

***Chironomus amissum* sp. n. (Diptera, Chironomidae) from southeastern Brazil**

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**Abstract:** The paper presents a description of larva, pupa, imago (male) and karyotype of *Chironomus amissum* from southeastern Brazil. It belongs to pseudothummi cytocomplex with  $2n=8$  and chromosome arm combinations: AE BF CD G. Several fixed homozygous inversions distinguished arm A of the new species from that of *Chironomus columbiensis* Wülker et al. 1989 and *Chironomus anonymus* Williston, 1896. One homozygous inversion of arm F differentiated it from *C. anonymus*. Species-specific characters were presented in the larva, pupa and imago.

**Keywords:** *Chironomus amissum* sp. n., morphology, karyology, Chironomidae, Diptera, Brazil.

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**Resumo:** Neste artigo é apresentada a descrição de larva, pupa, adulto macho e cariótipo de *Chironomus amissum*, uma nova espécie do sudeste brasileiro. Ela pertence ao citocomplexo pseudothummi com  $2n=8$  e cromossomos com combinações de braços: AE BF CD G. Várias inversões homozigóticas diferem o braço A da nova espécie em relação às espécies *Chironomus columbiensis* Wülker et al. 1989 e *Chironomus anonymus* Williston, 1896. Uma inversão homozigótica do braço F diferencia essa espécie de *C. anonymus*. Caracteres que diferenciam a espécie nova são também apresentados para larva, pupa e adulto.

**Palavras-chave:** *Chironomus amissum* sp. n., morfologia, cariologia, Chironomidae, Diptera, Brasil.

## Introduction

The cosmopolitan genus *Chironomus* Meigen, 1803 comprises several hundred species, some of which are separable only on cytological characters. In Brazil are known until the present 16 species, including 15 records to São Paulo State (Correia & Trivinho-Strixino 2005; Correia et al. 2005; 2006; Correia & Trivinho-Strixino, 2007). Some species of this genus are very abundant in heavily polluted standing or running waters; others commonly colonize small water bodies such as fish breeding tanks where they complete their life cycle in a few days. Because of its ability to inhabit different water bodies their larvae are reported in innumerable articles on aquatic ecology of Brazil, but species identification generally is not accomplished. The species identification is in general difficult because many of them are not distinguishable by larval external morphology, requiring analysis of the characteristics of all life stages or a combination of the morphology with cytotaxonomic analysis (Michailova 1989). In the present study are described the adult and immature stages as well as the karyotype of *Chironomus amissum*, sp. n., a new species from São Paulo (Brazil).

## Material and Methods

All specimens used in this study were obtained from reared egg masses collected in the field. The larval, pupal and adult specimens for morphology analyses were mounted in Euparal medium after being cleared in 10% potassium hydroxide solution. Adults male slides were mounted with associated larval and pupal exuviae if available. The measurements are given as range followed by the number of examined specimens in parenthesis if this is different from the number (n) stated at the beginning of the description. The terminology mainly follows Sæther (1980) and Langton (1994). The material examined (larva, pupa and male imago) is deposited in the Reference Collection of Laboratório de Ecologia de Insetos Aquáticos of the Universidade Federal de São Carlos (LEIA/UFSCar) São Carlos, SP, Brazil, with codification, as for example: B1-12, LEIA/UFSCar.

For the karyological analysis, larvae of 4<sup>th</sup> instar were fixed in alcohol/acetic acid - 3:1. Preparations of the polytene chromosome were obtained from squashes of salivary gland cells stained with aceto-orcein (Michailova 1989). Twenty-four preparations were made and the best of them (8) were used for the analysis of the polytene chromosomes. From one and the same individual was received both chromosome preparations and preparations of external morphology of the larva. All preparations are kept in Institute of Biodiversity and Ecosystem research, Bulgarian Academy of Sciences, Sofia.

Comparative karyological analysis was done with other American species (Wülker et al. 1989, Spies et al. 2002, Correia et al. 2005; 2006). The identification of chromosome banding patterns of the new species follows Keyl (1962) for arms A, E and F.

