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Unidentified male, perhaps of the *Chironomus decorus* group? Photo taken in the Madrona Marsh Preserve, California, USA. Photo: Emile Fiesler.

CHIRONOMUS Journal of Chironomidae Research

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Front page layout: Chironomid in title from photograph by Steve Marshall, Graphic design by Kolbjørn Skarpnes, NTNU Information Division.

Front page photo: Unidentified male, perhaps of the *Chironomus decorus* group? Photo taken in the Madrona Marsh Preserve in Torrance, Los Angeles county, California. Photo: Emile Fiesler, 2009.

Editorial

Keep the Fuel Burning

A few weeks back I was participating in a thesis defense. The topic was a far cry from chironomid work, but one message that came out of the defense is relevant to all of us, so bear with me a moment. The candidate had found that a very well-researched lizard was capable of vocalizing ultrasonically – something previously unknown to science. Towards the end of the defense, one of the referees asked the candidate how it was possible that this behavior was as-of-yet undescribed, especially considering that this lizard is considered as a model organism for reptilian studies. The candidate did well discussing several typical reasons – funding, time, technological needs, etc. The conversation took a turn, though; we began discussing how findings like these make the field of scientific research so fascinating. As researchers, I am certain we can all relate to this desire to discover; knowing that there is always "more" is the fuel that ignites our passion.

These new discoveries not only keep us going and keep our field fresh, but also act to attract new researchers to our community. I know that many students in my Entomology and Invertebrate Zoology courses are astounded by how easy (barring time and funding) it is to contribute new knowledge to science, particularly when considering insects and other invertebrates. This knowledge provides enough fuel for some of these students to continue on a research-based career path, and is likely what initially hooked many of us.

A quick glance through the many excellent contributions to this year's volume of *CHIRONOMUS* is evidence that this fuel continues to burn strong in our chironomid community. Step back to consider how fascinating it is that we can still easily discover new species and genera of chironomids (e.g. Andersen et al. 2017, Epler 2017). Then, when considering the species we do already know, bear in mind how much left there is to learn about their life history, development, home range, and patterns of richness (e.g. Baranov and Nekhaev 2017, Kuper 2017, Namayandeh and Beresford 2017). Lastly, when we utilize relatively new techniques, such as abilities to compare DNA sequences, or as we collect, curate, and closely study new specimens, we can add to or alter previous documentation and descriptions (e.g. Martin 2017, Lin and Wang 2017). Moral of the story – there is always more to discover. What an exciting field we have chosen.

We hope that you enjoy this year's collection of articles and that some of them aid in your research endeavors. Keep that fuel burning!

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CHIRONOMUS Journal of Chironomidae Research No. 30, 2017: 4-18. Current Research.

An annotated preliminary list of the Chironomidae (Diptera) of Zurquí, Costa Rica

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Abstract

An annotated list of the species of Chironomidae found at a four-hectare site, mostly cloud forest, in Costa Rica is presented. A total of 137 species, 98 of them undescribed, in 63 genera (17 apparently new), were found.

Introduction

The tropics have long been known as areas of great biodiversity (e.g. Erwin 1982), but our knowledge of many insect groups there remains poor. The two volume "Manual of Central American Diptera" (Brown et al. 2009, 2010) provided the first modern tools to analyze the diversity of one of the largest orders of insects, the Diptera (two-winged flies) of the northern portion of the Neotropics; Spies et al. (2009) covered the Chironomidae. These volumes offered the first family level identification keys for Central America (the first for any portion of the Neotropics) and also allow identification to the generic level of the described Central American Diptera. However, these volumes are just scratching the surface of Neotropical fly diversity. At both the genus and especially the species level, a tremendous amount of work remains to be done.

Art Borkent and Brian Brown conceived and developed a survey project for identifying all the Diptera species of a small area in Costa Rica. The study, patterned after an "All Taxa Biotic Inventory" (ATBI), but devoted entirely to the order Diptera, was called the Zurquí All Diptera Biodiversity Inventory (ZADBI). Fifty-nine collaborators took part in the project; I was fortunate to work with the Chironomidae. Borkent & Brown (2015) provided an overview of the project and its protocol.

As will be seen below, a large number of undescribed genera and species were found, as well as many new records for Costa Rica. This paper is also meant to update the generic synopses provided by Spies et al. (2009).

Material and methods

The majority of material came from the main site at Zurquí de Moravia (hereafter Zurquí), located at 10.047313°N, 84.008457°W, in San José Province, Costa Rica, collected from September 2012 to October 2013. The 150 by 266 m site, at an elevation of ~1600 m, is mostly cloud forest, with adjacent small pastures; the site has one permanent and one temporary stream, located in heavily forested ravines.

Collecting methods included two malaise traps run continuously and additional traps run three days each month: three additional malaise traps, several emergence traps (over leaf litter; over dry branches; over vegetation; over stagnant water; over running water), CDC light traps, bucket light traps, yellow pan traps, flight intercept traps and mercury vapor light traps. Some specimens were collected by sweeping and by hand.

Samples were sorted and prepared by technicians at the Instituto Nacional de Biodiversidad (INBio). Given the difficulties of female identification, for the most part only males were removed from the samples for identification. Thus parthenogenetic species, such as *Phytotelmatocladius delarosai* Epler, known from the USA (Florida) to Argentina (Epler 2010, Siri & Donato 2014) may have been missed.

Results

A total of 2,120 specimens were examined. Four subfamilies, 63 genera and 137 species were represented (Appendix 1). The majority of species (98), and 17 new genera, are apparently undescribed and were given letter/number designators. It is anticipated that a great deal of time will be necessary to describe all these new taxa. Workers whose work may involve some of these taxa are welcome to contact me with ideas for producing descriptions. Funding sources are also being sought.

All but one of the putatively new genera were orthoclads and are listed as "CHIRORTH-n", referring to "Chironomidae: Orthocladiinae" followed by a number; e.g., "CHIRORTH-1 sp. ZUR-1". The single CHIRCHIR-1 is a damaged specimen of a Chironominae, and may represent a known genus, but its combination of available characters does not allow generic placement. Undescribed species are listed with "ZUR-n", referring to new taxa from the Zurquí project (I also have numerous other undescribed species from Costa Rica, collected over the past several decades, that have been given different letter/number designators; with the possible exception of a single species of *Parametriocnemus*, none of these other undescribed taxa were found in the Zurquí material). A list of the taxa collected is presented in Appendix 1. An annotated accounting of these taxa follows below; any mention of morphological characters is not to be taken as a description of a taxon. Note also that the relative numbers or percentages given for species collected are not representative of the total catch, and pertain just to the material that was selected to be mounted.

PODONOMINAE

Parochlus - A single taxon belonging to the *Parochlus araucanus* group (Brundin 1966) was collected. Because pupae are necessary for species delimitation in this group, species-level identification of this taxon will have to wait until pupae are collected and associated.

TANYPODINAE

Procladius - To date, only one species of the subgenus *P. (Procladius)* has been reported from the Neotropics: *P. mozambique* Roback, from Colombia (Roback 1982b). The single species of *Procladius* collected at Zurquí, *P. (Procladius)* sp. ZUR-1, more closely resembles *P. (P.) paludicola* Skuse, an Australian species (Roback 1982a), but differs in coloration and morphology.

Thienemannimyia - *Thienemannimyia (Thienemannimyia)* sp. ZUR-1 may be the species referred to by Watson & Heyn (1993) as *Thienemannimyia* nr *barberi* (Coq). It resembles *T. barberi* (Coquillett), a western US species, but the wing pattern appears slightly different; comparison of more Costa Rican and Nearctic material is necessary.

Zavrelimyia - *Zavrelimyia (Paramerina) fasciata,* as *Paramerina fasciata* Sublette & Sasa, was described from Guatemala (Sublette & Sasa 1994); it is widespread in Costa Rica and was the most common tanypod collected in the study.

ORTHOCLADIINAE

Antillocladius - Three species were collected; *A. pluspilalus* was the most common (59 specimens). The genus has most recently been reviewed by Mendes & Andersen (2008) and Mendes et al. (2011).

Bryophaenocladius - Of the five *Bryophaenocladius* species collected, four are undescribed. The fifth species is listed here as *B. cf. psilacrus*. *Bryophaenocladius psilacrus* was described from South Carolina, USA, by Sæther (1982), based on a single specimen. A key character for this species was the absence of acrostichal setae. The Zurquí material (41 specimens) all had some acrostichal setae; otherwise the specimens appear to be *B. psilacrus*. The type was not available for examination.

Although a widespread and speciose genus, few *Bryophaenocladius* species are known from the Neotropics. Wang et al. (2006) and Donato (2011) provided the most recent treatments of the genus for the area. It is possible that some of the taxa that I've grouped here may belong elsewhere.

Compterosmittia - Three species were collected; the most common (104 specimens) was the undescribed *C.* sp. ZUR-1, followed by *C. nerius* (43 specimens). A single specimen of *C.* sp. ZUR-2 was collected. The genus was most recently reviewed, for taxa from the area, by Mendes et al. (2004).

Corynoneura - Two undescribed species were collected in addition to two described species: *C. ferelobata* was described from Guatemala by Sublette & Sasa (1994); *C. guanacaste* was described from northern Costa Rica by Wiedenbrug et al. (2012).

Cricotopus - About 15 species are recorded or described from the Central American-Caribbean area (Spies & Reiss 1996). All eight of the *Cricotopus* species that were collected at Zurquí appear to be undescribed; all belong with the subgenus *C.* (*Cricotopus*).

Diplosmittia - Two undescribed species were collected, in addition to *D. forficata*, described from La Selva, Costa Rica, by Andersen (1996). The genus has recently been reviewed by Pinho et al. (2009) and Wiedenbrug & Silva (2016) added an additional species from the Dominican Republic. The single specimen of *D.* sp. ZUR-1lacks antennae and bears a well developed inferior volsella; it may belong elsewhere but is placed in *Diplosmittia* until more complete material can be collected. *Diplosmittia* sp. ZUR-2 resembles *D. beluina* Andersen (described from La Selva, Costa Rica) but has 13 antennal flagellomeres, with an AR of 3.0-3.4, and a more sharply pointed anal point; *D. beluina* has 10 flagellomeres and an AR of 0.62. Both taxa have a long costal extension. The two taxa may be conspecific but more material is necessary to determine antennal vaiability.

Eukiefferiella - Three undescribed species that fit *Eukiefferiella* were collected. No described species are known from the Neotropics; Watson & Heyn (1993) recorded larvae from Costa Rica.

