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Chironomus suwai, a New Species of the *plumosus* Group (Diptera, Chironomidae) from Japan

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Abstract

Cytological examination of the larvae of the species previously referred to as *Chironomus plumosus* in Japan, reveals that it is actually a new member of the *plumosus* group. This species is described as *C. suwai* on the basis of the karyotype, since this is the most reliable method for recognizing the species. It belongs to the thummi cytocomplex, and is polymorphic in chromosome arms A, B and E. A B-chromosome was present in one of the two populations (Tsukuba) studied. The banding pattern of the polytene chromosomes indicates that *C. suwai* is most closely related to *C. borokensis*, from which it differs by the smaller centromeric bands, and the presence of three unique inversion polymorphisms. The difference in centromeric size is an important indicator that these are separate species, since such differences are common between species of *Chironomus*. Some notes are included on the larvae, particularly in relation to their use in lake typology, and the later life history stages.

Keywords: *Chironomus*, Chironomidae, cytology, new description.

Introduction

The *plumosus* group of sibling species is one of the most widespread and successful, as indicated by large population sizes, groups in the genus *Chironomus*. This group includes nearly a dozen closely related species: *C. agilis* Shobanov and Dyomin, 1988, *C. agilis* 2 Kiknadze, Siirin, Filippova et al., 1991, *C. balatonicus* Devai, Wülker, Scholl, 1983, *C. bonus* Shilova and Dzhvarsheishvili, 1974, *C. borokensis* Kerkis, Filippova, Shobanov et al., 1988, *C. entis* Shobanov, 1989, *C. sp.* J Kiknadze, Shilova, Kerkis et al., 1991, *C. muratensis* Ryser, Scholl, Wülker, 1983, *C. nudiventris* Ryser,

Scholl, Wülker, 1983, *C. plumosus* Linnaeus, 1758, *C. usenicus* Loginova and Belyanina, 1994. Identification of these siblings of *C. plumosus*, which was previously thought to be a single polymorphic species, has only been possible through karyological analysis (reviewed in Kiknadze et al., 1996; Butler et al., 1999). Many of these species have broadly sympatric distributions, and often larvae of more than one member of the group will occur in the same water body. Since these species are important in lake typology (Sæther, 1979; Butler et al., 2000), there have been attempts to differentiate these species by larval morphology (Shobanov, 1989; Kiknadze et al., 1991; Shobanov & Kerkis, 1995). In general this has shown that differences identified in one population may not apply in others. Even the commonly used *plumosus*- (long ventral tubules) and *semi-reductus*- (short ventral tubules) types are not always reliable because they can be polymorphic within species (Kiknadze et al., 1991; Kiknadze et al., 2000). Similar difficulties are encountered in the morphological separation of the other stages of the life cycle (Shobanov, in press a & b). Cytology therefore remains the most reliable method for correctly identifying the various species.

The majority of the species in the *plumosus* group are found in the Palearctic. Only two species, *C. plumosus* and *C. entis*, are found also in the Nearctic, and they can be considered as Holarctic species (Butler et al., 1999; Kiknadze et al., 2000; Golygina & Kiknadze, 2001). Consequently, the *plumosus* group in the Nearctic appears to be much smaller than in the Palearctic. The *C. vancouveri* described by Michailova and Fisher (1986) as a Nearctic member of the *plumosus* group is considered to be a diverged Nearctic population of *C. plumosus* (Butler et al., 1999). *C. plumosus* has also been recorded from North Africa and the Indian subcontinent (Ashe & Cranston, 1990) but no cytological studies have been made of these populations.