### *Chironomus amissum* sp. n.

**Type material:** Holotype male with pupal and larval exuviae. BRAZIL: SP, Luiz Antônio, Lagoa do Óleo (21° 35' 27" S, 46° 50' 12" W), leg. L. Correia, 26/vii/2001 (B1-01, LEIA/UFSCar). Paratypes: 3 males with pupal and larval exuviae (B1-02, B1-03, B1-04, LEIA/UFSCar), 2 males with pupal exuviae (B1-04, B1-06, LEIA/UFSCar), 4 larvae in two slides (B1-07, B1-08, LEIA/UFSCar), as holotype; 1 male with pupal and larval exuviae (B1-09, LEIA/UFSCar), 2 larvae in the same slide, (B1-10, LEIA/UFSCar), as holotype except, Lagoa do Infernã, 10/v/1996; 1 male with pupal exuviae, (B1-11, LEIA/UFSCar), as holotype except, São Carlos (21° 59' 10" S, 47° 52' 52" W), leg. S. Trivinho-Strixino, viii/2009; 3 larvae in the same slide, (B1-12, LEIA/UFSCar), São Carlos, leg. S. Trivinho-Strixino, 14/vii/2004.

**Etymology:** From Latin “*amissum*” in reference to the temporary loss of slides with the specimens.

### Morphological description

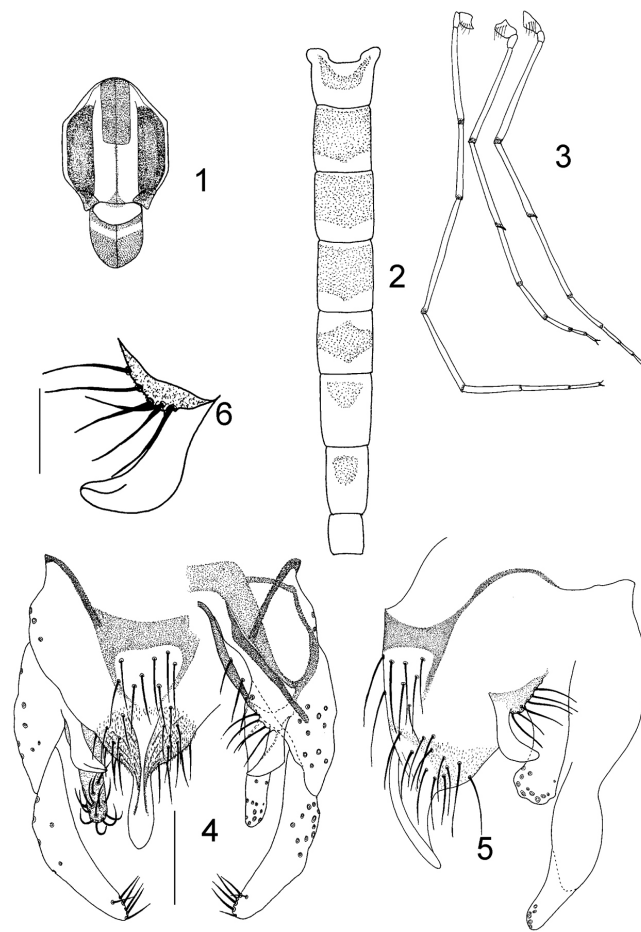
**Diagnostic characters.** The combination of abdomen and legs pattern of coloration and the shape of superior volsella distinguishes the male of *Chironomus amissum* sp. n. from Neotropical species.

**Adult male:** legs yellow with apex of femur, tibia, and tarsi brown; abdomen pale brown, tergites II–V with brown markings, tergites VI–VII with median brownish markings; superior volsella rather stout and strongly curving to ventromedial. **Larva:** abdomen with comparatively long lateral and ventral tubules, head with postmentum and frontoclypeal apotome light brown, antennal blade as long as flagellum, mandible with three inner teeth, mentum with 4<sup>th</sup> lateral tooth slightly shorter than 3<sup>rd</sup> and 5<sup>th</sup> lateral teeth. Internal apex of the ventromental plate bent down.

