. 14020-0011.01 stock, although they did not declare from which stock the specimen originated. We also carried out cross experiments between the No. 14020-0011.01 stock and *D. tani* from Zhejian, China (E-20801) and *D. rufa* from

Chiba, Japan (E-14801). In the cross with *D. tani*, F_1 and F_2 individuals were produced. In the cross with *D. rufa*, a small number of F_1 individuals were produced, but no F_2 individual was produced. Thus, the No. 14020-0011.01 stock is *D. tani*.

Laboratory stocks examined. ***D. tani***: iso-female lines; No. 14020-0011.01 maintained in DSSC-UCSD, Hangchow, China, collection date unknown, C. C. Tan; E-20801 maintained in NBRP-EU, Zhejian, China, 1979, H. Z. Chen. ***D. rufa***: iso-female line; E-14801, Chiba, Japan, 1978, Y. Oguma.

***Drosophila triauraria* Bock and Wheeler**

Drosophila triauraria Bock and Wheeler, 1972: 54.

Drosophila quadraria Bock and Wheeler, 1972: 55. **Syn. nov.**

Bock and Wheeler (1972) described *Drosophila triauraria* for specimens so far denominated as *D. auraria* Type C (Okada 1954; Kurokawa 1956, 1967) with the holotype from a laboratory stock, No. 1736.1 (newly numbered as 14028-0691.0). The collection locality of this stock was stated as Tokyo, Japan in Bock and Wheeler (1972), but as Hangchow, China in the Drosophila Species Stock List of National Drosophila Species Resource Center. Thus, a mistake occurred when the stock number or the collection locality was transcribed by Bock and Wheeler or to the Stock List. According to our examination, the phallic and periphallic organs of the No. 14028-0691.01 stock had characteristics of *D. triauraria* (illustrated by Bock and Wheeler (1972)) or *D. auraria* Type C (illustrated by Okada (1954) and Kurokawa

(1967)). The No. 14028-0691.0 stock has been lost.

Bock and Wheeler (1972) described *Drosophila quadraria* with the holotype from a single laboratory stock, No. 3075.1 (newly numbered as 14028-0651.00), collected in Chi Tou, Taiwan. Thereafter, repeated collections by the present authors and their colleagues were made in Taiwan, but no *D. quadraria* specimen has been collected. This species was differentiated from *D. triauraria* by a slight difference in the shape of the aedeagus (Bock & Wheeler 1972; Kimura 1983), but some specimens of *D. triauraria* from the Chinese continent had the *D. quadraria*-type aedeagus (Watada unpublished observation). In cross experiments with various geographic stocks of *D. triauraria*, this species always produced fertile F_1 females and males (Kimura 1987; Kim *et al*. 1989). In this study, cross experiments were carried out between the No. 14028-0691.01 (*D. triaruraria*) and No. 14028-0651.00 (*D. quadraria*) stocks; fertile F_1 and F_2 females and males were produced. In addition, the No. 14028-0651.00 (*D. quadraria*) stock did not clearly differ from *D. triauraria* (and also from *D. auraria*) in nucleotide sequences of the cytochrome oxidase I and II (COI and COII) or alcohol dehydrogenase (ADH) genes, or the internal transcribed spacers of ribosomal DNA (Schawaroch 2002; Miyake & Watada 2007; Watada unpublished data). On the basis of these results, *D. quadraria* is considered to be a junior synonym of *D. triauraria*.

Laboratory stocks used. No. 14028-0651.00 maintained in DSSC-UCSD, Chi Tou, Taiwan, collection date and collector unknown; No. 14028-0691.01, Tokyo, Japan or Hanchow, China, collection date and collector unknown.

