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***Drosophila (Sophophora) carrolli* n. sp., a new species from Brunei, closely related to *Drosophila (Sophophora) rhopaloea* Bock & Wheeler, 1972 (Diptera: Drosophilidae)**

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Abstract

We describe a new species in the *Drosophila melanogaster* species group, *Drosophila carrolli* n. sp., showing morphological affinities with *D. rhopaloea* Bock & Wheeler, 1972.

Keywords

Drosophila; *rhopaloea*-group; South East Asia; Brunei

Introduction

In 1972, in a landmark paper on the taxonomy of the *Drosophila melanogaster* species group, Ian Bock and Marshall Wheeler defined several species subgroups and described a number of new taxa. One of these new taxa was *Drosophila (Sophophora) rhopaloea*, from Thailand, which gave its name to a new species subgroup later defined by Toda (1991). The monophyly of the *rhopaloea* subgroup was later supported by a molecular phylogeny (Barmina & Kopp, 2007). *D. rhopaloea* has a broad geographic distribution in South East Asia, from India (Meghalaya, Madras) to Vietnam, and South to Java and Papua New Guinea (Chen et al., 2014; Krishnamurthy, 1973; Okada & Carson, 1983; Setoguchi et al., 2014). Okada and Carson (Okada & Carson, 1983) noted significant variation in the morphology of this species across its geographical range, but did not regard this variation as sufficient to separate new species. During field work in Brunei in 2003, A. Kopp and O. Barmina established an isofemale culture of a *Drosophila* species showing affinities to *D. rhopaloea*, but also a series of clearcut differences. This taxon, until now referred to as *Drosophila* sp. KB866, has been recognized as a distinct species and treated as such in the studies of genetics, behavior and phylogenetics (Arnoult et al., 2013; Barmina & Kopp, 2007; Kudo, Takamori, Watabe, Ishikawa, & Matsuo, 2014; Setoguchi et al., 2014). Attempts to cross it to *D. rhopaloea* from Vietnam invariably failed, ultimately leading us to conclude that it should be considered a separate species, and motivating its present description as *Drosophila (Sophophora) carrolli* n. sp.

Material and methods

Fly stocks.

All flies examined in this study are derived from cultures kept in the laboratory. All stocks were bred on standard cornmeal medium (as in Arnoult et al., 2013) at 20°C, 60% humidity under a 12:12 hours light cycle regime. A copy of the unique *D. carrolli* culture has been deposited at the Drosophila Species Stock Center (Cornell University, Ithaca, USA). A series of type specimens was derived from this culture, mounted on cardboard, and pinned (see type material below). The male holotype was dissected, its genitalia are mounted on a transparent plastic board, pinned with the specimen. All specimen carry 2 collection labels that read “Brunei Darussalam, Kuala Belalong, Ulu Temburong National Park, 4°58’30.40”N 114°53’27.68”E, hatched 7.III.2018 from culture of isofemale line KB866 collected in 2003, (A. Kopp/O. Barmina leg.)”, as well as a red label that reads “*Drosophila (Sophophora) carrolli* n.sp., Paratype/Holotype #m, N. Gompel/A. Kopp det. 2018”. The main *D. rhopaloa* stock used for comparison is BaVi0067, kindly provided by Dr. Hisaki Takamori. This stock, maintained at the Drosophila Species Stock Center (Cornell University, Ithaca, USA), was used for the reference genome of *D. rhopaloa* (Chen et al., 2014). We also used another line from the same locality, BaVi5327, kindly communicated by Dr. Takashi Matsuo.

Specimen preparation.

Adult flies for whole mount imaging were briefly anaesthetized with vapors of triethylamine, transferred to a microscope slide, then glued ventrally with a droplet of water-soluble glue. The appendages of these mounted flies were spread with a needle, their wings maintained opened by gluing their tip with a droplet of glue to the slide. The specimens were then imaged as described hereafter.

