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# The *Chironomus* species studied by Letha Karunakaran in Singapore, with a review of the status of selected South-East Asian *Chironomus*

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## Abstract

In the 1960s Letha Karunakaran studied the chironomid fauna of Singapore but faced a lack of sufficiently detailed descriptions to enable identification of her material with any certainty. She recognized seven species of *Chironomus* (s.s) but sent me fixed larval material of only four of these which she tentatively identified as *C. apicatus* Johannsen 1932, *C. costatus* Johannsen 1932, *C. javanus* Kieffer 1924, and *C. stupidus* Johannsen 1932. She sent fixed larvae to me for confirmation of her identifications, but died before I was able to determine accurate identities from morphology alone. With additional comparative material, along with polytene chromosome banding patterns and DNA barcode sequence from the mitochondrial COI gene, the species have been identified as a form of *C. flaviplumus* (auct, not Tokunaga)(here called *C. flaviplumus* Type B), *C. circumdatus* Kieffer 1916*,* probably *C. striatipennis* Kieffer 1910, and *Kiefferulus barbatitarsis* (Kieffer 1911), respectively. The identification of one species as a form of *C. flaviplumus* required an assessment of the present state of knowledge of this species where the name has been applied to at least five different species. Determination of a valid name for this species is not currently possible. The confusion of species identification is an indication that there are a number of closely related species which constitute a "*C. flaviplumus* group".

## Introduction

Letha Karunakaran worked on Chironomidae in Singapore from the 1960s to the early 1970s, when she tragically died in a fire that took her life and consumed her collection. When Letha began her studies, essentially the only taxonomic descriptions of Malaysian midges were those of Johannsen (1932), from which Letha concluded that four of the species she considered to belong in the genus *Chironomus* were *C. apicatus* Johannsen 1932*, C. costatus* Johannsen 1932*, C. javanus* Kieffer 1924 and *C. stupidus* Johannsen 1932 (transferred to *Stictotendipes* Lenz, 1937 by Sublette and Sublette 1973) and placed in *Nilodorum* Kieffer, 1921 by Alfred and Michael (1990), a resemblance noted by Johannsen (op.cit.), but considered to be *Kiefferulus barbatitarsis* (Kieffer 1911) by Cranston (2002). Her studies were included in her unpublished Ph.D. Thesis (1969) and in the report of nematode parasitism in an adult identified as *C. costatus* (Karunakaran 1966). In her thesis she also included *C. striatipennis* Kieffer 1910, correctly identified (see below), and *C. bicoloris* Tokunaga 1964, which may be an undescribed species.

In the hope that I might be able to confirm these identifications from cytological analysis of the polytene chromosomes of the larvae, she sent me samples of four of her species. At that time there was no information on the cytology of southeast Asian species, and they could not be identified morphologically. The slides and the fixed larvae (in 3:1 ethanol/acetic acid fixative) remained in my collection until the group of Prof. Rudolph Meier in Singapore began identifying the local chironomids by barcoding and contacted me to see if I knew the identity of the Karunakaran specimens. It was only at this time that I learned that all Letha's specimens had been lost and the few larvae that she had sent to me were probably all that remained. With this in mind, I began to study the material again.