On the basis of adult morphology, Palearctic *C. plumosus* seemed to have a very wide range from Western Europe to the Far East (Linevich & Sokolova, 1983). However, karyological analysis has shown that the siblings *C. borokensis* and *C. agilis* 2 replace *C. plumosus* in Eastern Siberia and the Far East (Kiknadze et al., 1996, and data of this paper). Some unknown member of the *plumosus* group was detected also in Kamchatka (Belyanina, pers. comm.). There were records that *C. plumosus* can be found in Japan (Yamagishi & Fukuhara, 1971; Sasa, 1978). Therefore it was of interest to study the karyotype of Japanese specimens to determine whether they were really *C. plumosus*, or one of its siblings.

The data of this paper suggest that the true *C. plumosus* does not occur in Japan. A new member of the *plumosus* sibling group was identified by cytotaxonomy, differing from true *C. plumosus*, as well as the other known siblings, by karyotypic characters. Although only larval specimens were available to the authors, it was felt desirable to describe this Japanese member of the *plumosus* group as a new species in order to provide Japanese workers with an appropriate name for use in their ecological studies (e.g., Yamagishi & Fukuhara, 1971; Hirabayashi et al., 1993a & b, 1997; Hirabayashi & Nakamoto, 2001). This is further justified in view of the fact, as indicated above, that the karyotype is the only reliable method for recognizing species in this sibling group. Consequently the Japanese species is designated as *C. suwai*.

Material and Methods

Mature larvae of 4th-instar *C. suwai* were investigated from two Japanese populations from waterbodies on Honshu Island: Lake Suwa, Nagano-Ken (population JP-SU, collection December 9, 1971; 9 larvae) and an experimental pond in Tsukuba (population JP-TS, collection 1990 and 1999; 7 larvae). Two populations of *C. borokensis* from the Far East near Vladivostok (Primorsk region), Chan Lake (population PRI-CH, collection May 7, 1999; 17 larvae) and Tumannaya River (population PRI-TU, collection May 28, 1998; 7 larvae), were studied in parallel to compare Japanese and Far Eastern members of the *plumosus* group. In addition the data of Kerkis et al. (1988) from Borok pond in the Yaroslavl region (Eastern Europe) (population YAR-BO, 100 larvae) are also given in Table 3 below for further comparison.

Larvae were fixed in a 3:1 mixture of 100% ethanol and glacial acetic acid, and each larva was used for both karyological and morphological study. Isolated salivary glands were squashed for polytene chromosome preparations, and the head capsule and larval body were mounted on slides for morphological analysis. Larval terminology and abbreviations follow Shobanov (1989). In addition, the existing morphological descriptions of Sasa (1978) were compared with those of other *plumosus* group siblings.

Polytene chromosome squashes were prepared by a routine aceto-orcein method (Keyl & Keyl, 1959; Kiknadze et al., 1991). Chromosomal mapping of arms A, C, D, E and F was done according to Keyl (1962) and Devai et al. (1989), using *C. piger* as standard. Arms B and G were not mapped because of the complex banding patterns in these arms. The abbreviation K indicates the centromere or kinetochore.

The banding patterns of each arm were designated by a symbol for the species, a symbol for the arm, and the number of the pattern, e.g., suw A1, suw A2, suw B1, bor A1 etc. The *C. suwai* karyotype was compared with the karyotypes of *C. plumosus* and *C. borokensis*, the members of the *plumosus* group whose banding patterns are most closely related to it. Since *C. plumosus* is a Holarctic species there are three categories of banding patterns in its karyotype: Holarctic (h' patterns), Palearctic (p' patterns) and Nearctic (n' patterns) (Butler et al., 1999).

Genotypic combinations of banding patterns of *C. suwai* were designated as suw A1.1, suw B1.1, etc., for homozygotes and suw A1.2, suw B1.2, etc., for heterozygotes.

Results

Chironomus suwai Golygina et Martin, sp. n.

Material

Holotype: (Lake Suwa, Sl. 2F, 9.xii.1971, coll. H. Yamagishi; chromosome squash with associated larval head) and paratype chromosome squashes in the collection of the Institute for Cytology and Genetics, Novosibirsk and the Australian National Insect Collection, CSIRO, Canberra.

