Chironomus strenzkei Fittkau, 1968 is a junior synonym of *C. striatipennis* Kieffer, 1910

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Abstract

Two species of *Chironomus* with essentially identical adult wing patterns, *C. strenzkei* and *C. striatipennis*, have been reported from Brazil. Specimens were collected from the same region in the Manaus area some 50 years apart. Morphological, cytological and DNA Barcode comparisons all confirm that these two species are inseparable on any of the characteristics studied. Moreover, for the mitochondrial *COI* region investigated, the sequences are completely identical, and polytene chromosome banding patterns are shared between *C. strenzkei* and *C. striatipennis* populations from Japan. I therefore argue that the former species must be a junior synonym of *C. striatipennis*. As a result of the synonymy, *C. striatipennis* now becomes a new record for California and North America, and hence has a Holarctic distribution. The DNA sequence comparisons suggest that the Brazilian population may have derived from China, rather than Korea as suggested previously, and that the Californian population may not have been introduced from South America, but could equally likely have come from Asia.

Introduction

In the 1960s, while researching in the area of Manaus, Amazonas, Brazil, Fittkau collected a species of *Chironomus* with patterned wings, a life cycle of about 10 days and amenable to being maintained as a laboratory colony. Such a colony was maintained in the laboratories at Plön, Germany for some years, with material being distributed for study of various aspects of its biology (Syrjämäki 1965, 1967, Platzer 1967, Platzer-Schultz 1968a, b, 1970, Platzer-Schultz and Welsch 1969). Some of these studies were published before Fittkau formally described the species as *C. strenzkei* in 1968 (Fittkau 1968). Wülker and Morath (1989) studied the polytene chromosomes noting that it belonged to the pseuothummi-cytocomplex with arm combination AE, BF, CD, G, but stating that the banding patterns showed no similarity to the other South American species they were studying. Subsequently Sublette and Mulla (2000) reported *C. strenzkei* from California on the basis of adult morphology, assuming it to be a recent migrant because it had not been collected in previous extensive surveys in the area.

About 40 years later, a Brazilian group collected specimens with patterned wings from the vicinity of Manaus (Amora *et al.* 2015). This material also had a life cycle of about 10 days and has been maintained in the laboratory since 2011. The species was identified initially as *C. kiiensis* Tokunaga, 1936 (Lacerda *et al.* 2014) on the basis of morphological studies of adults, pupae and larvae from the colony, and the mitochondrial *COI* DNA barcode region of 2 larvae and an adult by Amora *et al.* (2015). The latter authors used both the name *C. kiiensis* and *C. striatipennis* Kieffer, 1910, following the results of Pramual *et al.* (2016) who synonymised these two species. The *COI* results suggested that this Brazilian population had originated from Asia, probably from somewhere near Korea in recent times (Amora *et al.* (2015). At no stage was *C. strenzkei* mentioned despite the fact that it had been collected in the same area and the holotype and 5 paratypes are in the National Institute for Research in Amazonia (INPA) (Fittkau 1968), where the *C. striatipennis* colony is situated.

The similarity between the description of the two species, and their occurrence in the same general area, suggested that a detailed analysis should be undertaken to determine whether there were two quite similar species, or whether both collections referred to the same species, in which case the Kieffer name would have precedence.

Material and methods

Chironomus strenzkei: Fourth instar larvae and a reared adult male from the original Plön colony were provided by Dr. Frieder Reiss in 1970. Some salivary gland polytene chromosome squashes and slide mounts of larvae and the reared adult were made at that time. The remaining larvae were fixed in modified Carnoy's fixative (3 parts absolute ethanol: 1 part glacial acetic acid) and stored in a freezer at -20°C until the present time when three specimens were used for molecular analysis. It should be noted that these specimens had been reared at 25°C, which is not ideal for the study of

polytene chromosomes. Dr. Martin Spies provided five polytene chromosome squashes made from larvae from the original Plön stock by Dr. Wolfgang Wülker, and which had been lodged in the Bavarian State Collection of Zoology in Munich.