### Male (n = 8)

**Dimensions.** Length 5.4–6.7 mm. Wing length 2.25–2.88 mm.

**Coloration.** Head yellowish brown, flagellum and maxillary palp pale brown. Thorax yellowish brown with brown mesonotal stripes and posteromedian region darkened (Figure 1). Sternum yellowish brown; scutellum yellowish; postnotum brownish, with lighter anterior transverse stripe. Wing membrane transparent; most veins pale brown; RM brown, darker than FCu. Abdomen (Figure 2) pale brown, except for brown anterior 2/3 of tergites II–V, and median



**Figures 1-6.** *Chironomus amissum* sp. n., male imago. 1. Thorax. 2. Abdomen tergites I-VIII. 3. Front, middle and hind legs. 4. Hypopygium, dorsal (left), ventral (right). 5. Hypopygium lateral view. 6. Superior volsella. Scales: Figures 4, 5 = 100 µm; figure 6 = 50 µm.

**Table 1.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Chironomus amisum* sp. n., male.

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BR
p <sub>1</sub>	1138–1385	969–1092	1662–1969	831–1000	738–862	662–800	338–369	1.78	2.1–2.5
p <sub>2</sub>	1154–1508	1000–1415	631–831	323–415	123–308	154–185	108–169	0.67	
p <sub>3</sub>	1308–1431	1092–1400	800–1123	415–569	354–446	185–276	123–154	0.76	

brownish markings on tergites I, VI and VII. Legs (Figure 3) yellowish brown; femora, tibiae and tarsi brownish at apex.

**Head.** Eyes ratio 1.2–1.5. Frontal tubercles 21–23  $\mu\text{m}$  long. Antennal flagellum 907–1173  $\mu\text{m}$  long; AR=2.53–3.17. Palpomere 2–5 lengths: 31–43, 111–198, 185–241, 247–358  $\mu\text{m}$ . Temporal setae 26–32. Clypeus with 23–29 setae.

**Thorax.** Ac 9–13, biserial, beginning near antepronotum; Dc 14–17, partly biserial; Pa 5–6; Su 1; Scts 13–16 biserial, transversally arranged setae. Scutal tubercle short.

**Wing.** 0.60–0.64 mm wide. VR=1.03–1.14. WW=0.26–0.28. Membrane without setae. Brachiolum with 2–3 setae; R with 28–31 setae; R1 with 23–27 setae; R4+5 with 29–33 setae in distal 2/3. Squama with 9–12 setae. R2+3 ends halfway between R1 and R4+5.

**Legs.** Lengths and proportions of legs as in Table 1.

**Hypopygium** (Figures 4–6). Anal tergal bands fully enclosing 8–17 strong setae. Anal point 72–81  $\mu\text{m}$  long, narrow basally, apex curving ventrally (Figure 5). Superior volsella (Figure 6) of S-type (Strenzke 1959), rather stout and strongly curving to ventromedial; basal lobe with 4–10 long setae. Inferior volsella 105–115  $\mu\text{m}$  long, slightly clubbed, not extending beyond midpoint of gonostylus. Gonostylus elongate, 152–172  $\mu\text{m}$  long; distal part slender, with 5 inner marginal setae. HR 0.82–0.86.

