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Redescription of *Chironomus javanus* and *Chironomus kiiensis* (Diptera: Chironomidae) Larvae and Adults Collected from a Rice Field in Pulau Pinang, Malaysia

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Abstrak: *Chironomus javanus* (Kieffer) dan *Chironomus kiiensis* Tokunaga dihuraikan semula menggunakan specimen yang dikumpul daripada sebidang sawah padi di Pulau Pinang, Malaysia. Larva hanya boleh dibezakan selepas persediaan dan pemeriksaan specimen yang teliti menggunakan mikroskop majmuk, tetapi pupanya tidak sesuai digunakan untuk membezakan antara *C. javanus* dan *C. kiiensis*. Specimen dewasa menunjukkan ciri-ciri badan dan sayap yang jelas untuk pengecaman yang cepat dan tepat.

Kata kunci: *Chironomus kiiensis*, *Chironomus javanus*, Sawah Padi, Agas

Abstract: *Chironomus javanus* (Kieffer) and *Chironomus kiiensis* Tokunaga were redescribed from materials collected from a rice field in Pulau Pinang, Malaysia. The larvae can only be distinguished after careful preparation and examination using a compound microscope, but the pupae were not useful to differentiate *C. javanus* from *C. kiiensis*. The adult specimens showed clear body and wing characteristics for rapid and accurate identification.

Keywords: *Chironomus kiiensis*, *Chironomus javanus*, Rice Field, Non-biting Midges

INTRODUCTION

The non-biting aquatic midges of the family Chironomidae (Order: Diptera) include hundreds of species; the larvae inhabit almost all types of inland waters, such as ponds, lakes, swamps, rivers, sewage ditches, fish ponds and rice fields (Al-Shami *et al.* 2011, 2010a, b). Among these habitats, rice fields have long been recognised as a pre-eminent habitat for chironomids throughout the world, such as India, Australia and the USA (Darby 1962; Martin & Porter 1977; Chaudhuri & Chattopadhyay 1990; Stevens 1995; Stevens *et al.* 2002; Stevens & Warren 2003). Jones (1968) reported *Chironomus tepperi* Skuse as a pest of rice in Australia, and several other chironomid species have been reported to injure rice seedling in many countries, including Australia, India, Japan and the USA (Ishihara 1972; Martin & Porter 1977; Chaudhuri & Chattopadhyay 1990; Stevens 1995; Stevens *et al.* 2002). Hashimoto *et al.* (1981) gave a brief description of the

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adult morphology of 32 species of chironomids inhabiting rice fields in Thailand. In Japan, Sasa and Kikuchi (1986) provided descriptions of 34 chironomid species inhabiting rice fields and estimated that at least 6 more species breed in the rice-growing areas of Japan. In general, the knowledge of chironomid fauna in the Indo-Malaya ecozone is comparatively low compared to the Holarctic and Afrotropical regions (Ashe 1990). Among the Chironomidae subfamilies, only Tanypodinae, Orthocladiinae, Chironominae and Diamesinae are described in this area (Cranston 2004). Thienemann (1954) provided a list of chironomid species found in the rice fields of Sumatra and Western Java. However, very little is known about the distribution, taxonomy and bionomics of rice field Chironomidae in Peninsular Malaysia; some pioneering studies on rice field Chironomidae of Kedah and Selangor states are those of Lim (1990), Che Salmah and Abu Hassan (2002) and Al-Shami *et al.* (2010a).

Chironomus larvae display the characteristics striations at the base of the mandible, and molecular data confirmed that the striation is specific for all *Chironomus* species (Martin *et al*. 2007). According to Cranston (2007), genus *Chironomus* is one of the most difficult genera of chironomids to identify. The adult morphology is rather homogenous, and few efforts have been made to simplify the identification. Furthermore, although *Chironomus javanus* and *Chironomus kiiensis* possess some differences at the adult stage, Al-Shami *et al.* (2006) reported that these species were difficult to separate using immature specimens. Cranston (2007) reported that *C. javanus* (Kieffer) is somewhat atypical because it resembles the genus *Kiefferulus* in having numerous (6–7) premandibular teeth. In the same study, the remaining larvae with two-toothed premandibles were suggested to belong to what is undoubtedly a heterogeneous assemblage of this species. Adult males are rare, thus they cannot be discriminated or named with certainty. A pharate *C. kiiensis* Tokunaga male is mistakenly identified as *C. javanus* unless the distinctive wing pattern in the teneral wings is confirmed.

Both *C. javanus* and *C. kiensis* are widely described in several Asian countries, such as Thailand, India, Japan and Korea. However, no attempt was made to redescribe the larvae and adult materials from Peninsular Malaysia. Therefore, the present study was undertaken to redescribe these species based on selected larval and adult morphological characters.

