Kiefferulus barbatitarsis (Kieffer, 1911) and *Kiefferulus tainanus* (Kieffer, 1912) are distinct species, not synonyms

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Abstract

Morphological and molecular studies indicate that *Kiefferulus tainanus* (Kieffer 1912) is quite distinct from *K. barbatitarsis* (Kieffer 1911), and not a synonym of the latter species, as has previously been suggested by Chaudhuri and Guha (1987). The suggestion of synonymy seems to have been based on a comparison of the figures of *K. tainanus* in Sasa (1979), rather than an examination of the types. While the adults show some similarities, other characters, and mitochondrial COI sequence, clearly indicate that the two species are not identical, and not particularly closely related. Australian material previously considered to be *K. tainanus* is probably incorrectly identified and represents a separate, but closely related species.

Introduction

In his paper on chironomids of Thailand, Cranston (2007) noted that the identity of *Kiefferulus* species of South East Asia was not clear. One group for which uncertainty has existed in the past, is three species described by Kieffer: *K. barbatitarsis* (Kieffer 1911)*, K. tainanus* (Kieffer 1912), and *K. biroi* (Kieffer 1918). Kieffer originally placed *K. barbatitarsis and K. biroi in Chironomus*, but he described *K. tainanus* as a *Tendipes*. *Kiefferulus barbatitarsis*, described from India, remained in *Chironomus*, although Sublette and Sublette (1973) classed it as unknown Chironomini. Chaudhuri and Ghosh (1986) re-examined the types in the Indian Museum, along with rearings, and redescribed the species as *Kiefferulus.*

Kiefferulus tainanus was originally described from Tainan, Taiwan. It was variously placed in *Phytochironomus* (Kieffer 1921) and *Glyptotendipes* (Goetghebuer 1937-54), before Sasa (1979) re-described it for all stages from Japanese specimens. He placed it in *Chironomus*, although noting that it did not fit the strict definition. Hashimoto *et al*. (1981) also placed it in *Chironomus*, but noting that it was "rather related to *Kiefferulus* and *Glyptotendipes".* Chaudhuri and Ghosh (1987) placed it in *Kiefferulus*, when they placed it as a probable synonym of *K. barbatitarsis*, then Cranston and Martin (1989) placed it in *Nilodorum,* before restoring it to *Kiefferulus* in an analysis that included a syntype in the British Museum (Cranston *et al.* 1990).

K. biroi was originally described from Colombo, Sri Lanka, and later from Australia, India and Japan. Freeman (1961) placed it in the subgenus *Nilodorum* of *Chironomus*, and later as the genus *Nilodorum* (Freeman & Cranston 1980). Saxena *et al.* (1985) also referred Indian specimens to *Nilodorum.* Hashimoto *et al.* (1981) had synonymised it with *C. tainanus*, but the rather obscure publication was generally unknown. The synonymy was restated by Cranston and Martin (1989), and again when *K. tainanus* was returned to the genus *Kiefferulus* (Cranston *et al*. 1990). However, the possible synonymy of these species with *K. barbatitarsis* has been largely ignored. Chaudhuri and Guha (1987) apparently did not examine any type material of *K. tainanus,* but rather relied on the quite detailed re-description of Sasa (1979) since they attribute *K. tainanus* to Sasa in their listing of synonymies. The purpose of this paper is to provide morphological and molecular data to clarify that the two species, *K. barbatitarsis* and *K. tainanus* as re-described by Chauhuri and Ghosh (1987) and Sasa (1979) respectively, are not in question and that it is clear that they are morphologically distinct.

Material Examined

While the conclusions here are largely based on a comparison of previously published work, some additional specimens were examined.

Kiefferulus barbatitarsis:

1 male Mai Ping N.P., Lamphung Province, Thailand. 6 III.2002, coll: P.S.Cranston. Part of abdomen used for DNA extraction (BOLD CoTW018-08), and photo of hypopygium used in Figure 1.