Gravatamberus - The genus was established by Mendes & Andersen (2008); they included five species found in Brazil, Chile, Costa Rica, Guatemala, Mexico and Venezuela. *Gravatamberus curtus* Mendes & Andersen was described from material (three specimens) from Mexico and northwestern Costa Rica; *G. guatemaltecus* was described from a single specimen from Guatemala. The two species were diagnosed by the number of setae in cell m proximal to RM, number of setae on the subcosta, the length of the costal extension and the AR: *G. curtus* has <10 setae in cell m proximal to RM (*G. guatemaltecus* >10); subcosta with 6–15 setae (*G. guatemaltecus* 16), costal extension 68-86 µm (*G. guatemaltecus* 161); AR 0.64–0.69 (*G. guatemaltecus* 0.26).

Eighteen males of *Gravatamberus* were collected; unfortunately, antennae were missing on all of them. All had a costal extension, ranging from 128 to 200 µm (Table 1), greater than that recorded for *G. curtus*. However, the range of setae in cell m proximal to RM ranged from 0 to 24 in the Zurqui material, regardless of costal extension length, suggesting that this character does not aid in distinguishing *G. curtus* from *G. guatemaltecus*. All *Gravatamberus* collected in this study are considered to represent *G. guatemaltecus*.

Data also indicate that the midges had more setae in cell m and on the subcosta from February to May, with fewer setae present in those wing areas from May (one specimen) through October.

Irisobrillia - Eight specimens of the sole species of the genus, *I. longistyla*, were collected.

Krenosmittia - Two specimens were collected of a species that appears to belong with *Krenosmittia*. This would be the first record for the genus from the Neotropics. Examination of more material is necessary.

Limnophyes - *Limnophyes* was one of the more common of the small orthoclad taxa collected (~8% of total mounted catch). Two species were collected: *L. mariae* (38 specimens) and the much more common *L. guatemalensis* (142 specimens), both described from Guatemala by Sublette & Sasa (1994).

Lipurometriocnemus - Four species were collected, three of them undescribed. *Lipurometriocnemus* was first described from the British West Indies by Sæther (1981), with *L. glabalus* as the sole species. Sæther (1982) then described *L. vixlobatus* from South Carolina, USA. The two species were supposedly separated by the absence of setae on R_1, a weak inferior volsella and a "slight hump on tergite IX representing a reduced anal point" in *L. vixlobatus*. Cranston & Oliver (1988) reported *L. vixlobatus* from the Yukon Territory of Canada, and examined paratypes of *L. glabalus* and a sin-

Table 1. Selected measurements of *Gravatamberus guatemalticus*

Specimen #	Month collected	cell m setae	subcosta setae	costal extension, µm
4399193	OCT	0	5	175
4401710	JUN-JUL	1	4	195
4401650	JUN-JUL	0	6	200
4401625	OCT	0	5	155
4400249	JUN	1	5	180
4405861	MAY	1	5	153
4401624	OCT	0	9	200
4400246	JUN	1	7	188
4399225	JUN	0	5	155
4399224	JUN	9	5	175
4406521	APR	18	12	163
4370228	MAY	17	17	153
4363952	APR	24	14	128
4363944	FEB	11	7	188
4363957	APR	19	11	133
4399449	MAY	15	10	165
4406482	APR	13	6	150
4406453	MAY	16	10	138

gle specimen (which they thought to also represent *L. glabalus*) from Braulio Carillo National Park in Costa Rica (the Zurquí site is adjacent to a portion of the huge park). They noted that the two species were difficult to separate because of variation in the supposedly diagnostic characters, but gave no reason why they assigned the Yukon specimen to *vixlobatus*, which following its description should lack any setae on R_1, instead of assigning it to *L. glabalus*. I noted such variation in the volsellae and "anal hump" in the Zurquí material I've designated as *L. glabalus*; there was variation in the length/breadth of the gonostylus but much appeared due to the viewing angle. I saw no specimens in which R_1 was devoid of setae and considered all material I put in this group to be *L. glabalus* (63 specimens).

My *L.* sp. ZUR–1(eight specimens) has genitalia similar to those of *L. glabalus* but has a very low AR (~0.32–0.58) compared to what is being called *L. glabalus* today (following Cranston & Oliver 1988 and Andersen et al. 2016). In Sæther's (1981) original description of *L. glabalus*, all of the type material lacked antennae, but it is now apparently assumed that the species has an AR similar to that of *L. vixlobatus* – around 1.60–1.75. Zurquí material of *L. glabalus* had ARs > 1.70.

My *L.* sp. ZUR–2 (36 specimens) has an AR around 1.76–1.85 and genitalia very similar to those of *L. glabalus*, except the proximal portion of the gonostylus bears a hump that separates it from that of *L. glabalus*. Further work (i.e., measurements of other body parts, etc.) may show this taxon may also belong with *L. glabalus*.

My *L.* sp. ZUR–3 (two specimens) has an AR of about 1.0 or less and has a distinctively pronounced inferior volsella. Andersen et al. (2016) described two new *Lipurometriocnemus* species from Brazil and provided a key for the known species. Their *L. biancae* has a pronounced inferior volsella, but not the same as *L.* sp. ZUR–3, and an AR of 1.29-1.45. Their *L. amazonicus* (AR 1.23–1.45) is very similar to *L. glabalus*, but has fewer dorsocentral setae.

Litocladius - A single species (34 specimens), *L. chavarriai*, was collected.

Lopescladius - Two undescribed species, both belonging with the subgenus *L. (Cordiella)*, were collected (seven of *L.* sp. ZUR-1; two of *L.* sp. ZUR-2).

Mesosmittia - The widespread *M. patrihortae* (22 specimens) and one undescribed species (one specimen) were collected. The genus was most recently reviewed by Andersen & Mendes (2002). They noted that *M. truncata* Sæther, described from a single male specimen from Panama (Sæther 1985), was separable from *M. patrihortae* only by the length of the costal extension (116 µm in *M. truncata*; 8–62 µm in *M. patrihortae*). Costal extensions in Zurquí material ran from 50 to 140 µm, indicating a wide range of lengths that would include the single measurement from the Panama specimen. I consider *M. truncata* to be a junior synonym of *M. patrihortae*.

Metriocnemus - Three species, two undescribed, were collected. The named species, *Metriocnemus costatus,* was described from Guatemala by Sublette & Sasa (1994). Their description stated "genitalia with a midventral notched, somewhat quadrangular plate". One of the *Metriocnemus* species collected, initially designated *M.* sp. ZUR-2, was very similar to the description and illustrations for *M. costatus* (Sublette & Sasa 1994: fig. 88) except it apparently lacked this plate. I was able to examine 6 paratypes of *M. costatus* from the Sublette Collection, located at the University of Minnesota, St. Paul, MN. The specimens were all excessively squashed (typical of Sasa mounts) and the quadrangular plate was apparent. I then remounted the abdomen/genitalia of one of the Zurquí *M.* sp. ZUR-2 specimens and excessively squashed it – the quadrangular plate appeared! This plate is normally oriented in a dorsal-ventral manner and is visible as a thin sclerotized line when viewed in a typical genitalia mount that has not been excessively flattened. The structure may represent the aedeagus or an ejaculator (see Tuxen 1970: fig. 160).

Nanocladius - Eight specimens of a single undescribed species of *Nanocladius (Nanocladius)* were collected. The genus is poorly known from the Neotropics, with only three species described from the region to date (Epler 1986; Wiedenbrug & Silva (2013).

Onconeura - One undescribed species (six specimens) of *Onconeura* was collected. This would be the second species known from Costa Rica; Spies & Reiss 1996) recorded *O. semifimbriata* (Sæther) from the country. The most recent papers on *Onconeura* are Donato et al. (2012) and Wiedenbrug et al. (2009).

Parakiefferiella - The genus is unrecorded from Central America; Wiedenbrug & Andersen (2002) described several new species from South America and provided a review of the genus. Two specimens of an undescribed species were collected.

Parametriocnemus - Four species were collected; all appear to be undescribed. *Parametriocnemus* sp. ZUR-1 and *P.* sp. ZUR-2 resemble the Nearctic *P. lundbeckii* (Johannsen) but have a lower AR; *P*. sp. ZUR-1 was 0.67–0.72; *P*. sp. ZUR-2 was 0.52-0.68 (for *P. lundbeckii* see below). Both may represent a single species; more measurements of other body parts are necessary.

Sublette (1967: 539) gave an AR of 1.40 based on a specimen (a "metatype") from the type series; he also gave ARs of four Johannsen-determined specimens that ranged from 1.26–1.60, mean 1.36. Sæther (1969) gave a range of 0.90-1.30, mean 1.12, for the AR of *P. lundbeckii*.

Sublette & Sasa (1994) recorded larval, pupal and adult *P. lundbeckii* from Guatemala. I examined 4 females and one female pupal exuviae from this Guatemalan material (no males were available). None of these specimens could be reliably identified as *P. lundbeckii*.

Epler (2001: 7.117) discussed a *Parametriocnemus* from Great Smoky National Park that had genitalia similar to *P. lundbeckii* but had an AR of 0.40. Two other specimens resembling *P. lundbeckii* from the same area have an AR of around 0.84, and I've also examined *P. lundbeckii* from the same area with "typical" ARs (~1.40). It appears obvious that a thorough study of *P. lundbeckii* is necessary to determine just what that species is.

According to my notes, *P.* sp. ZUR-4 resembles a species I have seen from northwestern Costa Rica that I had designated *P*. sp. CR-3. Those specimens, and other Costa Rican *Parametriocnemus* species, are out on loan and were not available for comparison. All of the Zurquí *Parametriocnemus* specimens had an AR below 1.00.

Paraphaenocladius - *Paraphaenocladius exagitans longipes* was one of the most common orthoclads collected, with 113 specimens examined. The subspecies was described from Costa Rica (type locality, Cacao (a volcano) in Guanacaste Province), St. Vincent and Trinidad (Sæther & Wang 1995).

Pseudorthocladius - Two specimens of an apparently undescribed species were collected. Until this collection, *Pseudorthocladius* was not recorded from the Neotropical Region. The Zurquí species appears similar to *P. clavatosus* Sæther & Sublette, but has a smaller inferior volsella. *Pseudorthocladius clavatosus* was described from two males and a female from South Carolina, U.S.A. (Sæther & Sublette 1983); comparison with type material will be necessary to determine if the Zurquí specimens represent a new species.

Pseudosmittia - Three species were collected, one undescribed. *Pseudosmittia windwardensis* was the most common (75 mounted specimens), while 39 specimens of the widespread *P. forcipata* were collected. It should be noted that, pending further investigation, some of the putative new orthoclad genera found during this study may belong with *Pseudosmittia.* Ferrington & Sæther (2011) provided the most recent review of the genus.

Stictocladius - One specimen of an undescribed species was collected. In the Western Hemisphere, this genus has been mostly recorded from Argentina, Chile and Peru, but has recently been found in North America (Sæther & Cranston 2012). This would be the first record of the genus from Central America. The new Costa Rican species has clear wings and a very low AR of 0.21.