MATERIALS AND METHODS

Chironomidae larvae were collected in an experimental rice field at the Bukit Merah Rice Research station in Permatang Pauh, Seberang Perai, Pulau Pinang in Northern Peninsular Malaysia. The larvae were transferred to glass vials and immediately preserved in 80% ethanol. Taxonomic identification of the larvae was made using the keys of Cranston (2004), Morse *et al.* (1994), Merritt and Cummins (1996) and Epler (2001).

Some field-collected larvae were reared in the laboratory to the adult stage following the procedure of Chaudhuri *et al.* (1983). The reared adults were identified using the keys published by Sasa and Hasegawa (1983) and

Hasegawa and Sasa (1987). The identified materials have been maintained at the Aquatic Insect Collection of the School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia. The terminology used for the adult morphology was essentially that of Sasa and Hasegawa (1983), as provided in Table 1.

Table 1: Explanation of the abbreviations used in the standard measurements of midge adults (following Sasa & Hasegawa 1983).

Abbreviation	Explanation
BL	Body length, the combined length of the thorax and abdomen, in mm
WL	Wing length, from the base of vein R to the tip of wing, in mm
AR	Antennal ratio, the length of the last segment divided by the combined length of the preceding segments, not including the pedicel.
ER	Eye ratio, the distance between the eyes divided by the height of the eye.
so	Number of supraorbital setae
cl	Number of clypeal setae
dm	Number of acrostichals of the scutum
dl	Number of dorsolateral setae of the scutum
Pa	Number of prealar setae
sc	Number of scutellar setae
sq	Number of fringing setae of squamata
LR1	Length of the front tarsus I divided by the length of the front tibia
LR2	Length of the middle tarsus I divided by the length of the middle tibia
LR3	Length of the hind tarsus I divided by the length of the hind tibia
TR1	Length of the front tarsus V divided by the length of the front tibia
BR1	Length of the longest hair on the front tarsus I divided by the diameter of the front tarsus I at the base of the hair
BR2	As above, middle tarsus I
BR3	As above, hind tarsus I

RESULTS

***Chironomus javanus* Kieffer, 1924** (Fig. 1).

This species was first described by Kieffer (1924) from Buitenzorg, Java; later Johannsen (1932) described specimens from Lake Ranau, Sumatra. Specimens from various regions have subsequently been described by many authors, such as by Tokunaga (1964) from a number of Micronesian Islands, Hashimoto *et al*. (1981) from Thailand, Sasa and Hasegawa (1983) from Okinawa, Hashimoto (1984) from Shizuoka, Kikuchi *et al.* (1985) from rice paddies of Tokushima, Hasegawa and Sasa (1987) from Ryuku Island, Japan, Chaudhuri and Chattopadhyay (1990) from rice paddies of West Bengal, India, Kikuchi and Sasa (1990) from the Lake Toba area, Sumatra, Indonesia, and Sasa and Ogata (1999) from the Kurobe municipal sewage treatment plant, Japan.

Distribution: Caroline Island, Japan, Java, Sumatra, Marshall Island, Fiji, Papua New Guinea and Australia, Thailand, India.

Material Examined: 15 larvae from Seberang Prai, Pulau Pinang, Malaysia, collected in 2004.

Larvae (*n*=15): Body, 12–14 (mean 13.3) mm long, blood red in colouration. Two pairs of eyes present. Head, yellowish with dark postoccipital margin. Frontoclypeal apotome and labral sclerite 2 present; labral sclerite 1 absent. Pecten epipharyngis simple, consisting of 15 teeth of almost equal lengths. Premandibles with 7 teeth. Ventromentum plates, fan-shaped, separated medially by approx. 1/3 width of mentum plate. Anterior parapods with numerous pale claws, generally simple, but larger ones with accessory spikes near tips; posterior parapods with 16 dark brown claws, larger ones with 1 or 2 pointed spikes. Abdominal segment VII with a pair of caudolateral processes extending up to the middle of segment VIII [Fig. 1(h)]. Two pairs of ventral tubules present on abdominal segment VIII: the anterior pair long, extending beyond caudal extremity of body, and the posterior pair coils ventrally. Anal tubules relatively large, longer than half the length of the posterior parapods; anal setae with 7 long and 2 short hairs on a small tubercle.