#### **Pupa (n = 8)**

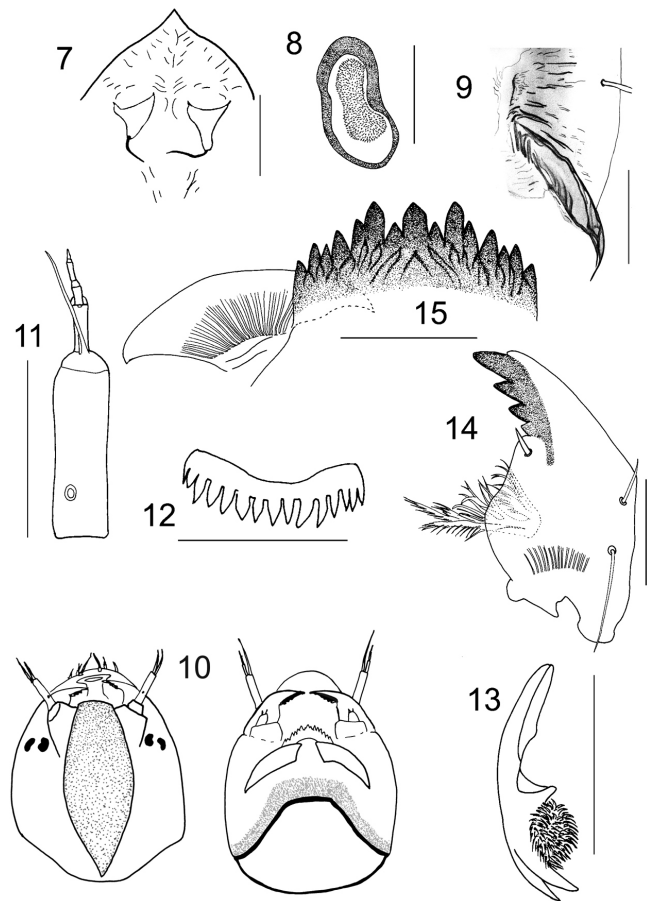
**Dimensions.** Length of abdomen 4.1–4.6 mm. Exuviae pale brown.

**Cephalothorax.** Cephalic tubercles conical (Figure 7); frontal setae 46–69  $\mu\text{m}$  long. Thoracic horn basal ring as in Figure 8. Thorax granulate in anteromedian dorsal region; scutal tubercle present. Thoracic setation: two lateral antepronotals (Laps); two precorneals (PC); four dorsocentrals (DC<sub>1-4</sub>); distance between DC<sub>1</sub> to DC<sub>4</sub> about 150  $\mu\text{m}$ . **Abdomen.** Tergite VI with a pair of small posterior patches of shagreen points; VI and VII with fine shagreen near anterior margin; VIII with pair of posteroventral patches of fine shagreen. V and VI with posterolateral point patches. Conjunctives IV/V, V/VI and VI/VII with fine shagreen. Hook row continuous, occupying 2/3 width of segment II. Pedes spurii B present on segment II. Pedes spurii A present on segment IV. Spur on segment VIII with 1 apical tooth (Figure 9). Segments I–IV with 0, 3, 3, 3 L setae, respectively; segments V–VIII all with 4 taeniae. Anal lobe with 1 stout dorsal seta and about 122 taeniata fringe setae.

#### **4th instar larva (n = 10)**

**Dimensions.** Total length 8.5–12.0 mm. Coloration: body red; head yellowish, head with postmentum and frontoclypeus light brown (Figure 10).

**Head.** Ventral head length 255–298  $\mu\text{m}$ ; head width 441–590  $\mu\text{m}$ . Antenna (Figure 11), 132–155  $\mu\text{m}$  long; AR=1.67–2.52; L1=89–100  $\mu\text{m}$ ; L2=29–31  $\mu\text{m}$ , W1=31–37  $\mu\text{m}$ ; L1/L2 = 3.07–3.38; L1/W1=2.54–3.22; ring organ near base; antennal blade surpassing segment five. Pecten epipharyngis (Figure 12), simple, consisting of about 15 subequal teeth. Premandible (Figure 13), with two subequal teeth and well-developed brush. Mandible (Figure 14), with yellowish brown dorsal tooth, apical and 3 inner teeth blackish, 3<sup>rd</sup> inner tooth slightly lighter; inner margin with 2 little spines. Mentum (Figure 15), with trifold median tooth and 6 pairs of blackish lateral teeth; 4<sup>th</sup> lateral tooth slightly shorter than 3<sup>rd</sup> and 5<sup>th</sup>. Ventromental plates separated