Synorthocladius - Two specimens of an undescribed species were collected. Watson & Heyn (1993) recorded "*Synorthocladius* sp." from Costa Rica.

The following orthoclad taxa appear to represent undescribed genera. These taxa are discussed below with mention of morphological characters in order to inform other workers that may have similar taxa and may wish to collaborate or borrow material. Please note again that any mention of morphological characters is not to be taken as a description of a taxon.

CHIRORTH-1 - A small species, represented by one specimen with bare eyes; 11 antennomeres; minute scalpellate (?) acrostichals; wing with strong punctation and bare squama; small rounded anal point; and each tarsal claw with 3 teeth.

CHIRORTH-2 - A small species with bare eyes; 13 antennomeres with low (0.43) AR; elongate thorax with a few decumbent acrostichals; 0-1 squamal setae, with long costal extension; abdomen with weak bands; no virga, no anal point.

CHIRORTH-3 - A small species with pubescent eyes; low AR (0.50); well developed scale-like virga; and a short, rounded anal point. Fifteen specimens were collected.

CHIRORTH-4 - There appear to be two species in this putative new genus, which differ from each other by having a radically different virga. The head has a U-shaped frons as in *Jururumberus* Mendes & Andersen (Mendes & Andersen 2013). This small-bodied genus also has bare eyes; 8/9 antennomeres; no squamal setae; R ends just before fCu and no anal point. Numerous specimens were collected.

CHIRORTH-5 - A genus similar to *Pseudosmittia*, represented by four specimens. It features antennae with 13 flagellomeres; low AR (0.55); weak decumbent acrostichals; one squamal seta; wing veins without setae except near base of R; long costal extension; no pulvilli and deeply bifid tarsal claws.

CHIRORTH-6 - This taxon, represented by four specimens, resembles *Saetheriella* Halvorsen, in having reniform, protruding hairy eyes and a rounded to triangular anal point. However, it has an extended costa; well developed pulvilli; a well developed, clear, scutal tubercle; lacks a virga; and has superior and inferior volsellae.

CHIRORTH-7 - This genus has pubescent eyes; no observable acrostichal or squamal setae; a very long costal extension; a scale-like virga; and a short rounded anal point. Three specimens were collected.

CHIRORTH-8 - A single specimen of this taxon was collected. It has eyes with very short pubescence; antennae with 13 flagellomeres and very low AR (0.19); no apparent acrostichals; no squamals, no pulvilli; apically bifid claws; no volsella and a gonostylus that is widened preapically.

CHIRORTH-9 - Represented by two specimens, this genus has pubescent eyes, with pseudo-ocelli present; a grossly enlarged globose palpomere 3; numerous scalpellate acrostichals; a small patch of short setae lateral to and above the apex of the antepronotal lobes; no squamals; a long costal extension; very recurved Cu1 and a *Mesosmittia*-like anal point.

CHIRORTH-10 - A single specimen of this moderately large midge was collected. It has 13 flagellomeres; bare eyes; reduced 4th and 5th palpomeres; about 20 scalpellate acrostichals that originate at about 1/3 the length of the scutum; finely punctate wing membrane; no squamals; sinuate Cu1; no anal point but a *Mesosmittia*-like median hump; abdominal tergites with dark posterior bands; and a well developed inferior volsella with a median swelling posterior and ventral to it.

CHIRORTH-11 - Two specimens were collected; both have lost their antennae. Eyes are bare; the acrostichals are absent or very minute; a weak virga is present; there are no volsellae present; and the gonostylus lacks a megaseta.

CHIRORTH-12 - A single specimen that lost its antennae was collected. It has hairy eyes; four palpomeres; no squamal setae; apparently no acrostichals; a virga; *Mesosmittia*-like anal point; and a small inferior volsella.

CHIRORTH-13 - This taxon has bare eyes; one palpomere; apparently no acrostichals; bare squama; elongate virga; and broadly spatulate anal point. Three specimens were collected.

CHIRORTH-14 - One specimen of this taxon was collected. It has bare eyes; 13 flagellomeres, with low AR (0.28) and a large seta on the last antennomere; no acrostichals; no squamals; a moderately long bare anal point and a well developed inferior volsella.

CHIRORTH-15 - A series of 10 specimens represents this genus, which has bare eyes; short palpomeres; two acrostichals at mid-scutum; long pulvilli; and no anal point. All specimens have lost their antennae.

CHIRORTH-16 - A single specimen of this midge was collected. It somewhat resembles *Pseudosmittia*, but has hairy eyes; no acrostichal or squamal setae; a long costal extension; weak virga; small inferior volsellae; arcuate gonostyli; and a short triangular/conical anal point lacking any large setae.

CHIRONOMINAE

Beardius - Two species were collected; *B. triangulatus* was described from Costa Rica by Andersen & Sæther (1996). One specimen of an undescribed species was collected. The genus was recently revised by Pinho et al. (2013).

Caladomyia - Two undescribed species were collected; one specimen of one species, two of the other. The genus was recently reviewed by Trivinho-Strixino (2012).

Cryptochironomus - Four specimens of an undescribed *Cryptochironomus* were collected.

Dicrotendipes - Fourteen specimens of an undescribed *Dicrotendipes* were collected. The species possesses an apically bifid inferior volsella. Although such a volsella is known from Afrotropical and Palaearctic species, as well as several Amazonian species (Epler 1988), this is the farthest north I have seen such a species in the Western Hemisphere.

Einfeldia - Five specimens of an undescribed *Einfeldia* were collected. The taxonomy of *Einfeldia* has been confused, with some species being allocated to other genera (Epler et al. 2013; Cranston et al. 2016). I examined the holotype of *E. atitlanensis* Sublette & Sasa, described from Guatemala (Sublette & Sasa 1994); this new species differs having a larger digitus of the superior volsella, a thinner gonostylus and darkened apices of the leg segments.

Endotribelos - Two described species and one undescribed species were collected. Fourteen specimens of *E. albatum* and 40 (including two intersex specimens) of *E. grodhausi* were examined; seven specimens of the undescribed species were collected. The undescribed species has a long thin anal point and appears similar to, but lighter than, an undescribed species from La Selva, of which I have several reared associations.

Endotribelos was established by Grodhaus (1987) for *Tendipes (Tribelos) hesperia* Sublette, 1960 (which thus became *Endotribelos hesperium* (Sublette)), a southern US species whose larva has an odd number of teeth on the mentum and a large gap (diastema) between the molar area and the proximal inner tooth of the mandible. Subsequently, Sublette & Sasa (1994) described two species from Guatemala, *E. albatum* and *E. grodhausi*; the larva of *E. grodhausi* has an even number of mental teeth and lacks the mandibular diastema (the larva of *E. albatum* remains unknown). Since then, several more species have been described from South America (Roque & Trivinho-Strixino 2008 and Trivinho-Strixino & Pepenelli 2015), the larvae of which display a variety of those character states. See also Epler et al. (2013).

Trivinho-Strixino & Pepenelli (2015) provided keys for the males and known larvae of *Endotribelos*. However, male *E. grodhausi* will not key correctly because couplet 4 gives one the choice of "base of superior volsella setose" or "base of superior volsella bare". Choosing "bare" eventually leads to *E. grodhausi* at couplet 14. However, the base of the superior volsella of *E. grodhausi* is clothed, ventrally and dorsally, with fine setae. Thus, *E. grodhausi* will key to *E. jaragua* Trivinho-Strixino & Pepenelli in couplet 5, from which it may be separated by the darker thorax of *E. grodhausi* (preepisternum and adjacent sclerites are dark; light in *E. jaragua*). Sasa and Sublette's illustration of the volsella (Sublette & Sasa 1994: fig. 141) does not indicate the presence of setae, which apparently led Trivinho-Strixino & Pepenelli (2015) to assume the structure was bare. Sublette & Sasa (1994: fig. 135) likewise illustrated the base of the superior volsella of *E. albatum* as bare, when it also is clothed with fine setae. I examined two paratypes of *E. albatum* and eight paratypes of *E. grodhausi*.

Nandeva - Two specimens of *N. latiloba* were collected; the species was described from Brazil by Sæther and Roque (2004). This is the first record of the genus from Costa Rica. Andersen et al. (2011) also recorded the species from Venezuela and confirmed the genus's position within the tribe Tanytarsini.

Nilothauma - Two undescribed species were collected. The genus was most recently reviewed by Mendes & Andersen (2009).

Parachironomus - Four specimens of an undescribed *Parachironomus* were collected. The Neotropical *Parachironomus* were documented by Spies et al. (1994).

Phaenopsectra - A single specimen of an undescribed *Phaenopsectra* was collected.

Polypedilum - A very speciose genus world-wide, *Polypedilum* was the most abundant chironomine genus collected in the study, with one undescribed species of *P. (Pentapedilum*), one described and three undescribed *P. (Polypedilum)*, three described and one undescribed *P. (Tripodura)* and one undescribed species of *P. (Uresipedilum)*.

The undescribed *P. (Pentapedilum)* species was the most abundant *Polypedilum* collected, with 88 specimens mounted. Mendes, Andersen & Jocqué (2011) described *P. (Polypedilum) panacu* from bromeliad phytotelmata in Honduras. Of the three described *P. (Tripodura)* collected, two were described by Sublette & Sasa (1994) from Guatemala. One of these, *P. (Tripodura) luteopedis* was the second most abundant *Polypedilum* collected, with 75 specimens mounted; the widespread *P. (T.) apicatum* was described from New Mexico, U.S.A., by Townes (1945) and was also recorded from Guatemala by Sublette & Sasa (1994).

Rheotanytarsus - One undescribed species, two described species and two tentatively identified described species were collected. *Rheotanytarsus guancastensis, R. scutulatus* and *R. subtilis* were described from northwestern Costa Rica by Kyerematen & Andersen (2002); *R. contrerasi* was described from Mexico by Andersen & Sæther in Kyerematen et al. (2000). Our specimens of *R. cf. contrerasi* and *R. cf. guanacastensis* differ slightly from their original descriptions and must be compared with type material for more positive identification.

Riethia - Fifteen male specimens of a genus from the putative tribe Pseudochironomini were collected; this genus is almost certainly *Riethia*, but there is a small chance they could be *Manoa*. Males of the two genera cannot at this time be separated utilizing morphological characters; only females and pupae may be separated at the generic level (Jacobsen & Perry 2002; Trivinho-Strixino et al. 2009). I'm calling the Zurquí specimens *Riethia* based on their close resemblance to *R. truncato-*

caudata (Edwards) as redescribed in Trivinho-Strixino et al. (2009) and *R. manauara* Neubern, Trivinho-Strixino & Silva (Neubern et al. 2011); the medial apices of the inferior volsellae of all three species have large, flattened, apically pectinate setae directed mediad (and dorsad in *R. truncatocaudata*). Such setae are unknown in *Manoa*. Note that the legs of the Zurquí specimens are unbanded and there are other differences, especially in the superior volsella; thus, they do not represent *R. truncatocaudata*. Likewise, differences in the genitalia indicate that *R*. sp. ZUR-1 is not conspecific with *R. manauara*.