Chironomus striatipennis: Many specimens of this species have been available from many areas, particularly India, Singapore, Korea and Japan. These have been used for morphological (e.g. Chaudhuri *et al.* 1992; Amora *et al.* 2015, Martin 2017), and molecular analysis, with some limited cytological studies (Nath and Lakhotia 1989, and Gupta and Kumar 1991, Martin 2017). Those used for molecular analysis are listed in Pramual *et al*. (2016) and confirmed the conclusion from morphological and cytological studies, that *C. kiiensis* (group B of Kondo *et al*. 2016) was a junior synonym of *C. striatipennis*. Kondo *et al.* (2016) considered only the Barcode sequences and concluded from these that they were identical or close relatives. Adults had been made available to Amora *et al*. (2015) for their studies, so were not available for further study.

Aside from chromosome squashes made from available larval material, Dr. Sumitra Saxena kindly made available her maps of the species with details of the extensive chromosomal polymorphism present in the Indian populations.

No larvae were available from Korea or China and the only specimens from Japan, kindly supplied by Dr. Koichiro Kawai, were mostly mid fourth instars, fixed in ethanol, and so not ideal for chromosomal analysis. However from 4 larvae, the banding patterns of some arms were sufficient to enable comparison with those of specimens identified as *C. strenzkei.*

Morphology: The morphology of all life stages of *C. striatipennis* has been well examined by Chaudhuri *et al*. (1992) and Amora *et al*. (2015), and as *C. kiiensis* by Sasa (1978); Sasa and Hasegawa (1983), with the identification problems raised by Pramual *et al*. (2016). The results are integrated by Martin (2017). Data from three additional Indian adult males is also included. The morphology of *C. strenzkei* was included in Fittkau (1968), with some additional specimens (1 adult male, 5 larvae) studied by the author.

Cytogenetical examination: Salivary gland chromosome squashes were prepared by the usual method (Martin *et al*. 2006), or had been prepared by W. Wülker, and suitable chromosomes were photographed on an Olympus Vanox microscope on film or as digital images. No clear photographs could be obtained for arm C.

Molecular analysis: Genomic DNA of the three larvae noted above was amplified by polymerase chain reaction (PCR), as Martin *et al*. (2007) for the "BARCODE" region of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) gene using the Folmer *et al*. (1994) primers: LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). PCR products were sent to Macrogen Inc. Seoul, Republic of Korea for sequencing, the resulting sequences being submitted to GenBank: Accession numbers KY454622-24.

Sequences were compared to some available in GenBank – those of Amora *et al*. (2015) (Acc. nos. KJ424334-336, as *C. kiiensis*), sequences from Korea and Japan (JF412086, KC407765 (as *C. kiiensis*), and MFD034354 (BOLD acc. COTW008-08 – a *C. striatipennis* specimen used for cytology), including one of the divergent Korean '*C. kiiensis*' sequences (JQ350720) and a more recent accession from China (KP902735), not studied by Amora *et al*. (2015). It was not considered necessary to duplicate all the samples used by Amora *et al.* (2015) as there is no reason to believe those results were incorrect and the scope of this analysis is essentially a comparison of the Brazilian sequences. Uncorrected and Kimura 2-paramter (K2P) pairwise distances were obtained using PAUP* v.4.10b (Swofford 2002), but only the uncorrected distances are presented in Table 1 since there was no significant difference between the two sets of results.

Results and discussion

Morphology

The morphology of Brazilian specimens of *C. striatipennis* and *C. strenzkei* cannot be directly compared because Amora *et al*. (2015) only make general comments on the morphology of their specimens. Therefore comparisons must essentially be made to Indian specimens, since data from Japan or Korea may be confused by the inclusion of two species.

The most obvious similarity is the wing pattern. However this is not a good character for species separation because the known *Chironomus* species with a patterned wing (*C. calipterus* Kieffer*, C. striatipennis*, and *C. pallidinubeculosus* Tokunaga), as opposed to darkening along the wing veins, have essentially the same pattern and there is more intra-specific variation in pattern than there is inter-specific variation.

Available data for the adult males is presented in Table 1, and indicates that there is overlap of all

characters between the two species. Insufficient data exists for the adult female of *C. striatipennis* to make any meaningful comparisons.