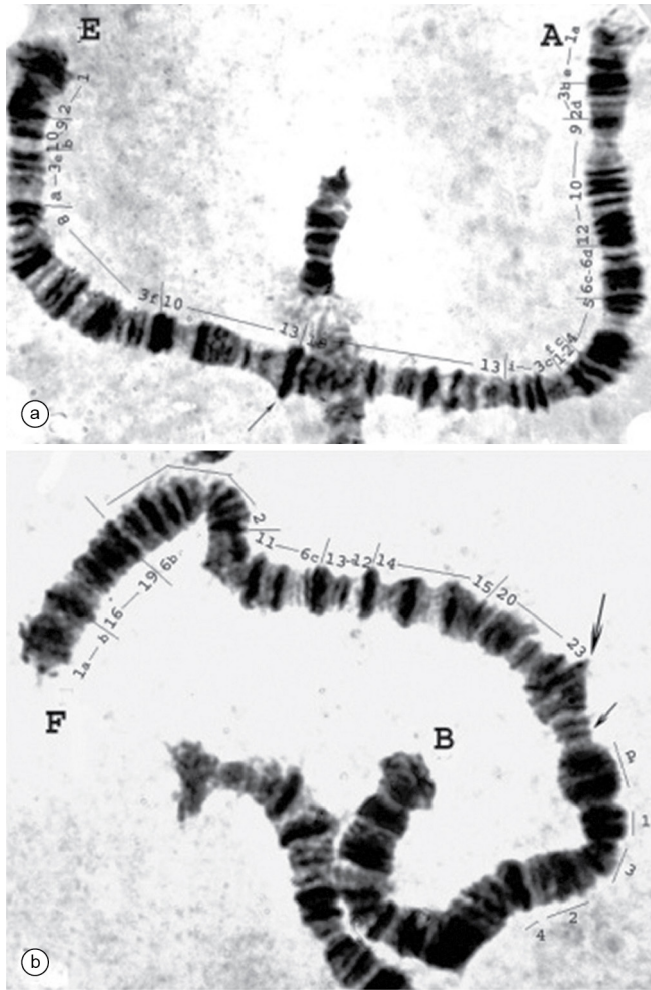
**Figures 7–15.** *Chironomus amisum* sp. n. Pupa. 7. Frontal apoteme and frontal tubercles. 8. Thoracic basal ring. 9. Anal spur. Larva. 10. Head, dorsal and ventral view. 11. Antenna. 12. Pecten epipharyngis. 13. Premandible. 14. Mandible. 15. Mentum and ventromental plate. Scales: Figure 12 = 50  $\mu\text{m}$ ; others = 100  $\mu\text{m}$ .

by about 1/3 width of mentum, anterior margin smooth. Internal apex of the ventromental plate bent down.

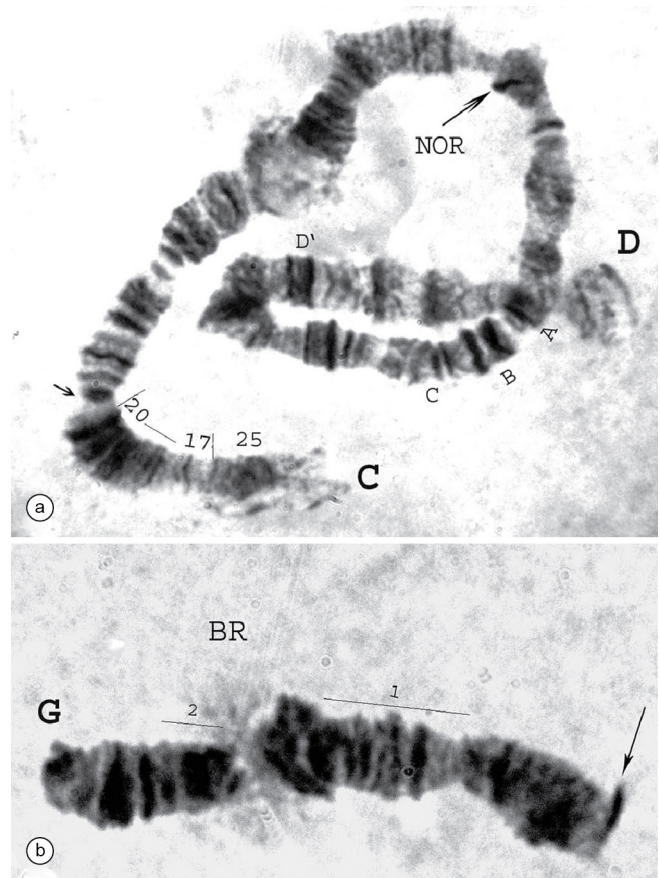
**Abdomen.** Lateral and ventral tubules of the plumosus-type (Andersen 1949); lateral tubules about 1/4 as long as 8<sup>th</sup> abdominal segment; ventral tubules, 1.5–2.4 mm long. Anal tubules with median constriction.