Kiefferulus tainanus:

1 male believed to be syntype, bearing three labels, respectively 'tainanus Kieff. det Kieffer','Formosa Sauter', 'Purchd. from Budapest Mus. BM 1922-72' (British Museum Natural History) - used to establish *K. tainanus* by Cranston *et al*. (1990), and a photograph of the hypopygium used in Figure 1.

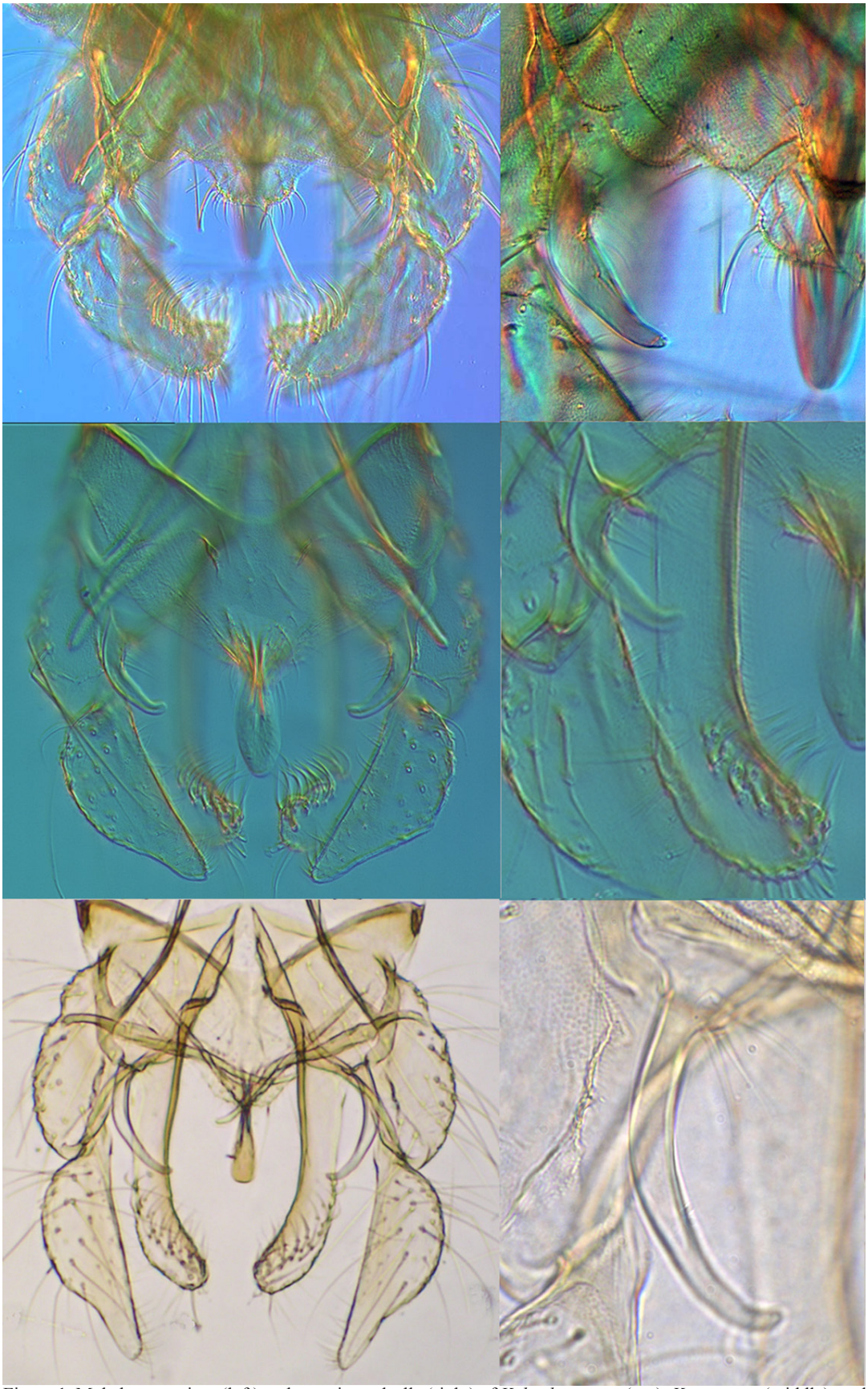

Figure 1. Male hypopygium (left) and superior volsella (right) of *K. barbatitarsis* (top), *K. tainanus* (middle), and Australian *K.* "*tainanus*" (below). Photos at top and middle courtesy of P.S. Cranston.