The species is named for Lake Suwa, the locality from which it was first identified.

Karyotype

We have found that the karyotype of the Japanese *Chironomus* larvae studied is closely related to that of the *plumosus* species group. *C. suwai* has $2n = 8$, with the arm combination AB CD EF G (thummi cytotocomplex (Keyl, 1962)). Chromosome 1 (arms AB) and chromosome 2 (arms CD) are metacentric; chromosome 3 (arms EF) is submetacentric and chromosome 4 (arm G) is telocentric (Fig. 1). The centromeric bands are moderately heterochromatinized and can be easily identified (Fig. 1, arrows). The nucleolus and two Balbiani rings (BRs) can be seen on arm G. An additional BR can be seen on arm B. The two homologues of arm G are tightly paired as a rule, although they can be unpaired to some extent with conjugation maintained in the region of the centromere or telomere. Banding patterns in *C. suwai* are closely related to those of *C. borokensis* and *C. plumosus*.

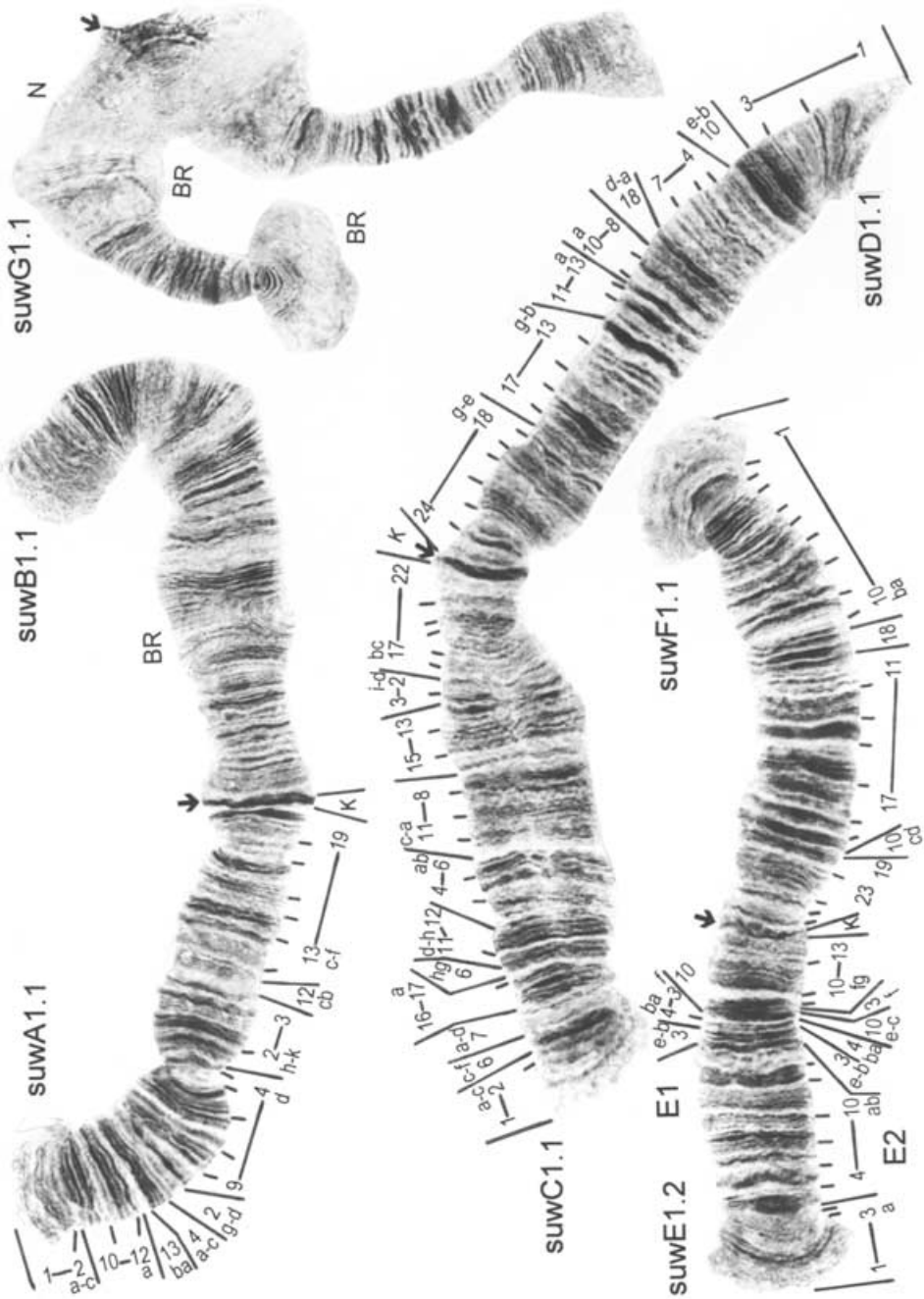


Figure 1. Karyotype of *Chironomus suwai*. Suw A1.1, suw B1.1, etc. — genotypic combinations of banding patterns; arrows show centromeric bands; BR — Balbiani ring; N — nucleolus. Mapping of arms A and F is according to Keyl (1962), arms C and D according to Devai et al. (1989), and arm E according to Butler et al. (1999).

Table 1. Number of larvae with particular genotypic combinations of banding patterns in populations of *C. suwai*.*

Population	Number of larvae studied	Genotypic combinations of banding sequences											
		 suw A1.1 bor A2.2	suw A1.2	 suw B1.1 bor B1.1	 suw B1.2 bor B1.2	 suw C 1.1 bor C 1.1	 suw D1.1 bor D 2.2	 suw E1.1 bor E1.1	suw E1.2	suw E1.3	 suw F1.1 bor F2.2	 suw G1.1 bor G2.2	