## Material & Methods

The samples comprised 3 larvae of presumed "*C. apicatus*", 7 larvae of "*C. costatus*"; 3 larvae of "C. *javanus*" and 1 specimen of *C. stupidus.* Specimens for comparison were available from India, Indonesia, Japan, Malaysia, Singapore and Thailand. Morphological and cytological analyses were by the usual methods (Martin et al. 2006). Where appropriate, the larval body was mounted on the same slide as the chromosome squash. A couple of Karunakaran's specimens were able to be barcoded for the conventional mitochondrial cytochrome c oxidase subunit I (*COI*) fragment (Hebert et al. 2003) using the Folmer et al. (1994) primers: LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). However, the condition of the larvae was such that the barcode region had to be amplified in two sections, using the unpublished reverse primer COI-Na-2 (5'- AGATAAAGGKGGATAAACWGTTCA-3') for the 5' section, and the forward primer of Carew et al. (2013) (5'- CCHCGAATAAATAATATAAGWTTYTG-3') for the 3' section, with PCR products sent to Macrogen Incorporated. Seoul, Republic of Korea for sequencing. The resulting sequences were compared to sequences in BOLD, including those mined from GenBank and sequences from my material that have been lodged in GenBank: the *C. costatus* sequence is ON406921 and the *C. apicatus* sequence is ON406926. Other sequences are GenBank accessions ON406917-920 and ON406921-928 for *C. flaviplumus* Type B, and AF192215, KT212957-976 for *C. circumdatus* Kieffer 1916. Sequences identified as *C. incertipenis* Chaudhuri and Das 1996, or *C. ramosus* Chaudhuri *et al.* 1992 were obtained from the BOLD database and from GenBank (KY835558, KY846714, MN934105-MN934321).

The slide mounted specimens will be lodged in the Lee Kong Chian Natural History Museum

(National Museum of Singapore). Morphological abbreviations follow Sæther (1980), and for some larval characters from Vallenduuk and Moller Pillot (1997).

## Results and Discussion

These studies have indicated that some of Letha's identifications were correct: An identification of *Kiefferulus barbatitarsis* was consistent with the subsequent synonymising of *C. stupidus* with that species (Cranston 2002). This species was readily recognised as a species of *Kiefferulus* by the presence of only one pair of ventral tubules, the sclerites of the dorsal head and the long narrow ventromentum. Amplification of DNA was unsuccessful, but the characters of the head matched those of *K. barbatitarsis* in Figs. 45 and 47 and the key of Cranston (2007). The immature stages were described by Chaudhuri and Ghosh (1986). The present specimen has a darkened posterior half of the gula.

The specimens noted as *C. costatus* proved, on the basis of the polytene chromosome banding patterns and the BARCODE sequence, to be *C. circumdatus.* This species has been well characterized for morphology (Martin and Saxena, 2009), polytene chromosome cytology (Alfred and Michael, 1990, Kumar and Gupta 1990, Pramual et al. 2009) and by mt*COI* barcoding (Pramual et al. 2016), and the *C. costatus COI* sequence had better than 90% homology, so the identification was quite simple. However, the actual identity of *C. costatus* has not been clarified and it seems likely that more than one species was included under this name since Lenz (1937) lists four larval types for the species.

The identity of the specimens called *C. apicatus* is not simple and reflects the general state of uncertainty over the identity of *Chironomus* species of Southeast Asia. *C. apicatus* was initially described as a variety of *C. costatus* (Johannsen, 1932), but the barcode results suggest it is not so closely related to that species. Rather, the mt*COI* sequence corresponds to those in a BOLD bin where most specimens are identified as *C. flaviplumus.* However, specimens identified as *C. flaviplumus* also occur in three other BOLD bins, indicating that the current concept of this species encompasses a number of species in the "*C. flaviplumus*-group". This group would also include other species such as *C. yoshimatsui* Martin and Sublette (1972) (one of the species incorrectly identified as *C. flaviplumus* in the BOLD database), *C. circumdatus, C. incertipenis* Chaudhuri and Das (1996), *C. ramosus* (Chaudhuri et al., 1992) and the Japanese concept of "*Chironomus samoensis*" (e.g. Kikuchi and Sasa, 1990).