Some comparisons are possible for the pupa and larva: For pupae, length of exuviae (4.8-7.6 mm cf. 5.5-7.6) is similar and both have pupal spurs with 1 main and 1 or 2 small subsidiary spines, but there are almost twice as many taeniae on the anal lobe in *C. strenzkei*. Amora *et al*. (2015) provide no information on the larva from their colony, so no direct comparisons are possible for specimens from the same locality. Metric comparisons are therefore of limited value due to ecological differences and the immaturity of available larvae from Japanese *C. striatipennis* and German *C. strenzkei* colony. What can be noted is that both have a plumosus-type larva, the basal antennal segment is about three times longer than wide, the third and fifth antennal segments are about the same length, and the AR is at least 1.6; the mentum of each has a central trifid tooth of type III and the mandible is type II of Webb and Scholl (1985). The mandible of each also has an unusually long dorsal tooth, i.e. there is no readily observable distinction between larvae attributed to the two species.

Cytogenetics

Both species belong to the pseudothummi-cytocomplex, with the chromosome arm combination AE, BF, CD, G. As noted above, Wülker and Morath (1989) could see no similarity between the

Table 1. Comparison of adult male characters of *C. striatipennis* and *C. strenzkei,* based on the published data of Chaudhuri *et al*. (1992) and Fittkau (1968) plus four additional specimens as in text. Abbreviations as in Sæther (1980); SV type as Strenzke (1959).

	Wing length	VR	AR	Ant. LR	Ant F/T	BR	Mid LR	Hind LR	SV type	Anal point
C. striatipennis	1.98-2.84	1.04-1.08	2.47-2.97	1.49-1.82	1.08-1.23	2.4-5.0	0.55-0.62	0.73-0.80	E(h)	Narrow at base
C. strenzkei	1.56-2.18	1.04-1.08	2.1-2.95	1.69-1.78	1.17-1.21	2.6-2.8	0.62-0.63	0.72-0.74	E(h)	Narrow at base