JP-SUW	9	9	–	8	1	9	9	7	1	1	9	9	
JP-TZU	7	5	2	4	3	7	7	7	–	–	7	7	

*Frequencies were not calculated because of small number of larvae studied.

Arm A is polymorphic (Table 1). Two banding patterns were identified. The predominant suw A1 (Fig. 1) is identical with bor A2 in *C. borokensis* and h⁺plu A2 in *C. plumosus* (Fig. 1):

suw A1 1–2c 10–12a 13ba 4a–c 2g–d 9–4d 2h–3 12cb 13c–19 K

The pattern suw A2 is rare. It was found only in the heterozygous state (Table 1) and is formed by a complex inversion, similar to p⁺plu A3 and p⁺plu A7 in *C. plumosus* (Butler et al., 1999; Golygina & Kiknadze, 2001). We could not map it in detail because of its complex structure and not very clear chromosomal morphology (Fig. 2a).

Arm B is polymorphic and has two patterns (Table 1). The pattern suw B1 is predominant, and is identical with bor B1 in *C. borokensis* (Fig. 1). The pattern suw B2 was recorded only in the heterozygous state (Table 1). It differs from suw B1 by a simple paracentric inversion (Fig. 2b) and is identical with bor B2 in *C. borokensis* and h⁺plu B2 in *C. plumosus*.

Arm C is monomorphic (Table 1). The pattern suw C1 is identical with bor C1 in *C. borokensis* and p⁺plu C1 in *C. plumosus* (Fig. 1):

suw C1 1–2c 6c–f 7 16–17a 6hg 11d–12 4–6b 11c–8 15–13 3–2d 17b–22 K

Arm D is monomorphic with the single pattern suw D1 (Table 1) identical with bor D2 and h⁺plu D2 (Fig. 1):

suw D1 1–3 10b–e 4–7 18a–d 8–10a 13a–11 13b–17 18e–24 K

Arm E is polymorphic with three banding patterns (Table 1). The pattern suw E1 is most common. It is identical with bor E1 and p⁺plu E1 (Fig. 1):