Terminalia of alcohol-killed specimens were briefly cleared using 10% KOH, then washed with water, and mounted in DMHF (Steedman, 1958) between a slide and a coverslip. Male genitalia were progressively repositioned as the DMHF hardened, using mounted minutien pins. Wings and antennae were mounted in DMHF with no previous treatment. Male prothoracic legs were dissected from live flies, mounted in Hoyer’s medium between two coverslips, and cleared overnight before imaging. Adult male testes were dissected from live flies in saline buffer and mounted in Fluoromount.

Type specimens were killed with vapors of ethyl acetate, glued on small cardboards and dehydrated in 100% ethanol for a day, then critical-point dehydrated in 2 successive bath (2×30 min) of HMDS (Brown, 1993). The solvent was left to evaporate under a chemical hood and the specimens were finally pinned and labelled for museum storage.

Microscopy and imaging.

Eggs, male testes, pupae and adult specimens were photographed using a Leica M420 Makroskop equipped with a Manta G-609B/C camera (GigE camera with Sony ICX694, Allied Vision, Exton, PA) driven by nVision software (Impuls Imaging GmbH, Türkheim).

All sclerotized dissected parts were imaged on a Nikon Ti2-Eclipse microscope equipped with a Nikon DS-Ri2 color camera.

Stacks of images were projected into single extended depth-of-field images using Helicon Focus software (HeliconSoft). All Images were enhanced using Adobe Photoshop. Measurements were made using a stage micrometer that was imaged under the same conditions, or directly on a Leica MZ6 stereoscope, using an eyepiece graticule.

Taxonomy

The following description of *Drosophila (Sophophora) carrolli* n. sp. is done in constant reference to the closely related species *Drosophila (Sophophora) rhopaloe* Bock & Wheeler, 1972.

***Drosophila (Sophophora) carrolli* Gompel & Kopp n. sp. (Figure 1A, B, E, G, I, K, M, Figure 2A, C, E, G, Figure 3A, C, E)**

Etymology.—The species is named after our former mentor, Sean B. Carroll, in appreciation of his inspiring approach to science in general, and evolution in particular.

Diagnosis.—This species resembles *Drosophila (Sophophora) rhopaloe* Bock & Wheeler, 1972, from which it differs by its darker overall color in male and female adults, a stronger stature, a region of gray pigmentation at the anterior distal tip of the male wing, a darker and brighter color of the male testes, and a strongly sclerotized plate at the end of the oviduct in females. *D. carrolli* appears to be closely related to *Drosophila (Sophophora) palmata* Takada, Momma & Shima, 1973, from Malaysia, another wing-spotted species from which it differs by its overall darker color and the nearly complete absence of dorsal bristles on the epandrium. It is unambiguously distinct from *Drosophila retnasabapathyi* Takada & Momma, 1975, from Malaysia as well, which has very different genitalia.

Description.—Adult dark, total length, from abdominal tip to frons: 2.4 mm (males), 2.6 mm (females).

Head. Dark brown. Antennae yellowish-dark, 3rd antennal segment ovoid (Figure 1K), distinctly longer than in *D. rhopaloe* (Figure 1L). Arista with 4-5 dorsal and 2 ventral branches, plus a terminal fork. Ocellar triangle and region of orbital setae darker, eyes bright red. Bristle pattern and lengths identical to that of *D. rhopaloe*, with 3 pairs of orbital setae (anterior proclinate, median and posterior reclinate), 3 pairs of vertical setae (anterior and posterior pointing toward midline, median reclinate), one pair of ocellar (proclinate) and one pair of vertical (reclinate) setae.