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Table 1 The number of teeth of a sex comb of the fore leg first tarsomere (SC), the number of rows of the acrostichal setulae (AC), the shape of the median process of the hypandrium (MH), the shape of the proximal margin of the tenth sternite (TS), the length of the paramedian setae on the median process of the hypandrium (SM), and the length of the apical portion of the aedeagus (AE) in the original description and examined specimens. N=number of individuals examined, R=round, C=concave, SC=slightly concave, NS=nearly straight, L=long, S=short

	Original dsecription		Present study					
			Tokunoshinma			Amami-oshima		
	D. asahinai	*D. yuwanensis*	Wild-caught males	Iso-female lines		Wild-caught males	Iso-female lines	
				TKN-M-1	TKN-I-4		AM-2k-1	AM-2k-3
N	-	-	3	10	10	9	10	10
SC	20	24	20-24	19-27	19-27	20-24	22-26	20-27
AC*	8	6	6-8	6-8	6-8	6-8	6-7	6-8
MH	R	C	C	C or R	C or R	C or R	C or R	C or R
TS	NS	C	C	NS	SC	C	SC or C	NS
SM	L	S	S	S	L	S	S or L	S or L
AE	S	L	L	S	S	L	L	L

* Few individuals had 7 rows in the achrostical hairs; i.e., 3 on a right or left side of the thorax and 4 in the other side.

Table 2. Results of cross experiments. F: F_1 females and males are fertile, f: F_1 females were fertile, but F_1 males were sterile.

Female	Male					
	D. asahinai		*D. neoasahinai*		*D. lacteicornis*	
	AM-2k-3	TKN-M-1	OKI-N2k-2	KM01-7	ISG-O-10	IR-96-1
AM-2k-3	F	F	f	f	f	f
TKN-M-1	F	F	f	f	f	f
OKI-N2k-2	f	f	F	F	f	f
KM01-7	f	f	F	F	f	f
ISG-O-10	f	f	f	f	F	F
IR-96-1	f	f	f	f	F	F

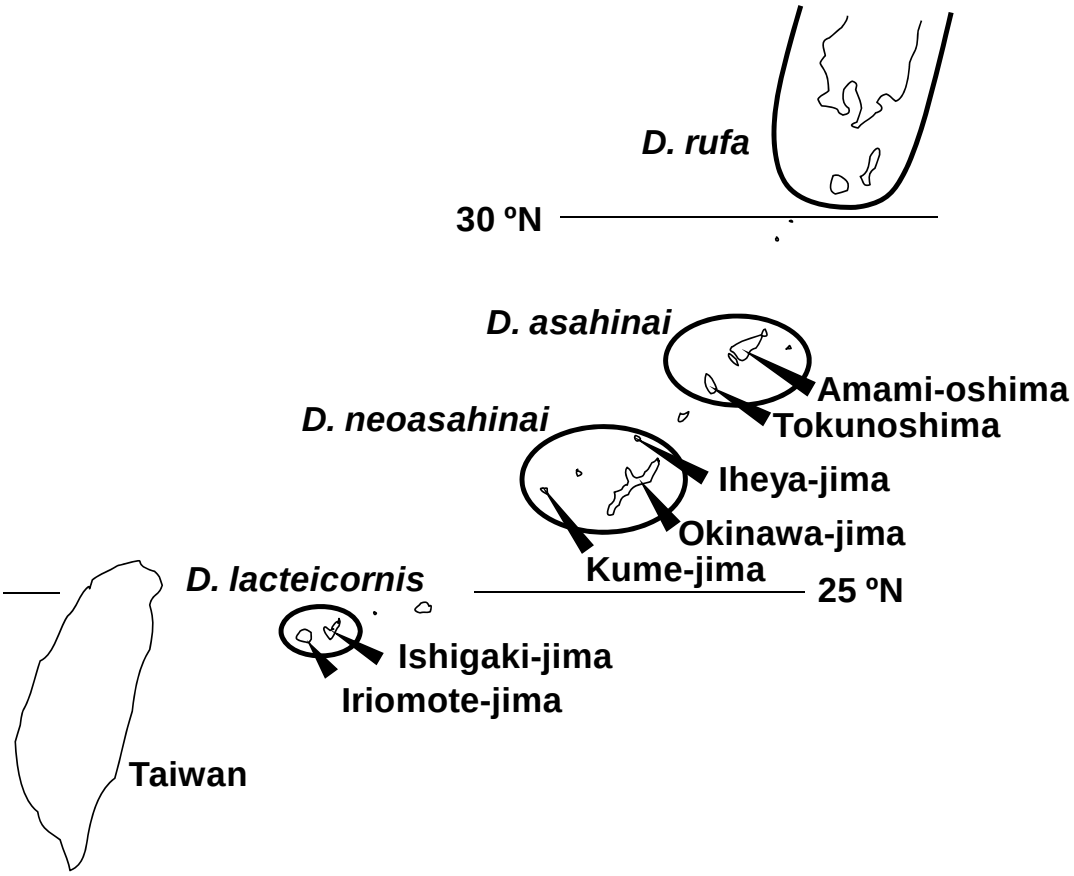
Figure legends

Figure 1 Map of the Ryukyu archipelago and Taiwan, showing locations where samples were collected, and the distribution ranges of *Drosophila rufa, D. asahinai*, *D. neoasahinai* and *D. lacteicornis*.