suw E1 according to Keyl (1962) 1–3e 5–10b 4–3f 10c–13 K

suw E1 according to Butler et al. (1999) 1–3a 4c–10b 3e–b 4b–3f 10c–13 K

The patterns suw E2 and suw E3 were recorded in the heterozygous state only (Table 1). Both inversions are very short and are formed from suw E1 by simple inversions (Fig. 2c, d) of bands 3f–10e in suw E2 and bands 6e–7c in suw E3:

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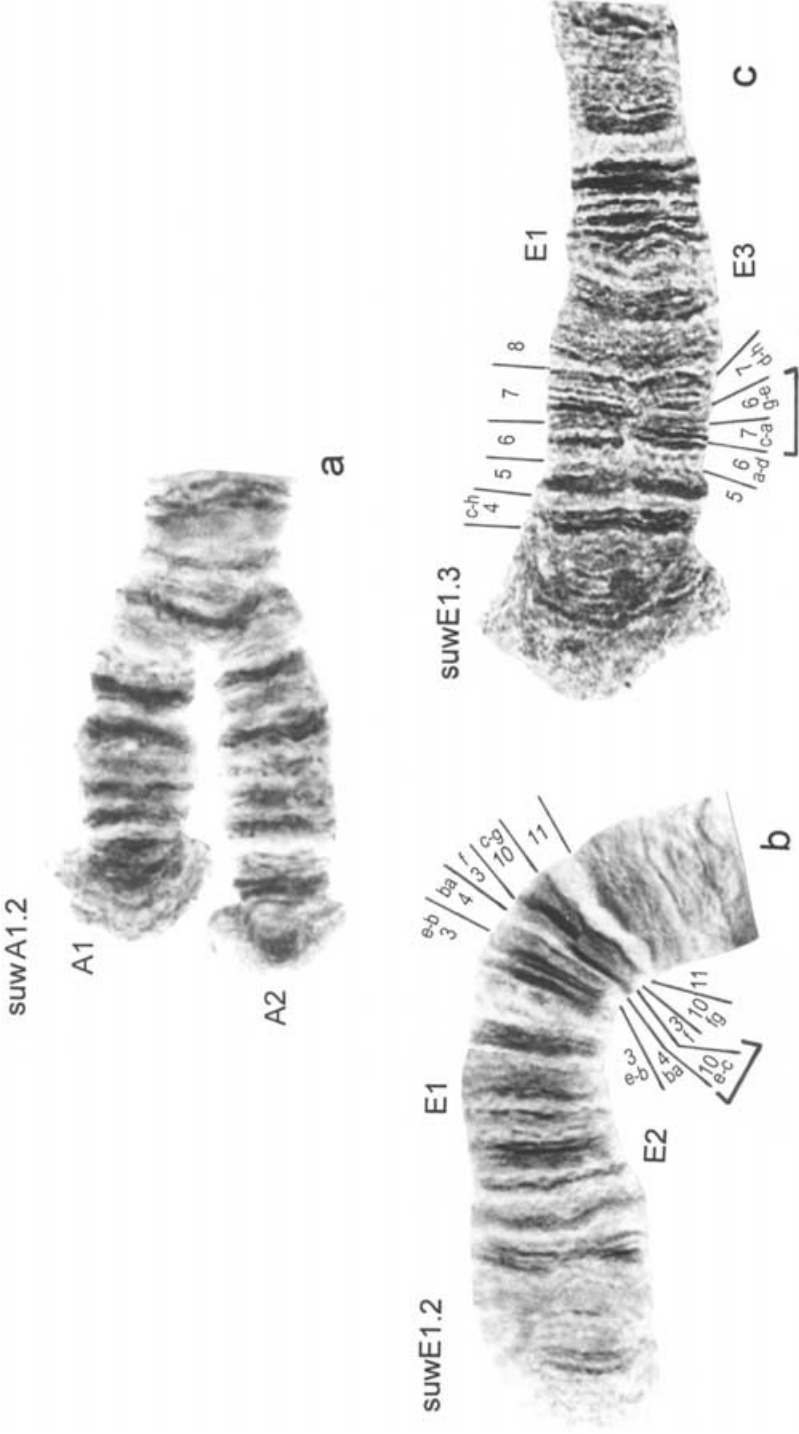
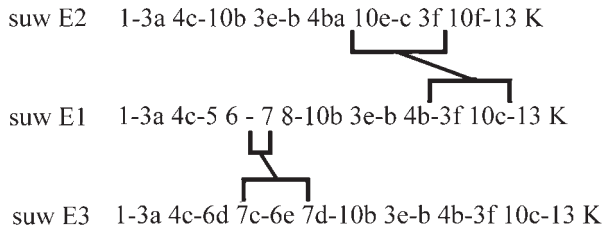
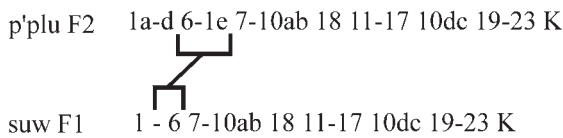