Thorax. Brown with a pattern of darker markings on notum disc: bilateral patches just anterior to scutellum; bilateral bands extending from wing hinges to intrascutal folds (but not reaching them); prescutal and intrascutal folds as well as scutellum darker. By contrast, *D. rhopaloe* has a uniformly light thorax. Legs brown, with apex of femora darker, unlike in *D. rhopaloe* where the legs are entirely yellow. Male foretarsae with 2 rows of sex combs on 1st and 2nd tarsomeres, similar to those of *D. rhopaloe* with typically 10-12 teeth per comb

(Figure 1M,N). Wing hyaline in both sexes, with a patch of gray pigmentation in males, extending from the anterior margin to the compartment between veins L3 and L4, and from the wing tip to the middle of the wing. This patch becomes distinct 24 hours after emergence and is absent from, or faint in *D. rhopaloa* males (Figure 1I, J; Setoguchi et al., 2014). *D. carrolli* females occasionally show a faint trace of darker pigmentation between veins L1 and L2 (Figure 1B). Notum with eight rows of acrostichal setae between dorso-central bristles.

Abdomen. Brown in males, where the darker pigmentation of tergites 5 and 6 typically found in males of many *melanogaster* group species is hardly distinguishable from the pigmentation of anterior segments (see variation in Figure 1E). By contrast, *D. rhopaloa* males are lightly pigmented on their tergites, except for a broad medial black band on segments 5 and 6, sharply interrupted laterally before sternopleural suture (Figure 1A,C, E-F). Female abdomen dark-brown, with stripes of darker pigmentation of variable width on each segment (Figure 1B, G). As in *D. rhopaloa* females, the overall abdominal pigmentation fades on the posterior segments (G, H).

Male terminalia. Similar conformation as in *D. rhopaloa*, but overall darker and bigger. Genital arch of epandrium almost devoid of dorsal setae (Figure 2A), in contrast to that of *D. rhopaloa* (Figure 2B). Anal plates elongated, with outer edges rounded and inner edges straight, covered with long bristles. Each anal plate is fused ventrally to a strong elongated black process, as in *D. rhopaloa*, which Bock and Wheeler (1972) interpreted as a secondary clasper, and is also present in *D. palmata* Takada, Momma & Shima, 1973. Primary clasper long (about twice as long as in *D. rhopaloa*), bearing 2 combs of stout bristles pointing inward. The dorsal comb carries 4-5 teeth and the ventral comb carries 6-7 longer teeth. 2-3 strong and long bristles are located ventrally to each dorsal comb and 1 long and 1-2 short bristles are located just dorsally to the ventral combs. These combs and bristles exist in *D. rhopaloa*, but they are smaller, and the combs have fewer teeth. Aedeagus similar to that of *D. rhopaloa*, only stronger and darker. Phallus with a strong basal sinuation, wider than in *D. rhopaloa* (blue double-arrows in Figure 2E, F). The palm-like process at the apex of the aedeagus reported in *D. palmata* Takada, Momma & Shima, 1973 is also seen in *D. carrolli* and *D. rhopaloa*. Testes orange-brown, darker than in *D. rhopaloa*, with 2 outer and 3 inner coils.

Female terminalia. Oviscape (egg guides) moderately sclerified, lined-up with single row of about 15 short and stout setae along its outer edge on each side, and an additional pair of longer and thinner bristles at its posterior tip. This configuration is similar to that of *D. rhopaloa*, which shows more irregularity in the alignment of the stout setae (Figure 3A, B). Anal plates well developed, stronger as in *D. rhopaloa*. Anterior to the anal plates lies a series of small brown sclerified plates that are not seen in *D. rhopaloa*. The distal tip of the oviduct is also characterized by 2 sets of strongly sclerotized plates not visible in *D. rhopaloa*. Spermathecae rounded and hollow, slightly bigger and darker than those of *D. rhopaloa* females.

Pupa. Length: 2.7 mm. Pupal case yellowish, with sides regularly rounded (more so than *D. rhopaloa* pupae, Figure 3E,F). Anterior spiracles similar to those of *D. rhopaloa*, with 7

(occasionally 8) terminal branches. Horn index ($100 \times$ length of the anterior spiracles/total puparium length) = 5.

Egg. Length: 0.7 mm. Two antero-dorsal respiratory appendages with a round basal stem and a flat spatula-like tip (Figure 3C).

