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Discovery of a predaceous drosophilid Acletoxenus indicus Malloch in South China, with descriptions of the taxonomic, ecological and molecular characters (Diptera: Drosophilidae)

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# Discovery of a predaceous drosophilid *Acletoxenus indicus* Malloch in South China, with descriptions of the taxonomic, ecological and molecular characters (Diptera: Drosophilidae)

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A drosophilid, *Acletoxenus indicus* Malloch, with larvae preying on *Aleurodicus dispersus* and *Aleurocanthus* sp. (Hemiptera: Aleyrodidae), is discovered from Guangdong and Hainan Provinces, China, and its morphological, ecological and molecular characters are described.

Keywords: Acletoxenus indicus; Aleurodicus dispersus; spiralling whitefly; natural enemy; Asia

# Introduction

Acletoxenus Frauenfeld 1868 is a small genus, including four species from eastern to southeastern Asia, Israel, Turkey, northern Australia, northern Africa and southern and central Europe (Brake and Bächli 2008). It has four recorded species; most of them are poorly known, except for Acletoxenus formosus (Loew, 1864), which has been studied in detail by Bächli et al. (2004). The Acletoxenus larvae of A. formosus and A. indicus are natural enemies of whitefly nymphs (Hemiptera, Aleyrodidae) and are used in the biological control (Ashburner 1981; Chiu et al. 1985; Gerling et al. 2001, 2004; Viraktamath 2002; Ulusoy and Ülgentürk 2003; Lambkin and Zalucki 2010). Acletoxenus indicus Malloch, 1929 was originally described from southern India; since then, it has been found widely in eastern Asia and discovered in infestations of Aleurocanthus (Aleyrodidae) and various other aleyrodid genera (Clausen and Berry 1932). Acletoxenus indicus was recorded as a predator of Aleurodicus dispersus Russell (Aleyrodidae) in India (Ramani et al. 2002).

Frauenfeld (1868) indicated only one character for diagnosis of this genus, e.g. arista microtrichose. Bock (1982) supplemented the diagnosis: eye very large; frons narrow, nearly parallel; ocellar setae absent; postocellar setae minute; all orbital setae long; carina absent; wing clear; costa exceeding apex of  $R_{2+3}$  vein (Figure 1B); cells bm and dm confluent; legs without preapical and apical setae. Bock (1982) pointed out that "the genus is probably most closely related to *Gitona* and the other steganine genera possessing micropubescent aristae". As can be deduced from the description (Malloch 1926), *Acletoxenus* is similar to *Luzonimyia* Malloch, 1926 in many respects,

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Figure 1. *Acletoxenus indicus* Malloch, male. (A) Head; (B) wing; (C) epandrium (epan), cercus (cerc) and surstylus (sur) (lateral view); (D) hypandrium (hypd), paramere (pm), aedeagus (aed) and aedeagal apodeme (aed a) (lateral view). Scale bars: 0.1 mm.

e.g. micropubescent arista (long micropubescent or short plumose in *Luzonimyia*), narrow gena, absence of carina, large prescutellar seta, clear wing without bm-cu crossvein, and black and yellow colouration. In addition, we can add the following common characters: eye very large; frons narrow, nearly parallel, lacking interfrontal setulae; ocellar setae absent or (sometimes in *Luzonimyia*) very weak; all orbital setae long, proclinate orbital setae shorter than the others (Figure 1A); legs without preapical or apical setae;  $R_{4+5}$  and  $M_1$  distally somewhat divergent (Figure 1B); epandrium and surstylus entirely fused (Figure 1C) (Cao and Chen 2008).

Okada (1989) assigned the genus *Acletoxenus* to his tribe Leucophengini, and also considered *Acletoxenus* as resembling *Luzonimyia* based on a phenetic analysis with 14 adult morphological characters. Grimaldi (1988, 1990) considered the genera *Acletoxenus* and *Mayagueza* as sister groups, possessing the following synapomorphy: dorsolateral tentorial apodeme arms parallel and very close together; facial and frontal region very narrow and parallel; anterior dorsocentral setae (on notum) barely longer than acrostichals, separated from posterior dorsocentrals by about one-half the length of anterior dorsocentral; ventral lobes of epandrium (male) tapered, with

pointed and sclerotized apices, pincers-like. The genus *Luzonimyia* was not included in Grimaldi's studies. It is well possible that *Acletoxenus*, *Luzonimyia* and *Mayagueza* form a monophyletic cluster of genera.

#### Materials and methods

#### Location of material and morphological terminology

The specimens examined were collected on tree leaves, except for a few that were reared from immature stages, and deposited in the Department of Entomology, South China Agricultural University, Guangzhou, China (SCAU). We followed McAlpine (1981) for morphological terminology and Zhang and Toda (1992) and Chen and Toda (2001) for the definitions of measurements, indices and abbreviations.

### DNA extraction, polymerase chain reaction and sequencing

Total DNA was extracted from a single male using the TIANGEN<sup>TM</sup> DNA extraction kit following the recommended protocol. The polymerase chain reaction (PCR)/sequencing primer pair for the fragment of the mitochondrial COI (cytochrome c oxidase, subunit I) gene was 5'-CGCCTAAACTTCAGCCACTT-3' (Wang et al. 2006) and 5'-CCTAAATTAGCTCATGTAGAC-3' (He et al. 2009). The PCR cycle programme comprised an initial 3 minutes of predenaturation at 94°C, 35 cycles of amplification (30 seconds of denaturation at 94°C; 1 minute of annealing at 56°C for *COI*; 1.5 minutes of extension at 72°C), and 5 minutes of sequence postextension at 72°. The PCR products were purified and then directly sequenced on an ABI3730 sequencer. The sequence generated in this study was submitted to GenBank, accession no. HQ701131.