Figure 2. Heterozygous inversions in arm A (a) and E (b, c) of *C. suwai*. Notation is as in Fig. 1.



Arm F is monomorphic with the pattern suw F1 (Table 1) which is identical with bor F2 (Fig. 1) and the arm F pattern found in a number of other Palearctic *Chironomus* species (Wülker, 1991). The pattern p'plu F2 is derived from it by a simple inversion 1e-6:



Arm G has one pattern suw G1 (Table 1), identical with bor G2.

Larval morphology

4th instar: Larval morphology of *C. suwai* is as in the earlier description of Japanese '*C. plumosus*' (Sasa, 1978). Larvae are large with two pairs of ventral tubules, anterior pair with an inflection in basal half (*plumosus*-type). Gular spot pigmented with a clear border and narrow coloured areas on either side coloured; mental teeth black. Morphometric data are given in Table 2.

Although differences between the larvae of members of the *plumosus* group have been described (Shobanov, 1989; Shobanov & Kerkis, 1995), in practice it is virtually impossible to separate the larvae of *C. plumosus* and *C. borokensis* (see Table 2). In view of the similarity of *C. plumosus* and *C. borokensis*, it is not surprising that the values for the larval characters of *C. suwai* fall within the same limits as those of the same characters in the other two species (Table 2). According to Yamagishi and Fukuhara (1971), and Sasa (1978) larvae of the Japanese '*C. plumosus*' differ clearly from larvae of other Japanese *Chironomus* species by their large body length and the presence of two pairs of long ventral tubules on body segment VIII. The only other large larvae among Japanese chironomids are those of the orthoclad *Propsilocerus akamusi* (Tokunaga), but these larvae have no ventral tubules. The presence of long ventral tubules in *C. suwai* is consistent with its occurrence in eutrophic lakes (Sæther, 1979) such as Lake Suwa.

Table 2. Comparison of morphometrical larval characters of *C. plumosus*, *C. borokensis* and *C. suwai*.

L	VT	HCW	SSm	mLC	CR	Mm	M1	M2	Str	A1	Pds	AR	FA
<i>C. plumosus</i> (<i>n</i> = 4)	0.50–3.00	0.69–0.99	319–394 (358)	406–515 (480)	1.23–1.42 (1.32)	54–67 (58)	96–113 (105)	131–157 (142)	79–107 (90)	162–201 (178)	270–360 (295)	1.81–2.36 (2.13)	303–393 (346)
<i>C. borokensis</i> (Shobanov & Kerkis, 1995) (<i>n</i> = 51)	–	0.73–0.85	287–352 (310)	–	1.20–1.47	49–63 (54)	81–107 (95)	113–148 (127)	90–109 (98)	163–210 (182)	224–316 (270)	–	303–339 (346)
<i>C. suwai</i> (<i>n</i> = 3)	1.00–1.70	0.86–0.92	309–350 (325)	397–443 (442)	1.26–1.38 (1.30)	61–63 (62)	102–133 (118)	135–151 (143)	86–99 (91)	167–198 (184)	268–299 (285)	2.17–2.24 (2.21)	319–330 (326)