Stempellinella - Four specimens of one undescribed species were collected. The genus was recently review by Ekrem (2007).

Stenochironomus - Ten species of *Stenochironomus* were collected, only one of which was positively identified as a previously described species: *S. nudipupa* is known from Brazil, Costa Rica, Ecuador and Venezuela (Borkent 1984; Reis et al. 2013). A single specimen of *S*. cf. *varius* appears to be *S. varius* but lacks any coloration on its fore femur; it does have a darkened fore coxa and trochanter, and may represent a slight color variation of the species. Since Borkent's (1984) excellent revision of *Stenochironomus* and its allies, numerous additional species have been described from the Neotropics; Dantas et al. (2016) provided the most recent key for Neotropical *Stenochironomus*. *Stenochironomus* sp. ZUR–1 (six specimens) is similar to *S. impendens* Borkent, but has a different abdominal color pattern; *S*. sp. ZUR–2 (one specimen) has a dark, broad anal point, but has different thoracic and abdominal color patterns from any described species; *S*. sp. ZUR–3 (16 specimens) will key to *S. sebastiao* Andersen et al. (Andersen et al. 2008) in Dantas et al. (2016) but has a shorter, thicker superior volsella and other hypopygial differences; *S*. sp. ZUR–4 (five specimens) and *S*. sp. ZUR–5 (one specimen) somewhat resemble *S. leptopus* (Kieffer) but differ in coloration and genitalic structures; *S*. sp. ZUR–6 (two specimens) is entirely pale like *S. palliaculeatus* Borkent, but has a stouter superior volsella; *S*. sp. ZUR-7 (two specimens) has an dark thorax like *S. varius*, but has numerous darkened abdominal tergites; and *S*. sp. ZUR–8 (two specimens) is unusual in having a globose/semi-pediform superior volsella.

Tanytarsus - *Tanytarsus* was by far the most common tanytarsine collected, with 140 specimens representing nine species. The majority did not fit descriptions of any known species, except *T*. cf. *capaira* (two specimens) resembles *T. capaira* described by Trivinho-Strixino & Strixino (2007) from Brazil, but appears a bit different; *T*. sp. ZUR-8 (one specimen) is also similar to *T*. cf. *caipira* but is much smaller. *Tanytarsus* sp. ZUR-5 (16 specimens) is similar to *T. cotopaxi* Giłka & Zakrzewska, described from Ecuador (Giłka & Zakrzewska 2013). The majority of specimens were *T*. sp. ZUR-1 (47 specimens), including an intersex specimen; 23 specimens of *T*. sp. ZUR-2; 34 specimens of *T*. sp. ZUR-3; 14 specimens of *T*. sp. ZUR-6; and one specimen of *T*. sp. ZUR-7.

Xestochironomus - Four species were collected, one undescribed. I examined the holotype of *X. ankylis*, described from Guatemala by Sublette & Sasa (1994); *X. gilvus* and *X. latilobus* were described from Venezuela by Borkent. *Xestochironomus* sp. ZUR-1, represented by seven specimens, will key to couplet 6 in Borkent (1984) but is neither *X. subletti* Borkent nor *X. gilvus* Borkent; it differs in having a very long, thin, anal point and almost straight superior volsellae.

CHIRCHIR -1 - This taxon is represented by one damaged specimen, missing its antennae and all tarsomeres. The wings are bare, the squama is fringed and the antepronotal lobes do not appear to meet medially. The gonostylus has an apical row of medially directed setae, all of which are broken off. The specimen also lacks a subapical seta on the superior volsella but has a subapical pit/sensillum, from which I can observe no indication of a broken or lost seta. This taxon may represent a new genus or be an aberrant *Stenochironomus* with separated tibial spurs (these spurs are fused in *Stenochironomus*); the specimen lacks the posteromedial patch of short setae on the gonostylus found on *Xestochironomus*. More, undamaged, material is needed.

Discussion

The Zurquí study has produced an impressive list of chironomid taxa, the majority of which (72%) are undescribed. Noticeable in their absence were some common and widespread taxa such as *Ablabesmyia*, *Chironomus*, *Coelotanypus*, *Goeldichironomus, Oukuriella* and *Tanypus*. This is no doubt due to a dearth of suitable habitats, as many of those genera are more common in ponds and lakes, especially those with a bit of eutrophication; no such water bodies were present at Zurquí.

Many of the taxa collected are considered terrestrial, semi-terrestrial or phytotelmatic; most of these belong with the subfamily Orthocladiinae: *Antillocladius, Bryophaenocladius, Compterosmittia, Lipurometriocnemus, Mesosmittia, Paraphaenocladius*, *Pseudosmittia* and probably *Diplosmittia*; at least one *Polypedilum, P. panacu*, is phytotelmatic.

It is unfortunate that the project's protocol did not include collecting the immature stages of Chironomidae, but a lack of funding (only half the requested amount for the grant was provided) made the laborious process of collecting larvae and pupae, and rearing them to adulthood, not possible. The likelihood that many of the taxa were probably terrestrial or semi-terrestrial (in wet moss, etc.), entailing an even more time-consuming process than collecting the immature stages from water, compounded the problem. One taxon collected in this survey, a member of *Parochlus araucanus* group, might have been identifiable to species if the pupal exuviae had been collected because the only species-specific characters for this group are found in the pupae (Brundin 1966).

As is demonstrated in this paper, the Neotropical Chironomidae (actually, all Diptera in the Neotropics) remain poorly known - and their immature stages, often used in environmental assessments in the Holarctic, even more so. Although Coffman et al. (1993) reported 266 species of chironomids from 13 streams in northwestern Costa Rica, based solely on pupal exuviae, none of those taxa have been supplied with an available name. Chironomid taxonomy relies mostly on characters of the male, especially the male genitalia, for species delimitation. Thus, collecting the immature stages *must* include rearing (or otherwise associating those stages, as with pharate pupae) to adulthood in order to associate those stages with the generally more better known adults, the bearers of the species' names. Alternatively, the use of suitable molecular markers (e.g. DNA bar codes), as shown by Trivinho-Strixino & Pepinelli (2015), may also help to associate life stages with available names.

While 137 species from a four-hectare area may seem impressive, it pales with the numbers from some of the other families from the ZADBI project. Chironomidae only placed eleventh on the list of total species collected. The Cecidomyiidae topped the list, with about 800 species found. A more complete analysis of these numbers, plus other aspects of diversity, biogeography, ecology, etc., will be presented in a series of multi-authored papers currently being written about the ZADBI project.

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APPENDIX 1

List of Chironomidae collected at Zurquí de Moravia, Costa Rica, 2012–2013

PODONOMINAE

Parochlus araucanus Brundin, 1966, group sp. ZUR-1

TANYPODINAE

Procladius (P.) sp. ZUR-1

Thienemannimyia (T.) sp. ZUR-1

Zavrelimyia (Paramerina) fasciata (Sublette & Sasa, 1994)

ORTHOCLADIINAE

Antillocladius antecalvus Sæther, 1981

Antillocladius arcuatus Sæther, 1982

Antillocladius pluspilalus Sæther, 1981

Bryophaenocladius cf. psilacrus Sæther, 1982

Bryophaenocladius sp. ZUR-1

Bryophaenocladius sp. ZUR-2

Bryophaenocladius sp. ZUR-3

Bryophaenocladius sp. ZUR-4

Compterosmittia nerius (Curran, 1930)

Compterosmittia sp. ZUR-1

Compterosmittia sp. ZUR-2

Corynoneura ferelobata Sublette & Sasa, 1994

Corynoneura guanacaste Wiedenbrug, Lamas & Trivinho-Strixino, 2012

Corynoneura sp. ZUR-1

Corynoneura sp. ZUR-2

Cricotopus sp. ZUR-1

Cricotopus sp. ZUR-2

Cricotopus sp. ZUR-3

Cricotopus sp. ZUR-4

Cricotopus sp. ZUR-5

Cricotopus sp. ZUR-6

Cricotopus sp. ZUR-7

Cricotopus sp. ZUR-8

Diplosmittia forficata Andersen, 1997

Diplosmittia sp. ZUR-1

Diplosmittia sp. ZUR-2

Eukiefferiella sp. ZUR-1

Eukiefferiella sp. ZUR-2

Eukiefferiella sp. ZUR-3

Gravatamberus guatemaltecus Mendes & Andersen, 2008

Irisobrillia longicosta Oliver, 1985

Krenosmittia sp. ZUR-1

Limnophyes guatemalensis Sublette & Sasa, 1994

Limnophyes mariae Sublette & Sasa, 1994

Lipurometriocnemus glabalus Sæther, 1981

Lipurometriocnemus sp. ZUR-1

Lipurometriocnemus sp. ZUR-2

Lipurometriocnemus sp. ZUR-3

Litocladius chavarriai Mendes, Andersen & Hagenlund, 2011

Lopescladius (*Cordiella*) sp. ZUR-1

Lopescladius (Cordiella) sp. ZUR-2

Mesosmittia patrihortae Sæther, 1986

Mesosmittia sp. ZUR-1

Metriocnemus costatus Sublette & Sasa, 1994

Metriocnemus sp. ZUR-1

Metriocnemus sp. ZUR-3

Nanocladius sp. ZUR-1

Onconeura sp. ZUR-1

Parakiefferiella sp. ZUR-1

Parametriocnemus sp. ZUR-1

Parametriocnemus sp. ZUR-2

Parametriocnemus sp. ZUR-3

Parametriocnemus sp. ZUR-4

Paraphaenocladius exagitans longipes Sæther & Wang, 1995

Pseudorthocladius sp. ZUR-1

Pseudosmittia forcipata (Goetghebuer, 1921)

Pseudosmittia windwardensis (Sæther, 1981)