*C. flaviplumus* was originally described by Tokunaga (1940) from Saga, Kyoto, Japan, but Sasa (1978) states that the description was very brief and not illustrated. Sasa (1978) redescribed the species from Japanese material, but not from the type locality. He lists the important features as a foreleg ratio of 1.6-1.8 and a relatively long anterior Ta5 which is about 0.35-0.4 of the length of the anterior Ti. However, in a later paper, Sasa and Hasegawa (1983) give a much broader range of values (including Ta5/Ti values of only 0.25) which could suggest that they had material of more than one species. Such a conclusion is supported by *COI* sequences attributed to *C. flaviplumus* from Japan being in two different BOLD bins. One is recorded only from Japan (called Type A), while the other is broadly distributed through Japan, Malaysia, Singapore, Thailand, India, Pakistan and also Israel (called Type B). Since both types occur in Japan, it cannot be determined with certainty which is *C. flaviplumus sensu* Tokunaga (1940), although Type A better fits the few known characters from Tokunaga's original description.

The situation is further complicated in that material of Type B from Pakistan is mostly listed as *C. incertipenis* and some Indian material as *C. ramosus* Chaudhuri, Das and Sublette (1992). I have a number of specimens of Type B from various locations, confirmed by the *COI* barcode sequence. A detailed comparison of

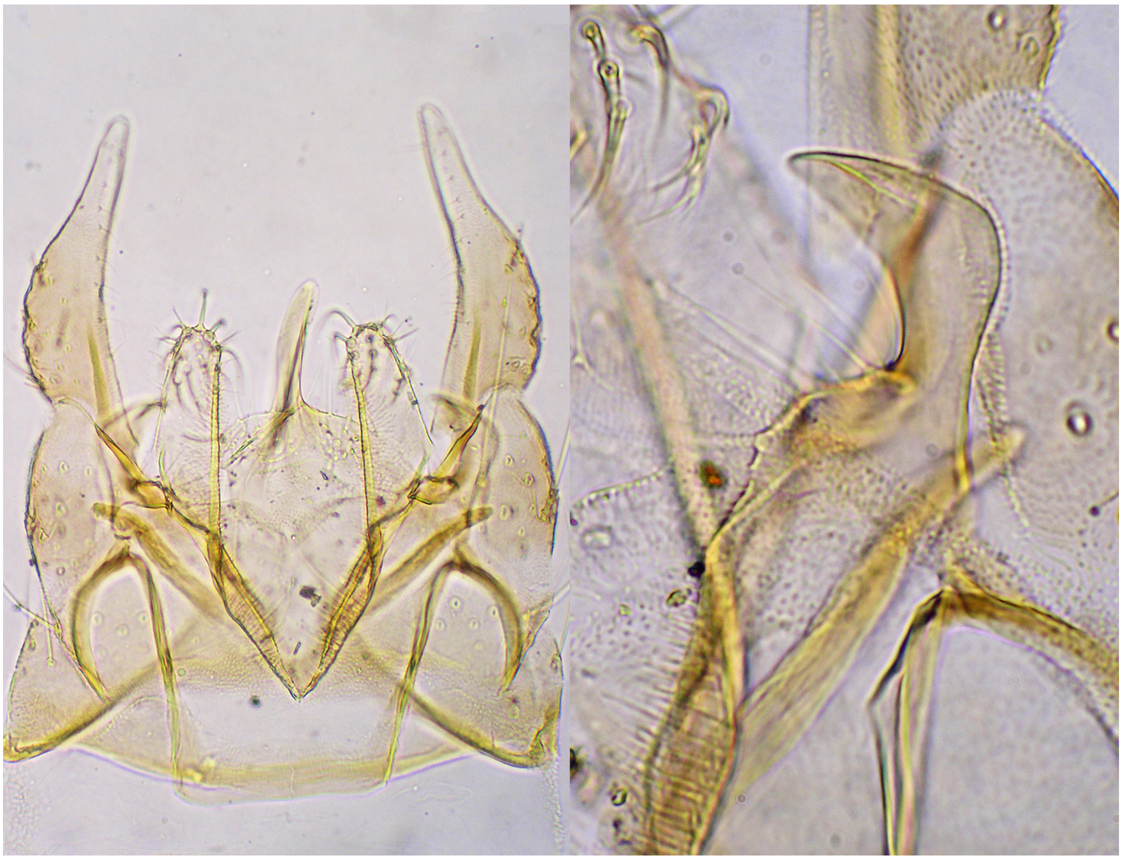

Figure 1. Male hypopygium of *Chironomus flaviplumus*-Type B. Note that the anal point is not black and not sharply turned-down, and that the SVo is beaked and not gently curved.

the *COI* sequences under these three names showed there were the same nine polymorphic bases (i.e. 1.2% variation) regardless of the name applied. Thus, there is no indication of multiple species in this material.