#### **Karyotype (n = 8)**

The chromosome set of the species is 2n=8. *Chironomus amisum* sp. n. belongs to *pseudothummi* cytochrome complex with chromosome arm combinations: AE BF CD G (Figures 16a,b; 17a,b). Chromosomes AE BF CD are metacentric and chromosome G - telocentric. Chromosome arm C has a Nucleolar Organizer (NOR), while chromosome arm G has the other key structure Balbiani Ring (BR), which is localized in the middle of this arm. The centromere regions of the polytene chromosomes of the species are distinct, looked like dark heterochromatin bands.



**Figure 16.** Polytene chromosomes of *Chironomus amissum* sp. n. **a.** Chromosome AE. **b.** Chromosome BF. Bar – 100 µm. A large arrow indicates the centromere region, a small arrow shows the band patterns by which this arm is recognized.



**Figure 17.** Polytene chromosomes of *Chironomus amissum* sp. n. **a.** Chromosome CD. **b.** Chromosome G. Bar - 100 µm. A large arrow indicates the localization of the centromere region, a small arrow shows the marker band patterns of the arm C.

**Chromosome AE (Figure 16a):**

**Arm A:**

1a - e - 3b - 2d - 9 - 10 - 12 - 6d - 6c - 5 - 4 - 2c - 1f - 3c - i - 13 - 19 *Chironomus amissum* sp. n.

1a - e - 3b - 2d - 9 - 6c - 6d - 12 - 10 - 5 - 4 - 2c - 1f - 3c - i - 13 - 19 **Inter 4**

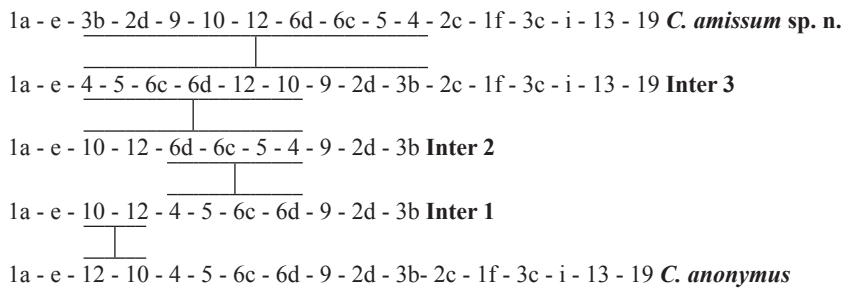
1a - e - 3b - 2d - 9 - 6c - 4 - 5 - 10 - 12 - 6d - 2c - 1f - 3c - i - 13 - 19 **Inter 3**

1a - e - 3b - 2d - 9 - 6d - 12 - 10 - 5 - 4 - 6c - 2c - 1f - 3c - i - 13 - 19 **Inter 2**

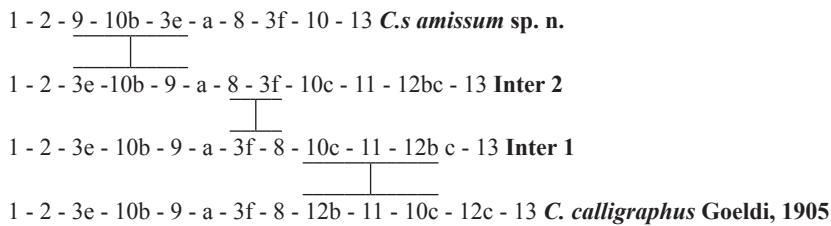
1a - e - 6c - 4 - 5 - 10 - 12 - 6d - 9 - 2d - 3b - 2c - 1f - 3c - i - 13 - 19 **Inter 1**

1a - e - 6c - 5 - 4 - 10 - 12 - 6d - 9 - 2d - 3b - 2c - 1f - 3c - i - 13 - 19 *C. columbiensis*

It is five inversion steps distant from the American species - *C. columbiensis*

**Arm A:**

It differs from *C. anonymus* (Wülker et al. 1989) by four inversion steps.