Pseudosmittia sp. ZUR-1

Stictocladius sp. ZUR-1

Synorthocladius sp. ZUR-1

CHIRORTH-1 sp. ZUR-1

CHIRORTH-2 sp. ZUR-1

CHIRORTH-3 sp. ZUR-1

CHIRORTH-4 sp. ZUR-1

CHIRORTH-4 sp. ZUR-2

CHIRORTH-5 sp. ZUR-1

CHIRORTH-6 sp. ZUR-1

CHIRORTH-7 sp. ZUR-1

CHIRORTH-8 sp. ZUR-1

CHIRORTH-9 sp. ZUR-1

CHIRORTH-10 sp. ZUR-1

CHIRORTH-11 sp. ZUR-1

CHIRORTH-12 sp. ZUR-1

CHIRORTH-13 sp. ZUR-1

CHIRORTH-14 sp. ZUR-1

CHIRORTH-15 sp. ZUR-1

CHIRORTH-16 sp. ZUR-1

CHIRONOMINAE

Beardius triangulatus Andersen & Sæther, 1996

Beardius sp. ZUR-1

Caladomyia sp. ZUR-1

Caladomyia sp. ZUR-2

Cryptochironomus sp. ZUR-1

Dicrotendipes sp. ZUR-1

Einfeldia sp. ZUR-1

Endotribelos albatum Sublette & Sasa, 1994

Endotribelos grodhausi Sublette & Sasa, 1994

Endotribelos sp. ZUR-1

Nandeva latiloba Sæther & Roque, 2004

Nilothauma sp. ZUR-1

Nilothauma sp. ZUR-2

Parachironomus sp. ZUR-1

Phaenopsectra sp. ZUR-1

Polypedilum (*Pentapedilum*) sp. ZUR-1

Polypedilum (*Polypedilum*) *panacu* Mendes, Andersen & Jocqué, 2011

Polypedilum (*Polypedilum*) sp. ZUR-1

Polypedilum (*Polypedilum*) sp. ZUR-2

Polypedilum (Polypedilum) sp. ZUR-3

Polypedilum (*Tripodura*) *apicatum* Townes, 1945

Polypedilum (*Tripodura*) *luteopedis* Sublette & Sasa, 1994

Polypedilum (*Tripodura*) *obelos* Sublette & Sasa, 1994

Polypedilum (*Tripodura*) sp. ZUR-2

Polypedilum (Uresipedilum) sp. ZUR-1

Rheotanytarsus cf. contrerasi Andersen & Sæther, 2000

Rheotanytarsus cf. guanacastensis Kyerematen & Andersen, 2002

Rheotanytarsus scutulatus Kyerematen & Andersen, 2002

Rheotanytarsus subtilis Kyerematen & Andersen, 2002

Rheotanytarsus sp. ZUR-1

Riethia sp. ZUR-1

Stempellinella sp. ZUR-1

Stenochironomus nudipupa Borkent, 1984

Stenochironomus cf. varius Borkent, 1984

Stenochironomus sp. ZUR-1

Stenochironomus sp. ZUR-2

Stenochironomus sp. ZUR-3

Stenochironomus sp. ZUR-4

Stenochironomus sp. ZUR-5

Stenochironomus sp. ZUR-6

Stenochironomus sp. ZUR-7

Stenochironomus sp. ZUR-8

Tanytarsus cf. caipira Trivinho-Strixino & Strixino, 2007

Tanytarsus sp. ZUR-1

Tanytarsus sp. ZUR-2

Tanytarsus sp. ZUR-3

Tanytarsus sp. ZUR-4

Tanytarsus sp. ZUR-5

Tanytarsus sp. ZUR-6

Tanytarsus sp. ZUR-7

Tanytarsus sp. ZUR-8

Xestochironomus ankylis Sublette & Sasa, 1994

Xestochironomus gilvus Borkent, 1984

Xestochironomus latilobus Borkent, 1984

Xestochironomus sp. ZUR-1

CHIRCHIR-1 sp. ZUR-1

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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^{o}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^{o}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

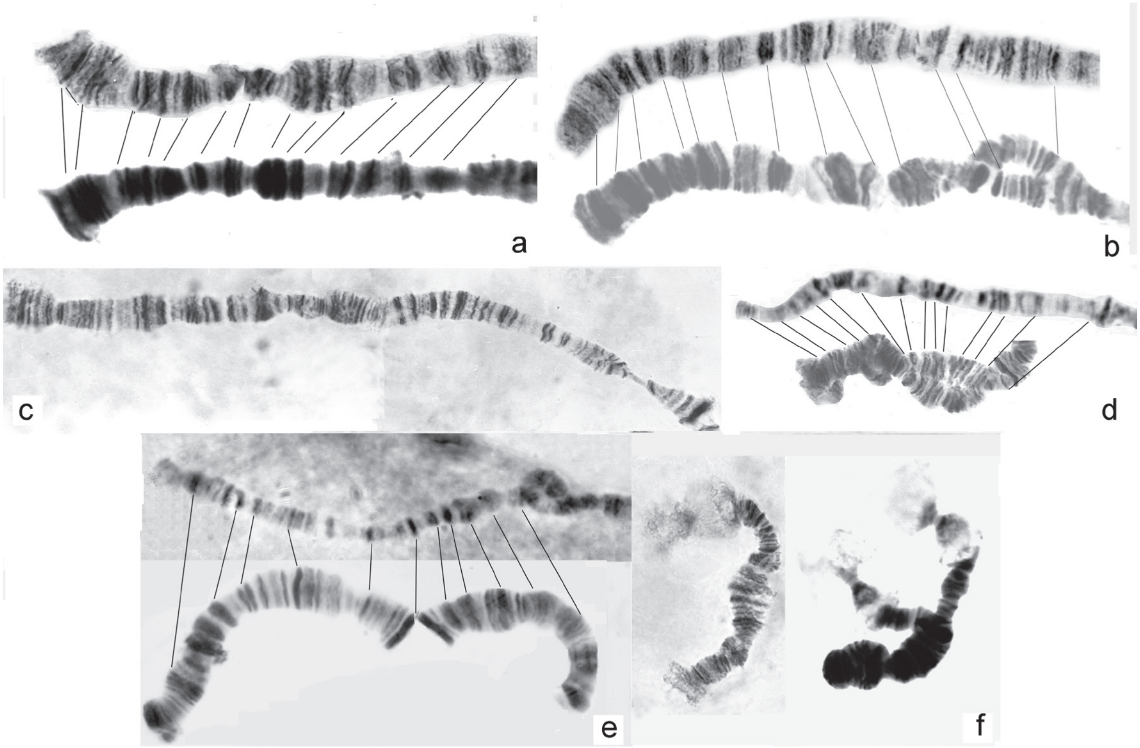

Figure 1. Comparison of polytene chromosomes of Brazilian *C. strenzkei* (above or left) and Japanese *C. striatipennis* (below or right). The centromere is to the right, except in arm G where it is at the top of the arm. Lines join homologous bands in the compared arms. a. Arm A, both A1.1; b. Arm E, 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).

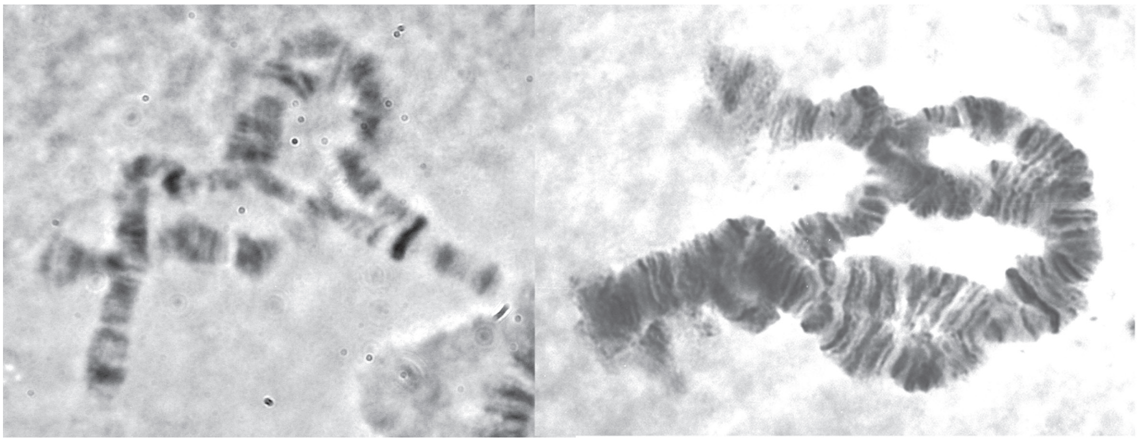

Figure 2. Heterozygotes for arm B of *C. strenzkei* (left) and *C. striatipennis* (right). It is likely that both have the sequences B1.5. The associated arm F of C. striatipennis is the same chromosome as Fig. 1,d.

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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TWO NEW NEOTROPICAL CHIRONOMINAE GENERA (DIPTERA: CHIRONOMIDAE)

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http://zoobank.org/4253DC36-B9D8-4D36-B71E-6116FF1E22AD

Abstract

Claudiotendipes n. gen. and *Sigmoitendipes* n. gen. are described and figured based on adults, pupae and larvae. Two species are included in *Claudiotendipes*: the type species, *C. froehlichi* n. sp. from Bahia, Rio de Janeiro, São Paulo and Santa Catarina States in Brazil; and *C. epleri* n. sp. from Costa Rica. Five species are included in *Sigmoitendipes*, all from Brazil: the type species, *S. susanae* n. sp. from São Paulo, Mato Grosso, Amazonas and Pará States; *S. fittkaui* n. sp. from Mato Grosso and Pará; *S. reissi* n. sp. from São Paulo; and *S. oliveirai* n. sp. and *S. spiesi* n. sp., both from Mato Grosso. A cladistic analysis grouped the two new genera with *Beardius*, *Oukuriella* and *Endotribelos*. Keys to the males of the two new genera are presented.

Introduction

The subfamily Chironominae comprises approximately 50% of the chironomid species described from the Neotropical region, which is more than twice the share of any other subfamily (e.g. Spies and Reiss 1996: table 1). In the fauna of Brazil, the largest country in the region, the proportion increases to about 60% (335 of 566 scientifically named species, Pinho 2016), whereas the second largest subfamily stands at less than 25% (134 species in Orthocladiinae). Chironominae larvae are found in a wide range of aquatic and semiaquatic habitats, but are relatively more abundant in standing and slow-flowing lowland waters than at lotic sites in mountainous areas.

During the first half of the 1960's, the late E. J. Fittkau made extensive collections in various parts of the Amazon region; this vast amount of chironomid material is now housed in the Zoologische Staatssammlung in Munich, Germany. Fittkau (2001) wrote: "the numbers of 'new species' and 'new genera' increased with each new light catch. I stopped this work when we had reached the number of 500 new species and 50 new genera, as well as a number of familiar genera". Fittkau (1971) suggested that at least 1000 species of Chironomidae live in the Amazon. Since then, many new species and several new genera have been described. In Brazil alone, for example, 32 Chironominae genera with more than 330 species are recognized (Pinho 2016). Even so, much of Fittkau's material still awaits analysis and publication, and the knowledge of Brazilian chironomids remains fragmentary.

Below we describe a new genus, *Sigmoitendipes*, based partly on material collected by Fittkau in the Amazon, and partly on more recent material gathered from several other Brazilian regions. The description is based on males, pupae and larvae, and five new species are recognized. *Claudiotendipes* n. gen. is described from material collected in the Brazilian Atlantic Forest (Mata Atlântica) as well as Costa Rica. Keys to the males of both genera are provided, and their relations among the Chironominae are evaluated based on a cladistic analysis.