Type material.—59 specimens derived from an isofemale line established in October 2003 (Artyom Kopp and Olga Barmina *leg.*), from a single female collected at Kuala Belalong, Ulu Temburong National Park, Brunei Darussalam [4°58'30.40"N 114°53'27.68"E]. This material, mounted on cardboard and pinned, will be dispatched as follows: 1 holotype (male, dissected) and 11 paratypes (4 males and 7 females): Universiti Brunei Darussalam, Institute for Biodiversity and Environmental Research; 11 paratypes (6 males and 5 females): British Museum of Natural History, London; 11 paratypes (6 males and 5 females): UC Davis Bohart Museum of Entomology; 11 paratypes (6 males and 5 females): Musée National d'Histoire Naturelle, Paris, France; 14 paratypes (7 males and 7 females): coll. N. Gompel, Munich, Germany.

Reproductive isolation.—Multiple attempts to cross *D. carrolli* (KB866) to *D. rhopaloa* (BaVi0067), in both directions, failed. Multiple replicate cultures consisting of 10-20 virgin females of one species, and 10-20 males of the other species, were maintained for several weeks. Since no larvae were ever observed, several females per replicate were dissected, and the absence of sperm in their reproductive tracts was confirmed.

Geographical range.—*Drosophila carrolli* is only known from Brunei Darussalam at Kuala Belalong, Ulu Temburong National Park [4°58'30.40"N 114°53'27.68"E] (Figure 1O), a locality that falls in the broad geographic range of *D. rhopaloa*.

Ecology, ethology.—*D. carrolli* was collected in primary rainforest, sweeping over a banana bait. It must be rare locally, as of over 1000 isofemale strains established over two weeks of field work, only two were of this species, one of which (KB866) is the origin of the specimens used here to describe the species. No *D. rhopaloa* were caught at that location. At 20°C, it takes 8 days after egg deposition for *D. carrolli* to enter metamorphosis and the total of 15-16 days for the adult fly to emerge from pupa, exactly like *D. rhopaloa*.

The male courtship behavior of *D. carrolli* has been described in detail and compared to that of closely related species (Setoguchi et al., 2014).

Molecular phylogeny.—Based on phylogenetic analysis of 12 nuclear and 2 mitochondrial loci, *D. carrolli* n. sp. belongs to the *rhopaloa* subgroup of the *melanogaster* species group, and may be the sister species to *D. rhopaloa*, Bock & Wheeler, 1972, within a clade that also contains more distantly related and morphologically different *D. prolongata* Gupta & Singh, 1978 and *D. kurseongensis* Gupta & Singh, 1977 (Barmina & Kopp, 2007; Setoguchi et al., 2014). No molecular information is available for another species morphologically similar to *D. rhopaloa* and *D. carrolli*, *D. palmata* Takada, Momma & Shima, 1973.

***Drosophila (Sophophora) rhopaloe* Bock & Wheeler, 1972 (Figure 1C, D, F, H, J, L, N, Figure 2B, D, F, H, Figure 3B, D, F)**

Drosophila rhopaloe Bock & Wheeler, 1972:69; Singh & Gupta, 1977: 35; Okada & Carson, 1983: 137; Toda, 1991: 81 *Drosophila (Sophophora) coonorensis* Reddy & Krishnamurthy, 1973: 262

This species has been extensively described by others, and the present work illustrates many aspects of its morphology in comparison to *D. carrolli* n. sp. Therefore, we have chosen to not include a redescription here.

Material examined.—*D. rhopaloe* line BaVi067 and BaVi5327 from Vietnam, Hanoi Ba Vi, near Vân Hòa [21°04'N, 105°22'E], March 2005, H. Takamori *leg.*