The adult males among these specimens have an AR of 2.94 (2.84-3.05) and an anterior LR of 1.65 (1.59-1.75), so any species to be considered for the name of Type B should also have similar values. *C. incertipenis* was created as a new name for *Chironomus niger* Chaudhuri, Das and Sublette (1992) since that name was preoccupied. While the AR and LR of *C. incertipenis* are within the range for Type B, the critical character that led Das *et al*. to originally call it *C. niger,* was the dark, sharply downturned anal point. Specimens of *C. flaviplumus* Type B do not have this dark anal point (Fig. 1), but a more usual yellow-brown one. As well, the SVo of *C. incertipenis* is described as gently curved, while that of *C. flaviplumus* Type B is strongly curved and beaked (Fig. 1). Therefore, an association of the name *C. incertipenis* with this taxon is unlikely, as noted by Pramual *et al.* (2016), although the types should be re-examined to confirm the accuracy of the original description of this species.

In the case of the name *C. ramosus*, as used by Laviad-Shirit et al. (2020) and Sela et al. (2021), the original description of the adult male indicates that it is a smaller species, with an AR of 3.86 (3.73-3.94) and an LR of 1.4 (Chaudhuri et al., 1992), outside the range of values for Type B. As well, the polytene chromosomes show some significant differences, notably that the nucleolus is on arm B (Nath and Godbole 1997), while in *C. flaviplumus* Type B it is on arm F, near the centromere (Martin, 2022). Therefore this name is not applicable to this species. Currently there is no obvious name for this taxon but the descriptions of many Oriental *Chironomus* species do not include the critical characters, so it is not appropriate to describe it as a new species until existing names, particularly *C. incertipenis,* can be ruled out.

The third type (Type C) was initially known only from *COI* sequence in GenBank (KP902730 & -31 from China and KT213029-038 from Thailand). However, in BOLD they have 99.5% homology to a sequence called ChironomidaeGC sp. 7 from Queensland, Australia. Other specimens from Australia indicate that these are not *C. flaviplumus* but a related species with the manuscript name of *"C. orientalis"* (Martin 2022).

With that explanation, we return to the question of the identity of the other two species sent by Letha Karunakaran. Her *C. apicatus* does not appear to fit the description of this species since LR1 is lower (1.59-1.75) c.f. 1.85 in *C. apicatus*, and the larvae of *C. apicatus* are found in salt ponds and a pool at 29°C and pH 2.83 (Lenz 1937). However, it can be easily placed as *C. flaviplumus* Type B on the basis of larval morphology, cytology and *COI* sequence.

The remaining species in the material sent to me was labelled as *C. javanus.* The original description by Kieffer (1925) other than being a greenish species is not definitive, but the redescription by Johannsen (1932) is likely correct. The Lenz (1937) description of the larva states only that it is "plumosus" type. Chaudhuri *et al*. (1992) listed *C. vitellinus* Freeman 1961 as a synonym, which is likely correct as the larvae characteristically have a premandible with 6 or 7 teeth and specimens identified with this premandible type have been recorded as *C. javanus* from Micronesia (Tokunaga 1964), through northern Australia (Freeman's original description of *C. vitellinus*), Singapore, Malaysia (Al-Shami et al. 2012), India (Chaudhuri et al. 1992) and to Malawi in Africa (larvae sent to me by A. McLachlan).