L: body length (mm), VT: ventral tubules (mm), HCW: head capsule width (mm), SSm–SSm: distance between SSm (micron), CR = mLC:SSm–SSm (mLC: minimal capsule length), Mm: width of central tooth (micron), M1: distance between first lateral teeth (micron), M2: distance between second lateral teeth, Str: number of striae on ventromental plate, A1: length of first antennal segment, Pds: distance between antennal sockets, AR: antennal ratio, FA: maximal width of frontal sclerite.

Adult and pupal morphology

There were no adults or pupae associated with the material available to us, but our material came from Lake Suwa and from the vicinity of Tsukuba. These are both localities from which the specimens described as *C. plumosus* by Sasa (1978) were collected, so his adult and pupal descriptions will refer to the present species. The adults and pupae of the members of the *C. plumosus*-group are very difficult to distinguish on the basis of morphology. Indeed, there has been no published material with which to make comparison. The existing descriptions of *C. plumosus* are likely to refer to one or more species of the complex, and the adults of *C. borokensis* were not included in the original description (Kerkis et al., 1988). Shobanov (in press a & b) has prepared keys to the adult males and pupae of this group, which permit correct separation of *C. plumosus* and *C. borokensis* in about 70% of cases. Sasa's description does not cover the detailed characters used by Shobanov, so that further morphological analysis of the adults and pupae will be required to determine whether any differentiating characters exist.

Discussion

A total of 11 banding patterns were found in populations studied. Three arms (A, B and E) were polymorphic, while four arms (C, D, F and G) were monomorphic. Genomic polymorphism was also observed in the populations. In addition to the inversion polymorphism, B-chromosomes were found in five of the seven larvae studied from the JP-TS population. As a rule, a single B-chromosome is present per nucleus. It has a net-like shape and is connected with arm G.

As noted, *C. suwai* has great similarity in banding patterns with *C. borokensis*, although the karyotypes differ greatly in the size of the centromeres: *C. suwai* has centromeric bands of average size, while *C. borokensis* has large heterochromatic centromeric bands (Kerkis et al., 1988).

Difference in centromeric band size between species is a very important karyotype characteristic (Keyl, 1957, 1962). Local amplification of centromeric DNA, changing the size of the centromeric bands, is one of the most widespread chromosomal mutations which accompanies species divergence. Several pairs of sibling species or closely related species characterized by difference of their centromeric bands, e.g., *C. piger* (small centromeres)/*C. riparius* (large centromeres), *C. plumosus* (small centromeres)/*C. borokensis* (very large centromeres), *C. agilis* (small centromeres)/*C. agilis 2* (very large centromeres) (Keyl, 1957; Kiknadze et al., 1991). Sometimes, a difference in centromeric size is the only cytogenetic difference between the karyotype of species (*C. piger*/*C. riparius*), while other times this difference is accompanied by several inversions (*C. plumosus*/*C. borokensis*, *C. agilis*/*C. agilis 2*).

In the case of *C. suwai* and *C. borokensis*, the difference in centromeric size is the main difference between the karyotype of the two species, although several new banding patterns were also observed in *C. suwai*.

To compare the banding patterns of *C. suwai* and *C. borokensis* more thoroughly we have studied the karyotypes of *C. borokensis* from two Far East populations. These Far East populations differ greatly from European and West Siberian populations of *C. borokensis* (Kerkis et al., 1988) by the presence of some additional inverted patterns such as bor G2, and by the much higher frequencies of patterns such as bor A2, bor B2 and bor F2 (Table 3).