Material and methods

Specimens, identification and terminology

Except for the material obtained on loan, specimens examined were preserved in alcohol and later mounted on slides in Canada balsam or Euparal following the procedure outlined by Sæther (1969); some slide-mounted specimens borrowed from Zoologische Staatssammlung München (ZSM) were not cleared in KOH. Since there are no identification keys which cover southern hemisphere chironomid fauna, identification of material was completed using keys for Central American and Holarctic genera (Pinder and Reiss 1986, Cranston et al. 1989, Spies et al. 2009, Epler et al. 2013). Comparison to descriptions of austral Chironominae genera not included in these keys, such as *Tapajos* Trivinho-Strixino, Silva *et* Oliveira (from Trivinho-Strixino et al. 2013), *Nilodosis* Kieffer, *Imparipecten* Freeman (from Cranston and Hardwick 1996), *Conochironomus* Freeman (from Cranston and Hare 1995) and *Xylochironomus* Cranston (from Cranston 2006) were also conducted. Diagnoses of both new genera were then prepared considering differential characters from all of these sources. The descriptive terminology follows Sæther (1980), with some additions and modifications from Epler et al. (2013). Measurement results are given as ranges, followed by the mean when four or more specimens were measured, followed by the number of specimens measured (n) in parenthesis.

Type material

The type materials are housed in the following museums (listed in alphabetical order):

INPA – Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil.

MZUFBA – Museu de Zoologia da Universidade Federal da Bahia, Salvador, Brazil.

MZUSP – Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil.

UFSC – Entomological Collection, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.

ZMBN – Department of Natural History, University Museum of Bergen, University of Bergen, Bergen, Norway.

ZSM – Zoologische Staatssammlung München, Munich, Germany.

Phylogenetics

To assess the phylogenetic positions of the new genera we used a matrix with 119 characters in 71 taxa (Appendix 1) that is based on the data in Cranston (2003, 2006) (matrix kindly made available by request) plus data on *Beardius* Reiss *et* Sublette (from Pinho et al. 2013), *Oukuriella* Epler (from Fusari et al. 2013), *Endotribelos* Grodhaus (from Grodhaus 1987, Roque and Trivinho-Strixino 2008), *Claudiotendipes* n. gen. and *Sigmoitendipes* n. gen. (from present study). The character states for *Nilothauma* Kieffer were revised according to Mendes and Andersen (2008). 'Gen. A' refers to an undescribed Afrotropical genus with wood mining larvae (Cranston 2006).

The matrix was edited in Mesquite (Maddison and Maddison 2009), the analyses were performed in TNT software (Goloboff et al. 2008b), and the character distribution was visualized in Winclada (Nixon 1999–2002). All characters were treated as unordered under the implied weighting searches (Goloboff 1993). Heuristic tree searches were run with the tree bisection and reconnection (TBR) branch swapping algorithm (1000 replications, random seed = 0). A TNT script (propk.run, written by Salvador Arias) calculated the appropriate value for the k constant (for details, see Goloboff et al. 2008a); it returned a value of k = 15.263673 for the data set selected for the analysis.

Characters and character states

Larvae

1. *Dorsal surface of head:* (0) S 3 on same plate as S 4 and S 5 (clypeus fused to frons); (1) S 3 not on same plate as S 4 and S 5 (clypeus separate from frons).

2. *Labral sclerites:* (0) only one sclerite expressed; (1) sclerites 1 and 2 present.

3. *Frontal fenestra or pit:* (0) present; (1) absent.

4. *Antennal segment 2:* (0) undivided; (1) divided.

5. *Antennal segments beyond style:* (0) three; (1) two; (2) four.

6. *Antennal blade extent:* (0) not to apex of flagellum; (1) to or beyond apex.

7. *Accessory blade:* (0) short; (1) subequal to segment 2; (2) absent.

8. *Blade insertion:* (0) apex of segment 1; (1) mid segment 2; (2) more apical.

9. *Style:* (0) short or absent; (1) extending to mid segment 3.

10. *Lauterborn organs:* (0) opposite; (1) alternate on segments 2 and 3; (2) absent.

11. *Lauterborn organ pedicel:* (0) absent; (1) < one third of segment 3; (2) > one third of segment 3.

12. *Antennal seta:* (0) absent; (1) present.

13. *Antennal pedestal:* (0) low; (1) elongate.

14. *Antennal pedestal apex:* (0) rounded; (1) with spur or palmate projection.

15. *Labral seta S I:* (0) simple; (1) palmate to plumose.

16. *S I bases:* (0) separate; (1) fused.

17. *Labral S II:* (0) simple; (1) plumose.

18. *Labral S IV:* (0) small (normal); (1) strongly developed, on pedestal.

19. *Labral lamella:* (0) comb-like; (1) reduced; (2) absent.

20. *Labral lamella:* (0) single; (1) divided; (2) absent.

21. *Seta praemandibularis:* (0) simple; (1) branched.

22. *Pecten epipharyngis:* (0) three separate scales; (1) one fused plate.

23. *Pecten epipharyngis:* (0) smooth; (1) toothed in one plane; (2) toothed in multiple planes.

24. *Premandible teeth:* (0) one to three; (1) > three.

25. *Premandible inner (bluntly rounded) teeth:* (0) none; (1) one; (2) two.

26. *Mandible dorsal tooth:* (0) present; (1) absent.

27. *Mandible inner teeth:* (0) four; (1) three; (2) two.

28. *Pecten mandibularis:* (0) present; (1) reduced; (2) absent.

29. *Seta subdentalis:* (0) simple; (1) bifid to plumose; (2) absent.

30. *Seta subdentalis extending to:* (0) proximal inner tooth of mandible; (1) middle inner teeth; (2) apex of mandible.

31. *Seta interna:* (0) present; (1) absent.

32. *Seta subdentalis:* (0) inserted dorsally; (1) inserted ventrally.

33. *Outer mentum:* (0) normal; (1) outer three teeth offset.

34. *Median mental tooth:* (0) trifid; (1) simple or double.

35. *Mentum coloration:* (0) all dark; (1) medially pale, contrasting with lateral parts; (2) all pale.

36. *Seta submenti:* (0) simple; (1) plumose; (2) bifid.

37. *Segment XI:* (0) without ventral tubules; (1) with ventral tubules.

38. *Mandibular basal striae:* (0) absent; (1) present.

39. *Mentum anterior margin:* (0) convex; (1) concave.

40. *Ventromental plate anterior margin:* (0) smooth; (1) crenulated.

Pupae

41. *Cephalic tubercles:* (0) absent; (1) present.

42. *Frontal warts:* (0) absent; (1) present.

43. *Frontal setae:* (0) absent; (1) present.

44. *Thoracic horn branches:* (0) one; (1) two to three; (2) four to eight; (3) multiple (plumose).

45. *Basal ring of thoracic horn:* (0) single, medially constricted; (1) kidney-shaped; (2) simple, rounded to elliptical; (3) absent.

46. *Tracheal branches:* (0) single; (1) double; (2) triple; (3) absent.

47. *Prealar tubercle:* (0) absent; (1) present.

48. *Scutal tubercle:* (0) absent; (1) present.

49. *Precorneal setae:* (0) two; (1) three.

50. *Antepronotal setae:* (0) none; (1) one; (2) two; (3) three.

51. *Dorsocentral setae:* (0) three; (1) four.

52. *Wing sheath "nose":* (0) absent; (1) present.

53. *Abdominal tergite I:* (0) bare; (1) with spinules.

54. *Hook row on tergite II:* (0) continuous; (1) interrupted.

55. *Extent of hook row on tergite II:* (0) > half segment width; (1) subequal to half segment width; (2) < half segment width.

56. *Conjunctive IV/V:* (0) bare; (1) with spinules.

57. *Pedes spurii A:* (0) absent; (1) on parasternite IV only; (2) on more than one parasternite.

58. *Pedes spurii B on segment I:* (0) absent; (1) present.

59. *Pedes spurii B on segment II:* (0) absent; (1) present.

60. *Segment I:* (0) with lateral projections; (1) without lateral projections.

61. *Segment VIII posterolateral spur/comb:* (0) absent; (1) with one more or less dominant tooth; (2) with multiple subequal teeth.

62. *Abdominal segment IV taeniate lateral setae (LS):* (0) absent; (1) present.

63. *Segment V LS:* (0) absent; (1) present.

64. *Segment VI LS:* (0) absent; (1) present.

65. *Segment VII LS:* (0) absent; (1) present.

66. *Segment VIII LS:* (0) absent; (1) present.

67. *Segment VIII LS number:* (0) three; (1) four; (2) five; (3) none.

68. *Abdominal segment O setae:* (0) absent; (1) one dorsal + one ventral; (2) two dorsal + one ventral; (3) two dorsal + two ventral; (4) one ventral only.

69. *Lateral setae origins:* (0) none on intersegmental conjunctives; (1) some L_4 on one or more conjunctives.

70. *Anal lobe fringe:* (0) absent; (1) single layer, sparse; (2) single layer, complete; (3) multiple layer, complete.

71. *Dorsal setae on tergite IX or anal lobe:* (0) present; (1) absent.

72. *Male genital sac length relative to anal lobe:* (0) shorter; (1) subequal; (2) longer.

73. *Tergite III (IV–) anterior transverse spine band:* (0) present; (1) absent.

74. *Tergite III (IV–) posterior transverse spine band:* (0) present; (1) absent.

Adults

75. *Male antennal flagellomeres:* (0) thirteen; (1) eleven or fewer.

76. *Frontal tubercles:* (0) absent; (1) present.

77. *Palp:* (0) normal (about as long as head); (1) distinctly shortened.

78. *Eye:* (0) bare; (1) hairy.

79. *Antepronotum lobes dorsomedially:* (0) lobes fused; (1) lobes separated.

80. *Antepronotal lobe dorsal narrowing:* (0) absent; (1) clearly present.

81. *Scutum anterior extension:* (0) absent; (1) present.

82. *Scutal tubercle:* (0) present; (1) absent.

83. *Acrostichal setae:* (0) present; (1) absent.

84. *Fore tibial scale:* (0) subtriangular; (1) rounded to flat and scarcely developed.

85. *Fore tibial scale:* (0) with projecting spur or spine; (1) without projection.

86. *Fore tibial apex:* (0) with comb; (1) without comb.

87. *Mid and hind tibial combs:* (0) separated; (1) contiguous.

88. *Mid tibial combs:* (0) each with one spur; (1) one with spur; (2) both unspurred.

89. *Hind tibial combs*: (0) each with one spur; (1) one with spur; (2) both unspurred.

90. *Mid leg sensilla chaetica:* (0) present; (1) absent.

91. *Hind leg sensilla chaetica:* (0) present; (1) absent.

92. *Pulvillus relative length:* (0) subequal to claw; (1) half-length of claw; (2) absent.