Previous records.—**Malaysia:** Borneo, Sabah, Mt. Kinabalu (Takada et al., 1973). **India:** Meghalaya, Shillong (Singh & Gupta, 1977); Madras, Nilgiri, Coonoor (type of *D. coonorensis* Reddy & Krishnamurthy, 1973). **Thailand:** Khao Yai [14°26'21.03"N 101°22'20.95"E], type locality. **Papua New Guinea:** Morobe Province, Mt Kaindi, Kunai Creek (1500 m.), 3.XI.1977 (H. Carson *leg.*) (Okada & Carson, 1983). Okada & Carson describe these specimens from Papua New Guinea as “exceedingly” different from those of Java, being in particular darker. The identity of these specimens is therefore questionable, and they may be assigned to *D. carrolli* n. sp., or to another new taxon. **Republic of Indonesia:** Java, (Okada & Carson, 1983). **Vietnam:** Vietnam, Hanoi Ba Vi, near Vân Hòa [21°04'N, 105°22'E], IX.2004 (line BaVi5327) and III.2005 (line BaVi067) (H. Takamori *leg.*) (Chen et al., 2014; Setoguchi et al., 2014).

Brake & Bächli (2008) also report *D. rhopaloe* from and **Sri Lanka**, without additional details.

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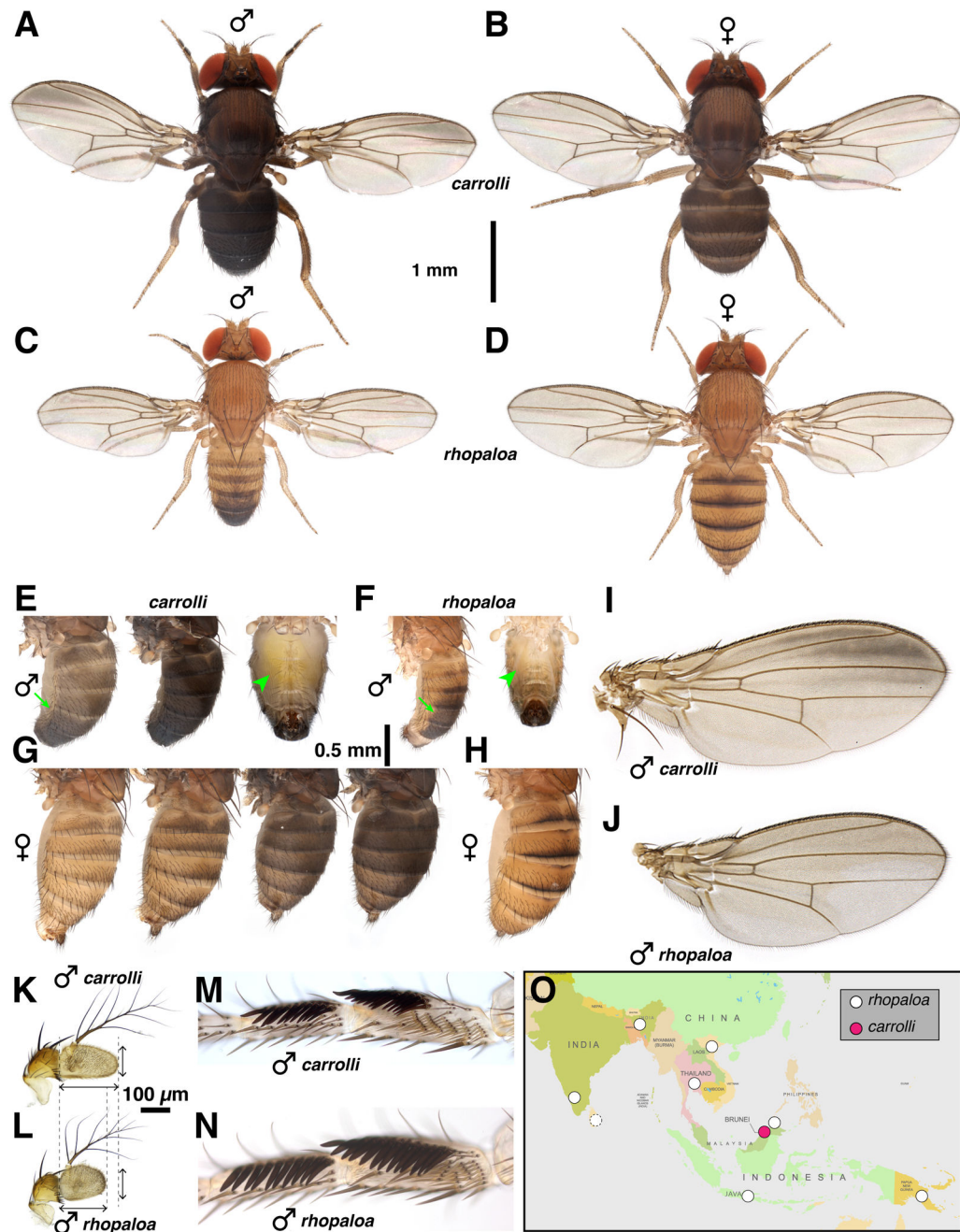