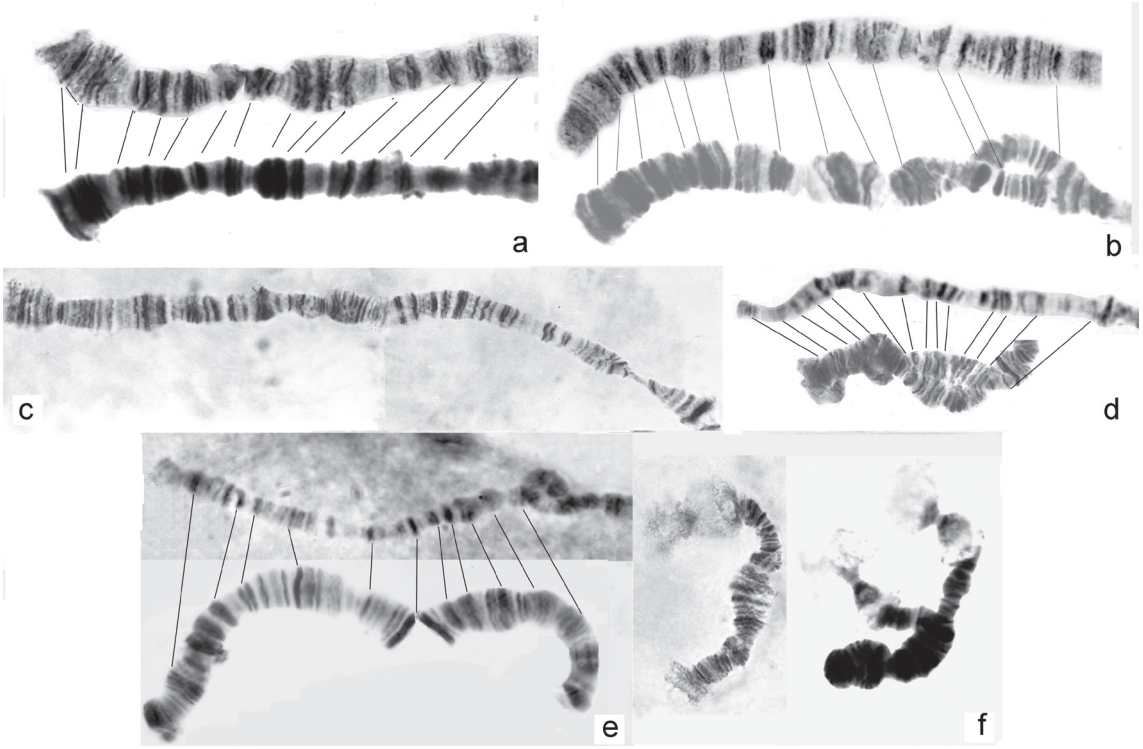

Figure 1. Comparison of polytene chromosomes of Brazilian *C. strenzkei* (above or left) and Japanese *C. striatipennis* (below or right). The centromere is to the right, except in arm G where it is at the top of the arm. Lines join homologous bands in the compared arms. a. Arm A, both A1.1; b. Arm E, both E1.1 (lack of pairing near centromere in Japanese specimen); c. Arm B of *C. strenzkei* only, B1.1; d. Arm F, both F1.1 (lack of pairing near centromere in Japanese specimen); e. Arm D, both D3.3; f. Arm G, both apparently G1.1 (only known sequence).

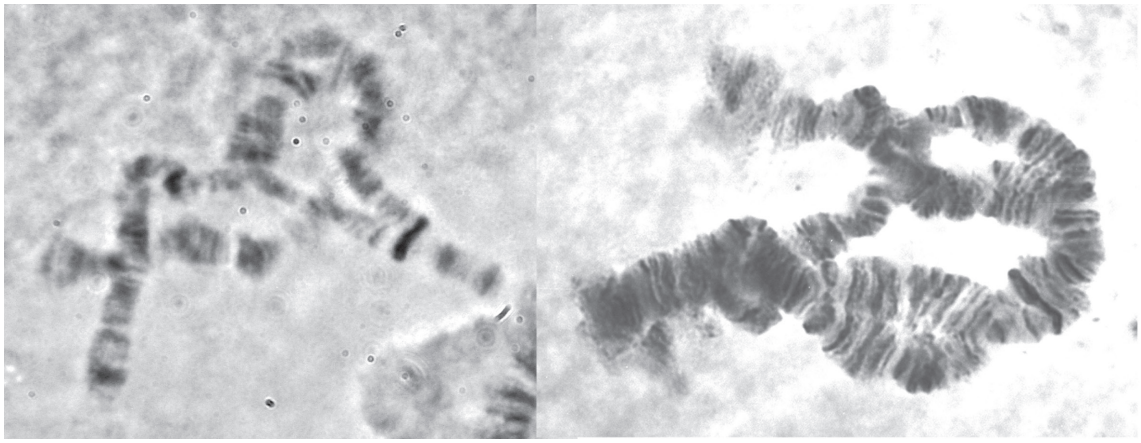

Figure 2. Heterozygotes for arm B of *C. strenzkei* (left) and *C. striatipennis* (right). It is likely that both have the sequences B1.5. The associated arm F of C. striatipennis is the same chromosome as Fig. 1,d.

banding patterns of *C. strenzkei* and those of the other South American species they were studying, but the banding pattern does show remarkable similarity to sequences observed in *C. striatipennis*, particularly those from Japan, as indicated below. Cytological comparison was difficult because of the general lack of cytological data for the Asian populations, only three descriptions from India, and the less than optimal suitability of specimens available to this study. Only Saxena (1995) has published photographs with patterns for arms A, E and F identified on the Keyl (1962) standards (as *C. calipterus*). Photographs provided by Saxena show that Indian populations of *C. striatipennis* are highly polymorphic (summarized in Martin 2017). In this study the banding patterns of five of the eight chromosome arms could be compared across populations. Japanese larvae investigated were heterozygous for arm B, possibly B1.5 (Fig 2b), but the actual banding patterns could not be determined with certainty. One larva from Brazil was also heterozygous for an inversion in arm B (Fig. 2a), which may well be the same sequences as seen in the Japanese specimens.