However, the larvae from Letha had the more usual two-toothed premandible of *Chironomus*, so do not fit the usual concept of *C. javanus.* The chromosomes were of very poor quality and the larvae were slide mounted before DNA sequencing was available. Therefore, while it seems that the material she sent was not *C. javanus* Kieffer, an accurate identification is not easy. More to the point, the morphology of the larvae do not fit that provided in her thesis – that description and the accompanying figures are much more like *C. javanus* but do not mention the premandibles, probably because the multitoothed nature in *C. javanus* was not recorded at that time. One possible explanation for the difference is that she accidentally sent larvae of one of her other species (*C. striatipennis* Kieffer 1910 or *C. bicoloris* Tokunaga 1964). She notes, for example that the anterior pair of ventral tubules are longer (true of *C. javanus*), while in the larvae I received the posterior pair of ventral tubules are longer – which is the situation in *C. striatipennis* and *C. bicoloris.* The darkened gula head coloration and other larval characters (e.g. mentum of Ty II, see below) strongly suggest it is *C. striatipennis* rather than *C. bicoloris*.

Further to the identity of these last two species: *C. striatipennis* should be easily recognizable by the patterned wing, but whether they were the more common Type 1 or the rarer Type 2 (Pramual *et al.* 2016) can currently only be determined from DNA analysis. *C. bicoloris* was described only on the basis of adults. I have a small number of reared specimens from northern Australia which fit Tokunaga's (1964) description of *C. bicoloris*. The two pupae have one and two spines (Fig. 2) on the spurs which are not spine-like as illustrated by Karunakaran (1969). Her illustration of the larval mentum is also slightly different - it is Type II of Vallenduuk and Moller Pillot (1997), i.e. 4th lateral tooth reduced to the height of the 5th lateral, while in the Australian larva is Type I (Fig. 2) i.e. 4th lateral in line with other lateral teeth. As well, *C. bicoloris* has not been identified elsewhere in south east Asia, so it is possible that Karunakaran's material was an undescribed species.

In summary: Letha Karunakaran did quite a commendable job in the identification of her *Chironomus* specimens given the difficulty even today of identifying many species and that she was largely working without specialist assistance. I have been able to confirm that her identification of *C. stupidus,* and quite possibly *C. striatipennis* and *C. javanus,* were correct. DNA sequence confirmed that her *C. costatus* was the well-known *C. circumdatus* and that her *C. apicatus* was Type B of the extensive but not well defined "*C. flaviplumus* group". If nothing else this analysis highlights the difficulties involved in trying to accurately identify the *Chironomus* species of southeast Asia on the basis of morphology and, even where DNA barcode data is available, the sequence may have been attributed to an incorrect species in the BOLD database.

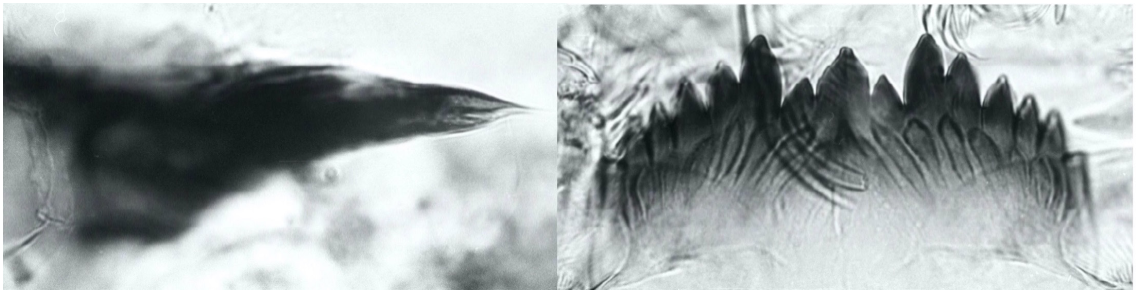

Figure 2. Pupal spur (left) and mentum (right) of *C. bicoloris.*

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