1 male Okhla, nr. Delhi, India, OK1, coll. S. Taneja (now Saxena); 1 male pupal exuviae Yamuna River, Okhla, nr. Delhi, India OK2; larva Honshu, Japan, 9.IX.2001 GenBank accession no. DQ648225), 1 male Mai Ping N.P., Lamphung Province, Thailand. 6.III.2002, coll: P.S.Cranston. Part of abdomen used for DNA extraction.

Australian specimens previously considered to be *K. tainanus*:

1 male Goanna Lagoon, Alligator Rivers Region, Northern Territory, 15.IX.1979, Coll. R. Marchant; 1 male Somerset Dam, Queensland AQ.20.10 M2, 26.V.1971, coll. J.Martin; Hutchins Lagoon, Ayr, Queensland, 14.VI.1974, coll: B.V.Timms; 4 larvae with associated chromosome squashes, Somerset Dam, Queensland, AQ.20.4, 23.I.1969, from egg mass #1, coll. J. Martin. Additional specimens were included in the morphological studies of Cranston *et al.* (1990) and the cytological studies of Saxena *et al*. (1985).

Observations

The published re-descriptions and the analysis of additional specimens leaves no doubt that material described as *K. barbatitarsis* is distinct from that described as *K. tainanus.* Both species were collected together at a site in Thailand, and there was no difficulty in separating them, as indicated by the molecular analysis of two such specimens (see below). On the other hand, there is no doubt that the adults of the two species are somewhat similar in gross morphology. Both have an AR around 4, and an LR around 1.25. The male hypopygium is also basically similar, including the presence of setae on the inner margin of the superior volsella (see Fig. 1).

Closer examination reveals that there are definite differences, in all life stages as seen in Table 1.

Possibly the most obvious is the relatively shorter palps of *K. tainanus,* as can be seen in Table 1, which had led to it being placed in the genus *Nilodorum* (e.g. Cranston and Martin 1989). Another obvious difference is in the shagreen pattern of the pupa. While both species have an anterior and posterior row of spines on tergite II, spines on other tergites of *K. barbatitarsis* are relatively small (Chaudhuri & Ghosh 1986), while those of *K. tainanus* are larger and more extensive (Fig. 2). The tergal spines of *K. tainanus* were well illustrated by Sasa (1979). It might be noted that material identified as *K. tainanus* in Australia (Saxena *et al*. 1985, as *N. biroi*; Cranston *et al*. 1990, Bugledich *et al*. 1999) is probably a distinct but closely related species. The most obvious difference is that the male superior volsella is longer and narrower

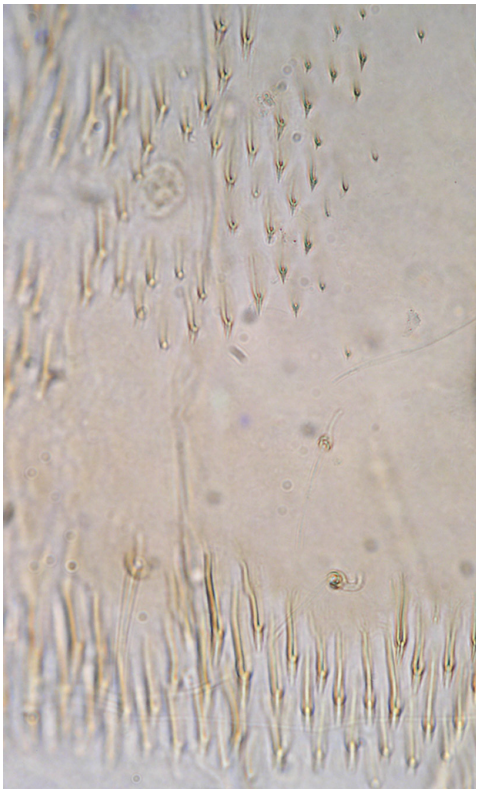

Figure 2. Spinose patches on posterior two thirds of tergite V of pupa of *Kiefferulus tainanus.* Note the shorter median spines and longer posterior spines.