Figure 1. Adult morphology and geographical range.

(A-D) dorsal views of 5-7 day old adult *Drosophila carrolli* (A, male; B, female) and *D. rhopaloo* (C, male; D, female). (E, F) male abdomens of *D. carrolli* (E) and *D. rhopaloo* (F) in lateral (left) and ventral (right) views, showing variation in pigmentation intensity and testes color in mature specimens. Note the sharp lateral boundary of the darkly pigmented area on segment A5 and A6 in *D. rhopaloo*, but not *D. carrolli* (green arrows). Also note the bright yellow testes of *D. carrolli*, contrasting with the paler testes of *D. rhopaloo* (green arrowheads). (G, H) female abdomens of *D. carrolli* (G) and *D. rhopaloo* (H) in lateral views, showing variation in pigmentation intensity in mature specimens. (I, J) male wings of

D. carrolli (I) and *D. rhopaloea* (J). Note the darker pigmentation at the anterior distal tip of *D. carrolli* but not *D. rhopaloea* wings. (K, L) right antennae of males, showing no notable difference in the arista branching between *D. carrolli* (K) and *D. rhopaloea* (L), but a relatively shorter 3rd antennal segment in *D. rhopaloea* (double arrows). (M, N) sex combs on the 1st and 2nd tarsal segments of forelegs in *D. carrolli* (M) and *D. rhopaloea* (N) showing no notable differences in teeth shape or pattern. Note that image on panel M is modified from Barmina & Kopp (2007). (O) map of South East Asia showing localities where *D. rhopaloea* (white circles) and *D. carrolli* (red circle) were found. The circle with dotted outline denotes an imprecise locality.