#### Results

#### Sequence aligning and characterization

The *COI* sequences of the *A. formosus* was obtained from GenBank with number EF576933 (Otranto et al. 2008); hence, we compare the *COI* sequences between *A. formosus* and *A. indicus*. The program MEGA 4.0 (Tamura et al. 2007) was used to visually inspect the alignment of sequences and to estimate the uncorrected pairwise divergence. The *COI* sequences were aligned using the CLUSTALW method (Thompson et al. 1994) and the alignments were edited manually to make it conform to the codon assignments. The *COI* sequence of *A. indicus* was 1536 base pairs in length, but the sequence of *A. formosus* obtained from the GenBank was only partial for the *COI* gene with 700 base pairs. When these were aligned, only 663 nuclear positions matched, and 10 amino acid variations (11.31% genetic divergence of the *COI* sequences) were found between *A. formosus* and *A. indicus* out of 221 amino acids.

#### Taxonomic characters

Acletoxenus indicus Malloch (Figures 1 and 2)

Acletoxenus indicus Malloch, 1929. Ann Mag Nat Hist, 31: 545.

#### Description

Some characters not mentioned in Malloch (1929) are given below. Measuring in  $5\sigma$  and  $5\varphi$  adults: BL 1.96–2.22 mm in  $\sigma$ , 1.94–2.35 mm in  $\varphi$ ; ThL 1.10–1.25 mm in  $\sigma$ , 1.08–1.30 mm in  $\varphi$ ; WL 2.08–2.27 mm in  $\sigma$ , 2.12–2.30 mm in  $\varphi$ ; WW 1.02–1.05 mm in  $\sigma$ , 1.04–1.13 mm in  $\varphi$ . Male terminalia: epandrium protruded anterad and posterolaterally much protruded ventrad, with *c*. 20 short setae and pubescence (Figure 1C). Cercus small, separated from epandrium, pubescent and setigerous (Figure 1C). Surstylus almost entirely fused with epandrium, with three small setae apically on inner surface (Figure 1C). Hypandrium anteriorly narrow and laterally broad, with five or six setulae per side, basally with a narrow, anterad rod-like process, submedially with lobe-like process bearing four or five setulae (Figure 1D). Paramere bilobed, sclerotized and pointed apically, lacking sensilla, basally fused to aedeagal apodeme (Figure 1D). Aedeagus basally with two pairs of lobe-like processes (Figure 1D, lop): one of them small; the other curved dorsad and pointed apically (Figure 1D), apically with gracile membranous tube (Figure 1D). Aedeagal apodeme small, lobe-like (Figure 1D).

#### Specimens examined

CHINA:  $4\sigma$ ,  $2\phi$  (SCAU, nos. 121002–121007), Guangzhou, Guangdong,  $23^{\circ}09'$  N, 113°21' E, 8 September 2003, from variegated laurel (*Codiaeum variegatum*), X.M. Wang; 11 $\sigma$ ,  $6\phi$  (SCAU, nos. 121008–121024), Zhanjiang, Guangdong, 21°16' N, 110°21' E, 17 April 2005, from *Hibiscus* sp., S.X. Ren;  $2\sigma$ ,  $2\phi$  (SCAU, nos. 120998–121001), Qionghai, Hainan, 19°16' N, 110°28' E, 19 June 2009, reared from immature stages collected on guava (*Psidium guajava*), G.Y. Yu.

#### Life history and habits

Larvae of A. indicus have been observed preying on whitefly nymphs in the following plants Acalypha wilkesiana, Terminalia catappa, Codiaeum variegatum, Psidium guajava, Hibiscus rosa-sinensis. In Hainan Province, China, A. indicus larvae prey on nymphs of spiralling whitefly Aleurodicus dispersus. The egg of A. indicus is covered with a white waxy incrustation which is roughened with irregular or indistinct reticulate markings. The eggs are laid singly upon the leaf surface, adjacent to the late-stage nymph or pupa of whitefly. The larva is translucent white in its early stages, and then changes to greenish as the body contents are visible through the transparent integument (Figure 2B,C). The larva is inactive because the colony of whiteflies around it provides sufficient food for its maturity. Noticeably, sometimes there are several larvae on one leaf. The larva secretes a mucilaginous fluid that covers the whole body in the early stages, leading to the body of larva, particularly on the dorsum, being covered with particles of extraneous matter in the later stages. Many mature larvae carry a large number of egg shells or wax of *Aleurodicus dispersus*, and these structures may also be found upon the puparium (Figure 2C). Pupation occurs on the underside of the leaf. The ventral surface of the puparium is flat and adheres strongly to the leaf surface. The greenish colour of the body contents is observable in the early pupal period, and then the large, deep red eyes of the developing pupa are also observable. Emergence is effected by the breaking away of the distinct lid at the anterior end. The empty puparium is white (Figure 2D).



Figure 2. *Acletoxenus indicus* Malloch. (A) Adult; (B) larvae (green) preying upon spiralling whitefly on the underside of guava leaf; (C) coarctate pupae of spiralling whitefly; (D) puparium after eclosion. [This figure can be viewed in colour online.]

### Distribution

China (Guangdong, Hainan; new record); India.

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