93. *Wing cell membrane setae:* (0) present; (1) absent.

94. *FCu position:* (0) proximal, opposite or slightly distal to RM; (1) distinctly distal to RM.

95. *Anal lobe of wing:* (0) distinct; (1) weak to absent.

96. *RM direction relative to R_{4+5}:* (0) continuous in more or less straight line; (1) oblique.

97. *Squamal fringe:* (0) complete; (1) reduced; (2) absent.

98. *Abdominal tergite setosity:* (0) dense; (1) sparse.

99. *Anal tergite bands:* (0) meeting; (1) separate; (2) absent.

100. *Median anal tergite setae:* (0) present; (1) absent.

101. *Apical anal tergite setae:* (0) present; (1) absent.

102. *Anal point:* (0) present; (1) absent.

103. *Superior volsella base:* (0) more or less transverse, pad-like, setose; (1) not pad-like.

104. *Median volsella:* (0) present; (1) absent.

105. *Inferior volsella:* (0) elongate, setose; (1) reduced, with few setae.

106. *Transverse sternapodeme anterolateral corners:* (0) with projections; (1) rounded.

107. *Inferior volsella isolated, strong, (sub)apical seta(e):* (0) present; (1) absent.

108. *R_{2+3}:* (0) ending halfway between R_1 and R_{4+5}; (1) ending nearer R_{4+5}; (2) weak or absent.

109. *R_{4+5} and costa ending:* (0) above or distal to apex of M_{1+2}; (1) proximal to apex of M_{1+2}.

110. *Dorsomedial extension of eye:* (0) strong; (1) weak; (2) absent.

111. *Female genital dorsomesal lobe, oromesal "group shagreen":* (0) absent; (1) present.

112. *Floor beneath vagina formed by sternite VIII:* (0) absent; (1) present.

113. *Apodeme lobe microtrichia:* (0) absent; (1) present.

114. *Segment X setae:* (0) present; (1) absent.

115. *Labial macrotrichia:* (0) absent; (1) present.

116. *Male superior volsella:* (0) present; (1) vestigial or absent.

117. *Inferior volsella:* (0) present; (1) vestigial or absent.

118. *Gonostylus proximal section:* (0) without distinct swelling; (1) distinctly swollen.

119. *Joint between gonocoxite and gonostylus:* (0) rigid; (1) flexible.

Results

Systematics

Chironominae relationships have been assessed by Sæther (1977) using morphological data, proposing the tribes Tanytarsini, Pseudochironomini and Chironomini (Fig. 1A). Cranston et al. (2012) analysed molecular data from four genes using mixed-model Bayesian and maximum likelihood inference methods, and suggested Chironomini is paraphyletic because it includes Tanytarsini and Pseudochironomini (Fig. 1B). Also, *Shangomyia* Sæther *et* Wang, sister to all remaining Chironominae, could deserve a tribe or subfamily rank together with *Xiaomyia* Sæther *et* Wang. The cladistic analyses performed here resulted in a cladogram (Fig. 2) where Tanytarsini and Chironomini are sister tribes. *Shangomyia* Sæther *et* Wang grouped with *Nandeva* Wiedenbrug *et* Fittkau within *Stenochironomus* complex as found in Cranston (2003) from a previous version of the present data matrix. Since information about *Nandeva* Wiedenbrug *et* Fittkau larva is unavailable and morphology of pupae and adults of *Shangomyia* Sæther *et* Wang are uninformative and very divergent, the inclusion of these genera in the *Stenochironomus* complex is very doubtful.

The clade (*Sigmoitendipes* n. gen. (*Endotribelos* (*Claudiotendipes* n. gen. (*Beardius*, *Oukuriella*)))) is recognized by the combination of some homoplastic synapomorphies in the pupa (absence of scutal tubercle, presence of pedes spurii B and abdominal segment VIII posterolateral spur with one more or less dominant tooth) and adult (mid leg without sensilla chaetica).

As 16 out of 40 of the larval characters could not be extracted from the single larval specimen of

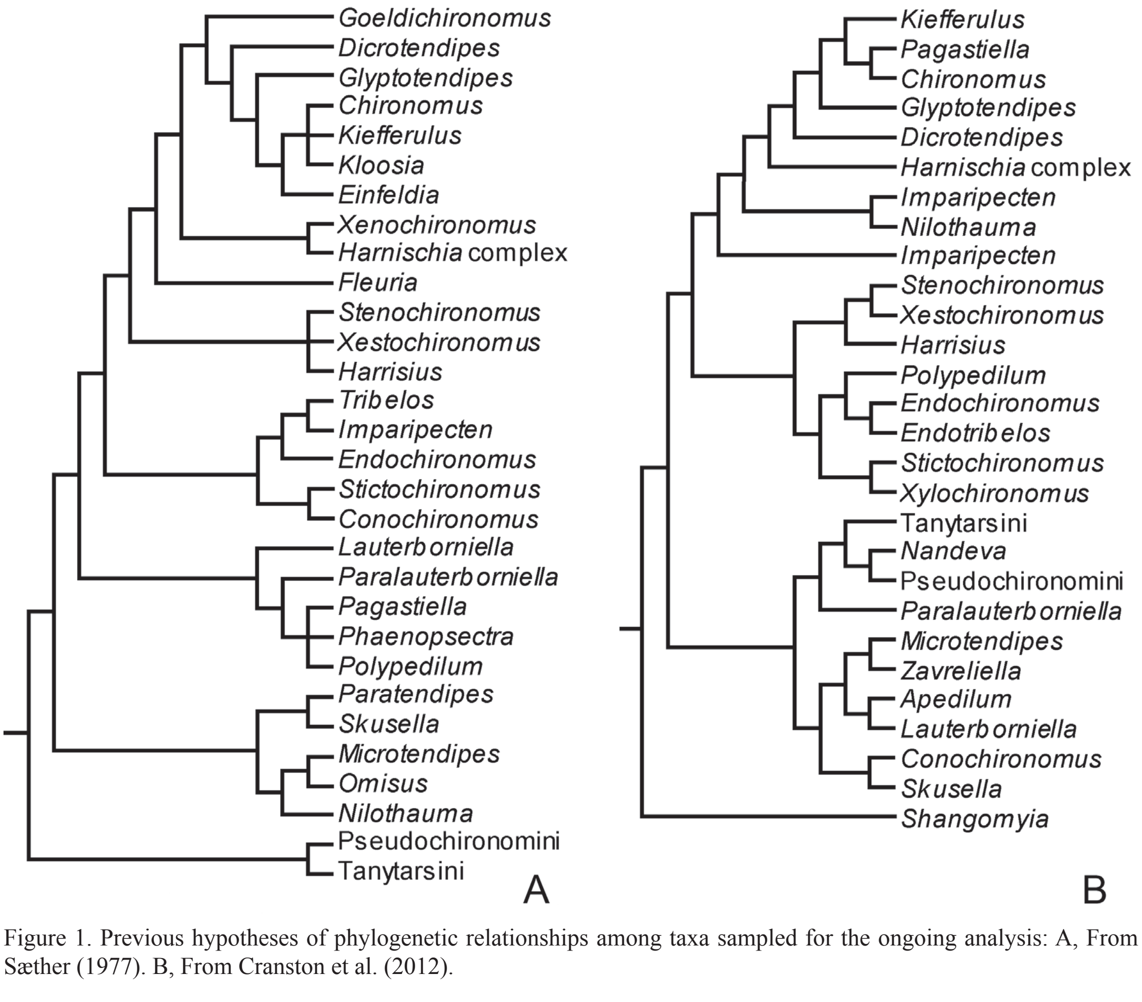

Figure 1. Previous hypotheses of phylogenetic relationships among taxa sampled for the ongoing analysis: A, From Sæther (1977). B, From Cranston et al. (2012).

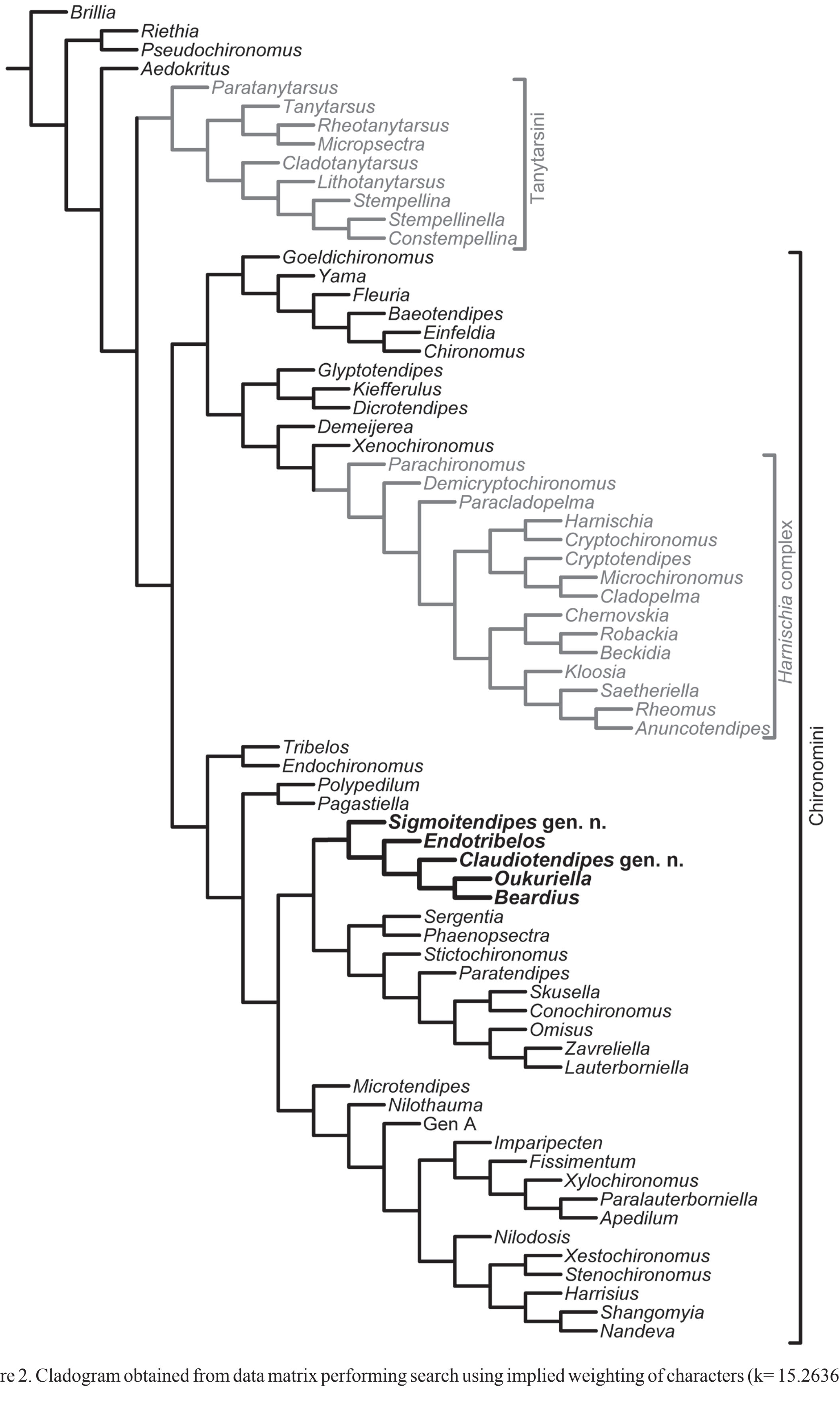

Figure 2. Cladogram obtained from data matrix performing search using implied weighting of characters (k= 15.263673).