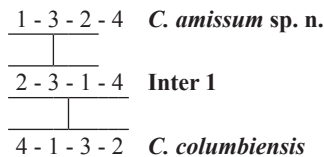
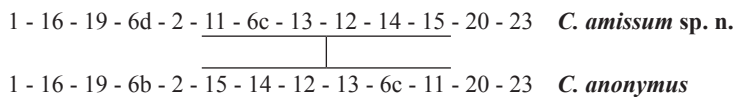
**Arm E:**

It coincided with band sequences of *C. columbiensis* and *C. anonymus* (Wülker et al. 1989), as well as those of *C. reissi* Correia et al., 2005 and *C. inquinatus* Correia et al., 2006. Three inversion steps distinguished it from *C. calligraphus* (Spies et al. 2002).

In one individual, a somatic heterozygous inversion was established only in few cells.

**Chromosome BF** (Figure 16b)**Arm B:**

There is a marker of this arm close to centromere region: a puff, characterized by group of dark bands on both sites. The band sequences, indicated in the pictures by 1-3-2-4 is similar to those of *C. columbiensis* Wülker et al. 1989 - they differ by two steps of inversions:

**Arm F:**

It is distinguished by one inversion step from the American species *C. anonymus*.

**Chromosome CD** (Figure 17a)**Arm C:**

There is a Nucleolar Organizer in the middle of the arm. This arm can be recognized by constriction in section, indicated by arrow (↓), a marker of this arm of the species in genus *Chironomus* (Michailova 1989, Kiknadze et al. 1991). The band sequences 25-17-20 are similar to those of European species: *C. muratensis* Ryser et al., 1983 (Kiknadze et al. 1991).

A somatic heterozygous inversion was detected in few cells of a single specimen.

**Arm D:**

The band sequences indicated as A B C are similar to those of *C. columbiensis* and are in an identical position, close to the centromere region. Double bands, indicated as D' are marker of the arm and can be seen in other species as *C. anonymus*, *C. columbiensis*, *C. inquinatus* and *C. reissi*.

**Chromosome G** (Figure 17b)

It has a Balbiani ring, both homologues are always paled. The band sequences 1-2, located on both sites of Balbiani ring are very similar to those of *C. anonymus*.

## Discussion

The male of *Chironomus amissum* sp. n. differs from most species of the genus in the Neotropical region by shape of superior volsella, except from *C. calligraphus*, *C. inquinatus* and *C. reissi*. It is distinguished from *C. calligraphus* and of *C. reissi* by the different coloration pattern of the legs and of *C. inquinatus* by different coloration of the abdominal tergites. The larva of *Chironomus amissum* sp. n. congregates the same morphological characteristics of *C. calligraphus* separating from this by the shape of the internal apex of the ventromental plate (as in Trivinho-Strixino 2011, Fittkau 1965 ); the new species also differs from *C. inquinatus* and *C. reissi* by different coloration of frontoclypeal apotome.

The new species belongs to pseudothummi complex, and has main similarities in band sequences of previously described North and South American species: *C. columbiensis*, *C. anonymus*, *C. calligraphus*, *C. reissi* and *C. inquinatus*. However, it has species-specific band patterns and differed from the above mentioned species by fixed homozygous inversions.

Arm A is more diverged in comparison with other species, and this is in agreements with Wülker & Morath's (1989) idea that this arm is mainly involved in the karyotype divergence of South American species. Very conservative is the band sequences of arm E - it is not changed in comparison with other American species. Also, groups of bands close to the centromere and telomere regions of arms A, B, C, D, E and F are constant and found in other American species (Wülker et al. 1989, Correia et al. 2005, 2006). Only one step of inversion in arm F distinguished this species from *C. anonymus*. It is important to underline that arm C shows some similarities in band patterns with the European species *C. muratensis* Ryser, Scholl & Wülker, 1983 (Kiknadze et al. 1991). The observed somatic alterations (heterozygous inversions in arms C and E) are possible indicators for existence of some pollutants in the water basins (Lagadic & Caquet 1998, Michailova et al. 2009).

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