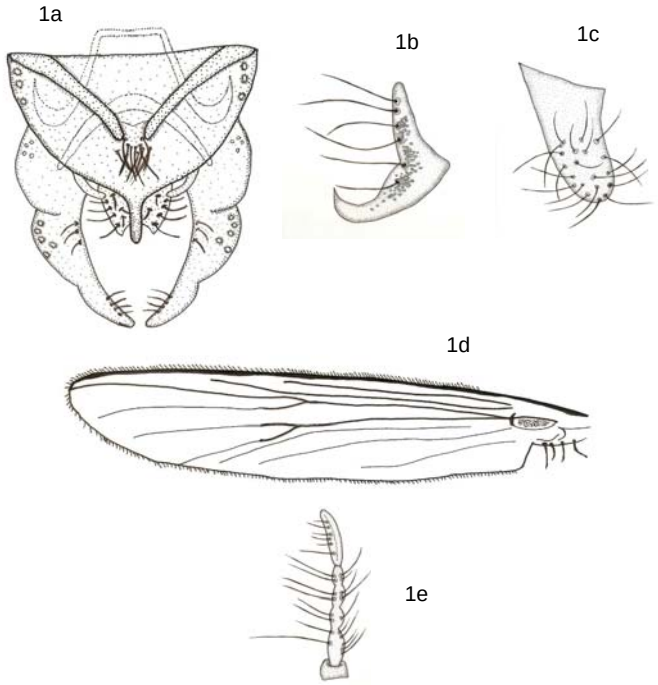

Figure 1: *Chironomus javanus* Kieffer 1924, adult: (a) hypopygium; (b) superior volsella; (c) ventral appendage; (d) wing; (e) antenna (female).

Adults (*n*=12):
Male (*n*=7): Mean body length, 5.9 mm. Body colouration largely greenish yellow; antennal shaft brown, hair grey; ground colour of scutum greenish yellow; stripes reddish brown, scutellum yellow, postnotum brown, halteres yellow; abdominal tergites I-VII greenish yellow without dark masks, abdominal tergite VIII and hypopygium brown; wings unmarked, r-m darker than the remainder of veins; in the forelegs, femur, tibia and tarsus I nearly white, both ends of tarsi II and III dark brown and their middle parts white, tarsi IV and V entirely dark brown; in the middle and hind legs, femora yellowish brown, tibiae and proximal 4/5 of tarsi I white, distal end of tarsi I and both ends of tarsi II and III dark brown, middle of tarsi II and III yellowish brown, tarsi IV and V entirely dark brown. Frontal tubercles well developed. Acrostichal setae of scutum usually absent. Scutal setae 10–16 in a single transverse row. Fore tarsus III longer than fore tarsus II (Table 2).
Female (*n*=5): All characters, in general, similar to the male, with usual sexual differences and the presence of 20–26 acrostichal setae on scutum.

Remarks: The specimens collected in the rice fields conformed to the description of Hashimoto *et al.* (1981) and Sasa and Hasegawa (1983). The body colouration, various measurements and structure of the male hypopygium were almost identical with those reported by previous authors for *C. javanus* (Kieffer) from Southeast Asia and the Micronesian regions. The absence of acrostichal setae on the scutum in most of the male specimens and the presence of long setae on the basal pad of the superior volsella of the males were characters of this species not referred to by previous authors.

***Chironomus kiiensis* Tokunaga, 1936** (Fig. 2).
C. kiiensis was first described by Tokunaga (1936), Hashimoto (1977) from Japan, Sasa (1978) from Japan, Hashimoto *et al.* (1981) and Kikuchi *et al.* (1985) from rice fields of Tokushima, Japan, Hirabayashi *et al.* (2004) from a filtration plant in Japan and Jeong *et al.* (2004) from rice fields of Korea.

Distribution: Japan, Thailand, Korea.

Material Examined: 41 larvae and 22 adults from Seberang Perai, Pulau Pinang, Malaysia, collected in 2004.

Larvae (*n*=41): Length ranged 11 to 15 mm (13.1 mean), body red in colour. Head almost yellow with dark postoccipital margin. Premandible consisting of two teeth. Frontoclypeal apotome and labral sclerite 2 present; labral sclerite 1 absent. Abdominal segment VII with a pair of short caudalteral processes; abdominal segment VIII with two pairs of long ventral tubules, the anterior pair relatively straight, the posterior ones longer and coiled. Four anal tubules elongated, almost equal, about half the length of the posterior parapods.