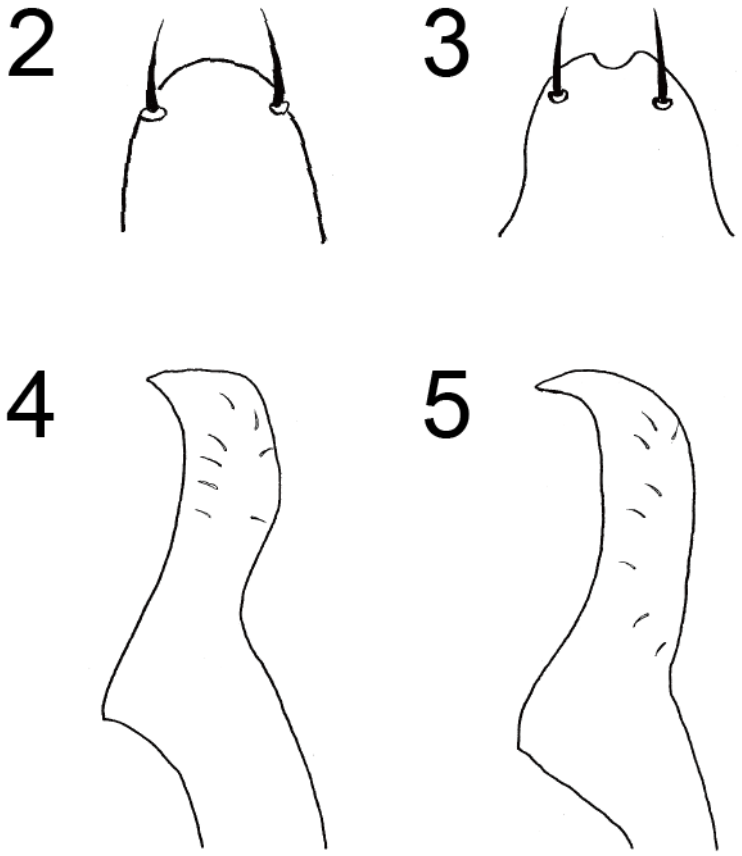
Figures 2–5 **2, 3** Variation of median process of hypandrium in an iso-female line of *D. asahinai*, AM-2k-3; **4, 5** aedeagus of males from iso-female lines of *D. asahinai*, TKN-M-1and AM-2k-3, respectively.

Figures 6–18 *Drosophila* (*Sophophora*) *neoasahinai* Watada & Kondo, sp. nov. **(6–14),** *Drosophila asahinai* **(15, 16)** and *Drosophila lacteicornis* **(17, 18).** **6** Peripharic organs in lateral view; **7** 10th sternite in ventral view; **8, 15, 17** pharic organs in ventral view; **9, 16, 18** aedeagus in lateral view; 10 gonopods in dorsal view; **11, 12** ejaculatory apodeme; **13** female terminalia in lateral view; **14** spermatheca. aed = aedeagus, cerc = cercus, epand = epandrium, hypd = hypandrium, par = paramere, sur = surstylus. Scale line: 0.1 mm. Collection data on *D. asahinai* and *D. lacteicornis* were as follows: *D. asahinai*, Kinsakubaru, Amami-oshima, 12-19.vii.2006, M. Kondo; *D. lacteicornis*, Ara-dake, Iriomote-jima, 22.ix.2007, M. Kondo

Fig. 1


D. rufa
30 ºN
D. asahinai
Amami-oshima
Tokunoshima
D. neoasahinai
Iheya-jima
Okinawa-jima
Kume-jima
D. lacteicornis
25 ºN
Ishigaki-jima
Iriomote-jima
Taiwan

Figs. 2-5



Figs. 6–18