Table 1. Listing of most obvious differences between *Kiefferulus barbatitarsis* and *Kiefferulus tainanus*. Abbreviations here and in text as in Sæther (1980).

Character	*Kiefferulus barbatitarsis*	*Kiefferulus tainanus*
Adult male		
Anal tergal band	H-type	Y-type
Palp ratios	4: 3: 7: 11: 15	4: 3: 5: 8: 10
IV (Fig. 1)	more swollen distally	less swollen distally
Pupa		
Spines of tergites III-VI	relatively small	larger & more extensive (Fig. 2)
Larva		
S1 setae	deeply feathered	palmate

(Fig. 1), while further evidence can be drawn from the cytological comparison of Indian and Australian specimens (Saxena *et al*. 1985), where a small number of fixed differences were noted in the banding patterns of the salivary gland chromosomes in the two continents. Further study will be required to clarify the situation.

In the larvae, the main difference is in the S1 setae (Table 1), which differ in the manner previously used as a distinction between the genera *Kiefferulus* and *Nilodorum* (Cranston *et al.*1990).

Finally, the DNA barcode sequence of the mitochondrial COI gene of *K. tainanus* from Japan has been published (Martin *et al.* 2007), and a further sequence was obtained from an adult from Thailand. These can be compared with the equivalent data for *K. barbatitarsis* (Fig. 3). As previously noted, the *K. barbatitarsis* sequence came from the same specimen as the hypopygium in Figure 1.

This comparison shows that, while there are 14 polymorphic sites between *K. tainanus* from Japan and India (2.3%), there are 42 (6.8%) and 39 (6.3%) respectively between the Japanese and Thai sequences of *K. tainanus* and the sequence of *K. barbatitarsis*. While the difference between the two *K. tainanus* samples is well within the arbitrary five percent limit for intraspecific variation of this sequence, the difference of the *K. barbatitarsis* sequence falls outside that limit. In a Neighbor-joining tree of *Kiefferulus* species (not shown), the two species do not cluster together.

It therefore must be concluded that the gross similarity of some adult characters does not indicate any particularly close relationship. Differences exist for larvae and pupae, as well as for the adults. Indeed the two species were previously considered to be in different genera (see Introduction). In a subsequent listing of Indian Chironomidae, Chaudhuri *et al*. (2001) did not mention *K. tainanus* at all, but included *K. biroi* in the genus *Nilodorum.* Since *K. biroi* is accepted as a synonym of *K. tainanus* (see Introduction), this provides further confirmation that this species is quite distinct from *K. barbatitarsis.*

Acknowledgements

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                                 1111111111111122222222222233333
                  1144568888993455666677789923345566888990111 2
                 1693651234847024803673587396258062306958834 95
tainanus(Jpn):   ATACATTAATATCTTTTATTTTAATACTTATAAAATAAACTTGTA
tainanus(Thai):  ......................G...CC.........GT.....
barbatitarsis:   GAGACCATGATATAACCTCCCCT.CTTCCCCTTCTATG.TCATAT


                 3333333333333333444444445555555555555555566666
                 3344556667778899347889990001223345778888900011
                 1401281243795917988173790257395970170369824739
tainanus(Jpn):   TGAGACTCTGGATTATAATTTTCTTACAAAATTTCCATATTCTTTC
tainanus(Thai):  ......C..A.G.........C....T........T.C..C...C.
barbatitarsis:   AACTTT.TATATACTATTAAA.TACTTCTCTCACTTT.TG.TACCT
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Figure 3. Polymorphic sites in 621 bases of the mitochondrial COI sequences of two populations of *K. tainanus,* and *K. barbatitarsis*

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