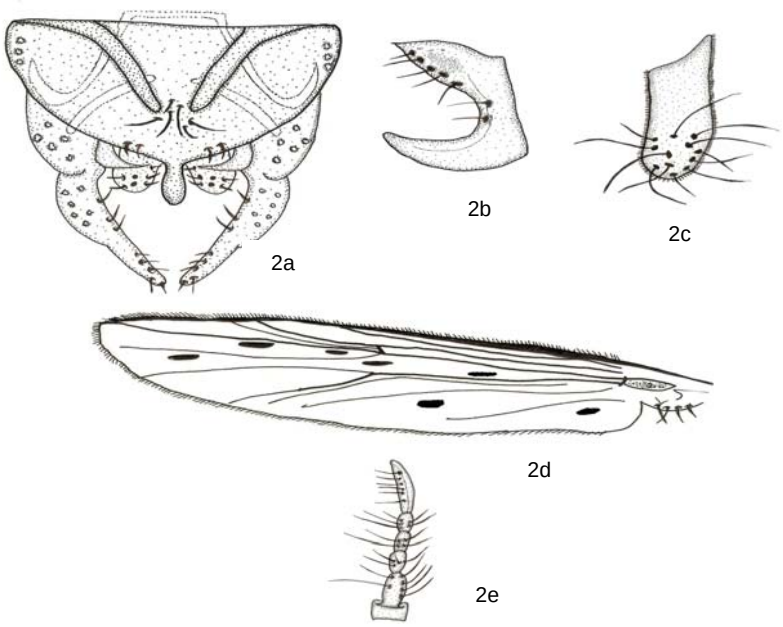

Figure 2: *Chironomus kiiensis* Tokunaga 1936, adult: (a) hypopygium; (b) superior volsella; (c) ventral appendage; (d) wing; (e) antenna (female).

Adults (*n*=22):
Male (*n*=12): Mean body length, 6.4 mm. Head yellowish, frontoclypeal apotome darker. Antennal hair brown, shaft dark brown; ground colour of scutum yellow, scutal stripes reddish brown, scutellum yellow, postnotum dark brown, halteres yellow. Tarsi I, II and III largely yellow and each with an apical dark ring, tarsi IV and V largely dark brown; ground colour of abdominal tergites greenish yellow. Tergites VIII and hypopygium dark brown. Standard measurements are shown in Table 2. This species is morphologically very similar to *C. javanus*. However, this species is unusual as a member of *Chironomus* in the strict sense that the wings have fuscous marks and the superior volsella have high basal pads somewhat resembling the *Einfeldia* group. The wings of *C. kiiensis* have a marks or spots that are characteristics to distinguish it from *C. javanus*.
Female (*n*=10): The body colouration is similar to the male; presence of 20–25 acrostichal setae on scutum.

Remarks: This species is most similar to *C. javanus*, *C. samoensis*, and *C. crassiforceps*, especially in the pupal stage in having a transverse band of spinules on tergite VII. Moreover, this morphological similarity has been reported for rice field specimens from Japan (Sasa 1978) and India (Chaudhuri & Chattopadhyay 1990; Chattopadhyay *et al.* 1991).

DISCUSSION

Many species of *Chironomus*, such as *C. javanus*, *C. kiiensis*, *C. samoensis* and *C. crassiforceps*, have been reported from rice fields throughout the world, including Japan (Sasa 1978) and India (Chaudhuri & Chattopadhyay 1990; Chattopadhyay *et al.* 1991). However, no previous efforts to redescribe *C. javanus* and *C. kiiensis* from Malaysian rice fields were demonstrated. These specimens were compared with reported extant species in Asian countries. According to the present observations, the characters of the Malaysian specimens matched well with the Japanese specimens, with the exception of the body length of *C. kiiensis*, which was longer than what has been reported for Japanese specimens by Sasa and Hasegawa (1983). Although *C. javanus* is somewhat atypical because it resembles *Kiefferulus* in having numerous premandibular teeth (5–7 teeth), the striations at the base of the mandible are characteristic only for *Chironomus* (Cranston 2007).

C. javanus and *C. kiiensis* showed very strong similarities that hinder a rapid identification of the larval materials. Under microscopic examination, only the number of teeth on the premandibles separated these two species (Sasa & Hasegawa 1983; Cranston 2007). The difference in the ventral tubule on abdominal segment VIII was clear because it was longer and highly coiled in the *C. kiiensis* larvae. The colouration of the mandibular teeth was also a valuable character for the identification of the larvae. However, *C. kiiensis* and *C. javanus* were very similar in their pupal morphology in having a transverse band of spinules on tergite VII.

The adults are more easily differentiated, as the body length of *C. kiiensis* was longer than *C. javanus*. In addition to the standard measurements, there are some other characters that could be used effectively to distinguish between *C. javanus* and *C. kiiensis*. The acrostichal setae of the scutum in the males of *C. javanus* were absent, an observation that was also reported by Sasa and Hasegawa (1983). The appearance of dark spots on the wings of *C. kiiensis* was also a characteristic to identify adult specimens.

It is concluded that the differentiation between *C. javanus* and *C. kiiensis* using larval material would be possible under microscopic examination. However, this procedure was rather tedious because the slide preparation was time consuming. The pupae were not useful materials for species differentiation, whereas the adult materials showed clear differences between *C. javanus* and *C. kiiensis*, with a satisfactorily high level of accuracy. Future investigations, including cytological and molecular identification, would further confirm the morphological identification of all of the *Chironomus* species, particularly *C. javanus* and *C. kiiensis*.

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