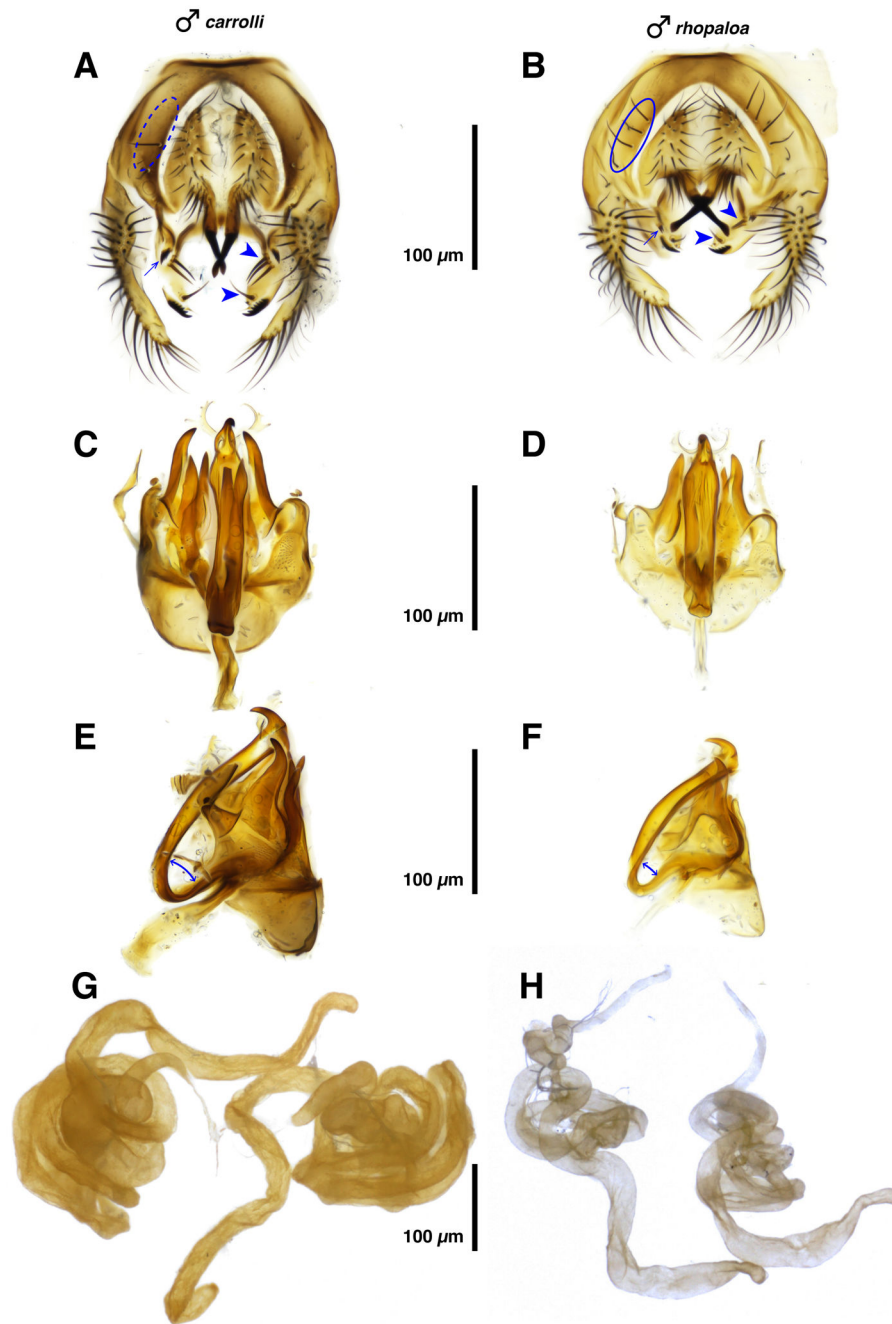


Figure 2. Male terminalia.

Dissected parts from *D. carrolli* (A, C, E, G) or *D. rhopaloa* (B, D, F, H). (A, B) epandrium in dorsal view. Note the differences in sensory bristle length and distribution (blue ovals and arrowheads) as well as genital comb size (blue arrows) between species. The epandrium is always darker overall in *D. carrolli*. Phallus in ventral view (C, D) and side view (E, F). The phallus conformation in *D. carrolli* (C, E) is similar to that of *D. rhopaloa* (D, F), but shows quantitative difference in size and appears sturdier. The looping of the aedeagus base is more open in *D. carrolli* than in *D. rhopaloa* (blue double-arrows). (G, H), testes of *D. carrolli* (G) and *D. rhopaloa* (H) have similar shapes and coiling, but they appear bigger in *D. carrolli*,

and show a characteristic bright yellow color compared to the dull yellow testes of *D. rhopaloa*.