One Brazilian larva was homozygous for arm B and carried the B1 sequence (Fig. 1c). For arms A, E, D and F (Fig. 1a, b, d and e), the pattern was common across Indian and Japanese, as well as *C. strenzkei* individuals, with the sequences stpA1, stpE1, stpD3 and stpF1. It should be noted however that sequence stpE1 corresponds to a "basic sequence" of Wülker (1980) and occurs in many species across continents and in both the thummi- and the pseudothummi-cytocomplexes (Kiknadze et al. 2008), so that sequence on its own would not provide evidence of species identity. The other two sequences are known only from *C. striatipennis*. It also seems likely that there are shared sequences across the three regions for arm C, where Japanese *C. striatipennis* and *C. strenzkei* both appear to have Saxena's sequence stpC4 of Indian popula-

Table 2. Uncorrected ("p") distance matrix for *C. strenzkei* larvae (1-3) compared with *C. striatipennis* from Brazil (4-6), China (7), Korea (8 & 9), Japan (10-12) and the distinct '*C. kiiensis*' from Korea (13). Note that sequences 4-9, 11 and 12 are entered in GenBank as the synonym *C. kiiensis*.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	strenBraz1F	-												
2	strenBraz15	0.00000	-											
3	strenBraz16F	0.00000	0.00000	-										
4	kiiBraz334	0.00000	0.00000	0.00000	-									
5	kiiBraz335	0.00000	0.00000	0.00000	0.00000	-								
6	kiiBraz336	0.00000	0.00000	0.00000	0.00000	0.00000	-							
7	kiiChinT1L	0.00655	0.00654	0.00654	0.00654	0.00654	0.00654	-						
8	kiiKorD002	0.00818	0.00817	0.00817	0.00817	0.00817	0.00817	0.00817	-					
9	kiigg276	0.00818	0.00817	0.00817	0.00817	0.00817	0.00817	0.00817	0.00000	-				
10	striJpnJM	0.00981	0.00980	0.00980	0.00980	0.00980	0.00980	0.00980	0.00163	0.00163	-			
11	kiiJpn321	0.08661	0.08660	0.08660	0.08660	0.08660	0.08660	0.08987	0.08824	0.08824	0.08824	-		
12	kiienJpn404	0.08989	0.08987	0.08987	0.08987	0.08987	0.08987	0.08987	0.08824	0.08824	0.08824	0.00490	-	
13	KiienKoD 4	0.09805	0.09804	0.09804	0.09804	0.09804	0.09804	0.09967	0.09804	0.09804	0.09804	0.02124	0.01961	-

tions. Arm G has not been mapped, but the banding patterns appear identical (Fig. 1f).

Mitochondrial COI sequences

The sequence obtained from DNA of *C. strenzkei* from Brazil was identical to the sequence of *C. striatipennis* from Brazil (Amora *et al.* 2015) and less than 1% different to the other *C. striatipennis* sequences, but 9.8% different to the divergent Korean sequence (Table 2). The closest of the Asian sequences was that from China, at 0.65%.

Overall, the morphology, cytology, and particularly the COI sequences, clearly point to the conclusion that *C. strenzkei* is the same species as the one Amora *et al.* (2015) collected from the same region of Brazil and demonstrated to be *C. striatipennis*. Consequently the name *C. strenzkei* should be regarded as a junior synonym to *C. striatipennis*.

There are two other minor points that arise from the foregoing analysis. The inclusion of a Chinese *COI* sequence suggests that the Brazilian population of *C. striatipennis* may have originated from China, rather than Korea as suggested by Amora *et al.* (2015). The synonymy of *C. strenzkei* means that the material described by Sublette and Mulla (2000) in California is actually *C. striatipennis,* a new record for North America. In the absence of any DNA sequence for the Californian material, it can no longer be concluded that it was introduced from South America, as it could as easily be an independent introduction from Asia. A further consequence of this synonymy is that *C. striatipennis* now has a Holarctic distribution, although this distribution is almost certainly the result of unintentional human transport.

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