These last three patterns were found in European and West Siberian populations in the heterozygous state only but homozygotes were observed in Far East populations. The higher frequency of bor A2, bor B2 and bor F2 suggests a higher adaptive value of these patterns in Far East. A small sample of *C. borokensis* from Yakutia (East Siberia) also showed the presence of bor A2.2, bor B2.2 and bor G2.2, as in Far East populations (Kiknadze et al., 1996). Thus, Yakutian and Far East populations are divergent from European and West Siberian populations of *C. borokensis*.

At the same time, Far East and Yakutian *C. borokensis* is more similar to Japanese *C. suwai* due to the presence of common patterns: bor A2.2 = suw A1.1, bor B1.2 = suw B1.2, bor F2.2 = suw F1.1, bor G2.2 = suw G1.1. However, in *C. suwai* the frequency of B2 is lower, and it has several new banding patterns not found in *C. borokensis*: suw A2, suw E2 and suw E3.

The presence of shared banding patterns in all chromosome arms allows us to conclude that the Japanese *C. suwai* is closely related to Palearctic *C. borokensis*, and differs from it mainly by the smaller size of the centromeric bands. The comparison of the *C. suwai* and *C. plumosus* karyotypes indicates the presence of common banding patterns in arms A, B, C, D and E, but arms F and G have no identical patterns. The most important difference between the *C. suwai* and *C. plumosus* karyotypes is the presence of a fixed inversion in arm G, because chromosomal polymorphism in arm G has not been found in *C. plumosus* despite the large number of populations studied. As well, the homologues of arm G in *C. plumosus* are never paired, as opposed to those of *C. suwai*. Thus, the *C. suwai* and *C. plumosus* karyotypes differ more than the *C. borokensis* and *C. suwai* karyotypes.

Conclusions

Data obtained show that the large *Chironomus* from Japan is not really *C. plumosus*, but a new species, *C. suwai*, which belongs to the *plumosus*-species group. The karyotype of *C. suwai* is closely related to that of *C. borokensis* as indicated by the many common banding patterns, but it differs by the much smaller size of the centromeric bands. The *C. suwai* karyotype differs from that of *C. plumosus* by the absence of any common patterns in arms F and G.

Mature larvae of *C. suwai* are morphologically similar to *C. plumosus*, as are those of many of the members of the *plumosus* group.

Table 3. Number of larvae with certain genotypic combinations of banding sequences in populations of *C. borokensis*.

Population	Number of larvae studied	Genotypic combinations of banding sequences														
		bor A1.1	bor A2.2 = sww A1.1	bor A1.2	bor B1.1 = sww B1.1	bor B2.2	bor B1.2 = sww B1.2	bor C1.1 = sww C1.1	bor D1.1	bor D1.2	bor E1.1 = sww E1.1	bor F1.1	bor F2.2 = sww F1.1	bor F1.2	bor G1.1	bor G1.2
YAR-BO	100	100 (1.00)*	-	-	99 (0.99)	-	1 (0.01)	100 (1.00)	64 (0.64)	36 (0.36)	100 (1.00)	27 (0.27)	22 (0.22)	51 (0.51)	100 (1)	-
PRI-CH	17	16 (0.94)	1 (0.06)	-	9 (0.53)	2 (0.12)	6 (0.35)	17 (1.00)	6 (0.35)	11 (0.65)	17 (1.00)	-	15 (0.88)	2 (0.12)	8 (0.47)	9 (0.53)
PRI-TU	7	-	4 (0.06)	3	7	-	-	7	4	3	7	3	-	4	7	-

*The frequency of the genotypic combination is in parentheses. In the case of population PRI-TU, frequencies were not calculated because of the small number of larvae studied.

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