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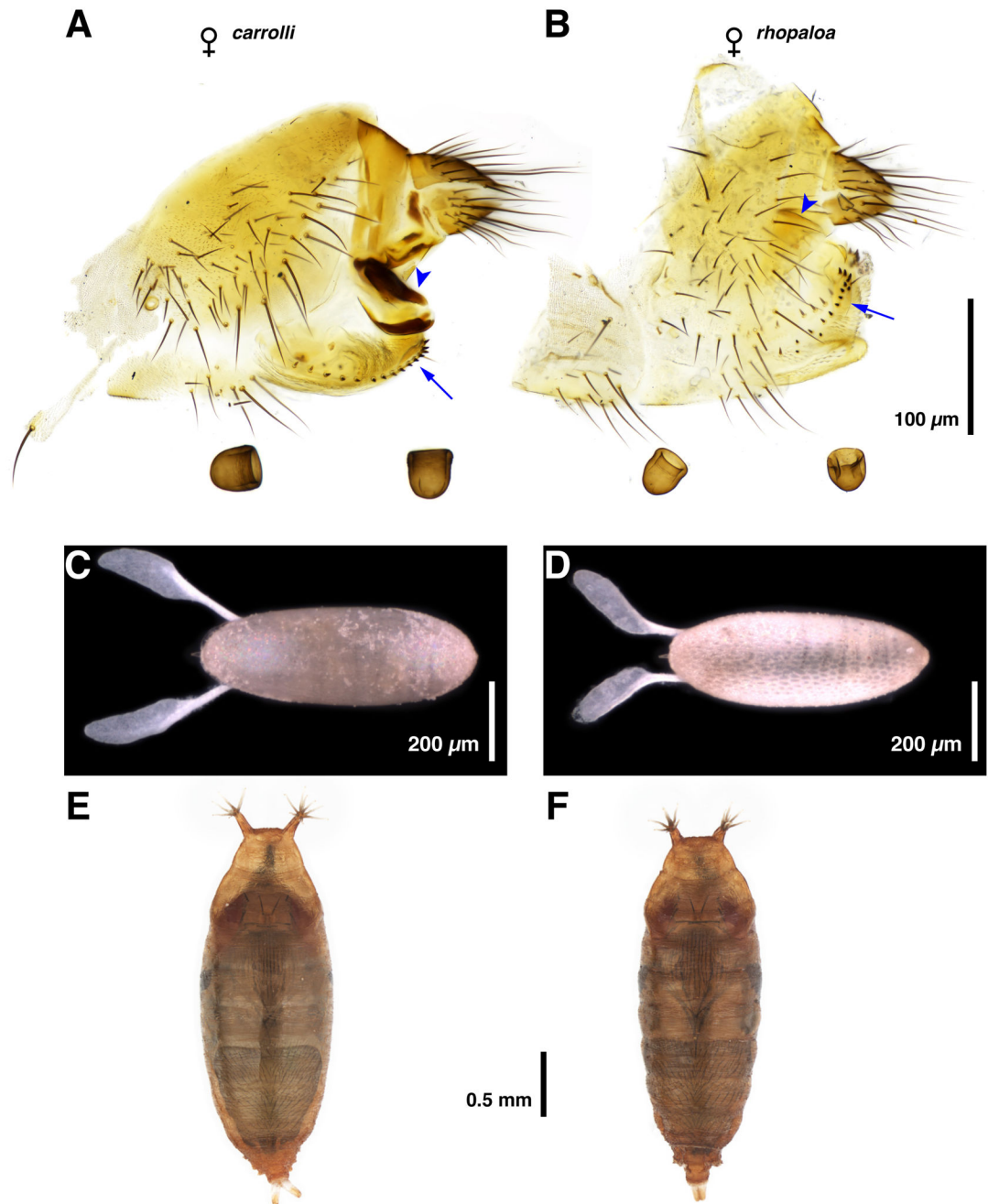


Figure 3. Female terminalia, eggs and pupae.

(A, B) last abdominal segments and spermatheca of *D. carrolli* (A) or *D. rhopaloa* (B) females. Note the strongly sclerotized parts (blue arrowheads) at the end of the oviduct in *D. carrolli*, but not *D. rhopaloa*. Also note the teeth (bristles) on the egg-guides are slightly more numerous and stouter in *D. carrolli* (blue arrows). The spermathecae are marginally bigger and darker in age-matched *D. carrolli* compared to *D. rhopaloa*. (C, D) eggs of *D. carrolli* (C) and *D. rhopaloa* (D) in ventral view. Both species show characteristic spatula-shape respiratory appendages. These appendages vary in shape within species but show no

notable difference between species. (E, F) pupae of *D. carrolli* (A) or *D. rhopaloea* (B) males about 24 hours before adult emergence. *D. carrolli* pupae are more rounded on their sides.

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