Sigmoitendipes studied, this relationship hypothesis should be viewed as tentative and will likely improve as more larval material becomes available and more Chironomini genera are included in the data matrix.

Taxonomy

Claudiotendipes new genus

http://zoobank.org/4377891C-B29C-4CB5-8171-180517119AA4

Type species: *Claudiotendipes froehlichi* n. sp.

Other included species: *Claudiotendipes epleri* n. sp.

Etymology: Named in honor of Professor Dr. Claudio G. Froehlich for his many important contributions to aquatic entomology and for initiating, with all his kindness and knowledge, the formation of many Brazilian research groups in ecology and systematics of aquatic insects. The suffix *-tendipes,* is a common ending among Chironominae genera. For the purposes of nomenclature, the gender of the genus name is masculine.

Diagnostic characters: The males can be separated from all other Chironomini by the combination of an antenna with 13 segments, antennal ratio 0.3–1.1; squama bare; wings lacking markings; fore tibia with weakly developed, shallow scale without spur; mid- and hind tibiae with short, fused combs, with single spur; anal tergite bands separate; setae on tergite IX restricted to posterior margin; anal point long, parallel-sided to weakly spatulate; superior volsella digitiform without basomedial group of setae; median volsella reduced to single setae; and inferior volsella apically forked.

The pupa can be separated from all other Chironomini except *Paratendipes* by the combination of a thoracic horn with few branches; cephalic tubercles and frontal setae present; tergites II–VI with anterior bands of weak shagreen; large pedes spurii B on segment II; segment II with 2–3 non-taeniate L setae, segments III–IV each with 3 non-taeniate L setae; and anal lobe with fringe and dorsal seta. It can be separated from *Paratendipes* by the bare conjunctive III/IV.

The larva can be separated from all other Chironomini except *Omisus* and *Paratendipes* by the combination of a plumose S I; mentum with four pale median teeth, the inner pair of which are smaller and shorter; and antenna with 6 segments and alternate Lauterborn organs. It can be separated readily from *Omisus* and *Paratendipes* by the presence of 3 inner teeth on the mandible; the shape of the ventromental plates; and the simple pecten epipharyngis with about 12 teeth.

Generic description

Male. Small to medium sized species, with wing length 1.0–2.0 mm.

Coloration. Whitish to pale yellowish, wing translucent without dark markings.

Antenna. With 13 flagellomeres. AR 0.28–1.13.

Head. Eyes bare, with well-developed dorsomedial extension containing either 5 or 6 ommatidia in each lateral facet diagonal. Frontal tubercle absent. Temporal setae 9–13, uniserial, composed of 3–5 strong inner verticals, 2–4 weak outer verticals and 2–4 weak postorbitals. Clypeus with 8–14 strong setae. Palp with 5 segments, not shortened; third palpomere with 2–6 sensilla subapically, sometimes grouped in pit, longest 11–20 µm long.

Thorax. Antepronotal lobes separated medially; each lobe narrowing dorsally, with 0–1 lateral seta. Scutum overreaching antepronotum, tubercle absent. Acrostichals 6–13, all decumbent, starting close to antepronotum. Dorsocentrals 7–15, uniserial; prealars 2–3; supraalars absent. Scutellum with 5–9 setae, uni- to partly biserial.

Wing. VR 1.16–1.26. Wing membrane bare, with fine punctation. Anal lobe absent to weakly developed. Costa not extended, reaching wing tip; R_{2+3} ending close to apex of R_1; FCu distal to RM. Brachiolum, R, R_1 and R_{4+5} with setae, remaining veins bare. Squama bare.

Legs. Fore tibia with weakly developed, shallow scale without spur. Mid and hind tibiae with short, fused combs, with single spur per tibia. Pseudospurs and sensilla chaetica absent. Pulvilli vestigial. LR_1 1.33–1.46.

Abdomen. Tergites and sternites with sparse, irregular setation. Segment VIII subtriangular, tapering anteriorly.

Hypopygium. Anal tergite bands separate, ending slightly above or reaching base of anal point. Tergite IX in dorsal view (Figs 3F, 6F) with posterior margin subtriangular, with few marginal setae to each side of anal point only. Anal point parallel-sided to weakly spatulate, one-third to one-fourth the length of gonostylus, with microtrichia at base only. Laterosternite IX without or at most with three setae. Transverse sternapodeme well developed, nearly straight, with slightly higher lateral corners. Phallapodeme narrow. Superior volsella without setose base, curved to nearly straight, tapering towards apex, without microtrichia, with two dorsal-lateral and two distal-medial setae. Median volsella possibly represented by one to two setae on single, small protuberance. Inferior volsella apically foot-shaped, with microtrichia, with 5–7 simple setae on main branch and 3–5 simple or apically split setae on side branch. Gonostylus well developed, with simple setae along inner margin, and with or without apical field of elongate trichia along inner margin. HR 0.85–1.13.

Female. Unknown.

Pupa and larva. See description under *C. froehlichi*.

Remarks

Larvae of *Claudiotendipes* n. gen. have been recorded repeatedly in studies of Brazilian lotic systems as morphotypes unassigned to genus, provisionally named "aff. *Omisus*" (Sanseverino and Nessimian 1998; Suriano and Fonseca-Gessner 2004; Henriques-Oliveira et al. 2003a, 2003b), "cf. *Omisus*" (Henriques-Oliveira et al. 1998), "cf. *Paratendipes*" (Sanseverino and Nessimian 2008) or "prox. *Paratendipes*" (Trivinho-Strixino 2011). However, such overall resemblance to *Omisus* and *Paratendipes* does not reflect close phylogenetic relationship (Fig. 2).

Key to males in *Claudiotendipes* n. gen.

1. Anal point weakly spatulate, wing length about 1.7 mm, AR = 1.0. Bahia, Rio de Janeiro, São Paulo, and Santa Catarina States, Brazil. ………..................………… *C. froehlichi* n. sp.

– Anal point parallel-sided, wing length about 1.0 mm, AR = 0.28. Guanacaste Province, Costa Rica ………….................…………. *C. epleri* n. sp.

Claudiotendipes froehlichi new species

http://zoobank.org/087B77F5-597F-46B0-A5B9-0A96804EBC71

Type material: *Holotype:* BRAZIL, Bahia, Camacan, Serra Bonita, córrego 1, 15°23'28"S, 39°33'56"W, 820 m a.s.l., 31.vii.2008, Malaise trap, AR Calor, L Lecci, LC Pinho, RA Moretto, 1 male with larval and pupal exuviae (reared) (MZUSP). *Paratypes:* BRAZIL, Bahia, Camacan, Serra Bonita, córrego 3, 15°23'02"S, 39°34'10"W, 805 m a.s.l., 01–04.viii.2008, Malaise trap, AR Calor, L Lecci, LC Pinho, RA Moretto, 2 males (MZUSP); as previous except córrego 2, 15°23'10"S, 39°34'03"W, 819 m a.s.l., 01.viii.2008, hand net, 1 male (MZUFBA); as previous except Barreiras, Rio de Janeiro, Cachoeira Acaba Vida, 11°53'40"S, 45°36'06"W, 722 m a.s.l., 14.x.2008, light trap, AR Calor, RL Mariano, S Mateus, 1 male (MZUFBA). Rio de Janeiro: Teresópolis, P4A-P514, 22°26'51"S, 43°00'48"W, 1453 m a.s.l., E Shimabukuro, 1 male (MZUSP). São Paulo: Ubatuba, Pico do Corcovado, P1M-C50, 23°27'52"S, 45°11'55"W, 222 m a.s.l., E Shimabukuro, 1 male (MZUSP); Estação Ecológica Boracéia, Salesópolis, Rio Claro, poço Verde, 10.x.2004, light trap, CG Froehlich et al., 3 males (ZMBN); as previous except 18.ix.2002, AS Melo, CG Froehlich, RL Mariano, A. Prather, R Blatnik, 1 male (ZMBN); as previous except córrego Venerando, 12.xii.2001, CG Froehlich et al., 1 male (MZUSP); as previous except Parque Estadual Campos do Jordão, Campos do Jordão, córrego Galharada, 22°41'40"S 45°27'36"W, 757 m a.s.l., 03.x–03.xi.2005, Malaise trap, MR Spies, 1 male (INPA); as previous except 20.viii.2006, light trap, LS Lecci, 1 male (INPA). Santa Catarina: Parque Estadual da Serra Furada, Grão-Pará, 28°11'28"S 49°23'30"W, 508 m a.s.l., #01, 13.x.2012, light trap, LC Pinho, AC Ganzer, LS Gomes, AG Parise, 1 male (UFSC); Urubici, Rio Canoas, 28°01'41"S 49°22'36"W, 1014 m a.s.l., #29, 08.i.2013, light trap, LC Pinho, MC Novaes, MF Haddad, 3 males (UFSC).

Etymology: Like the genus name, the specific epithet honors Dr. Claudio G. Froehlich.

Diagnostic characters: See key.

Description

Male (n = 8–10, except when otherwise stated). Total length 2.33–3.05, 2.79 (6) mm. Wing length 1.39–1.99, 1.69 mm. Total length / wing length 1.63–2.05, 1.79 (6). Wing length / length of profemur 1.72–2.02, 1.88.

Coloration. All whitish, wings hyaline without markings.

Antenna. AR 0.91–1.13, 1.04. Ultimate flagellomere 407–514, 461 µm long.

Head (Fig. 3A). Temporal setae 9–13, 12; including 3–4, 4 inner verticals, 2–5, 4 outer verticals, and 3–4, 4 postorbitals. Clypeus with 9–14, 11 setae. Frontal tubercle indicated as paler area to small knob. Tentorium, stipes and cibarial pump as in Figure 3B. Tentorium 115–152, 136 µm long; 29–37, 33 µm wide. Stipes 106–118, 113 µm long; 27(1) µm wide. Palp segment lengths (in µm): 24–30, 27; 26–38, 35; 118–158, 140; 104–148, 128; 159–230, 197. Third palpomere with 2–4, 3 sensilla, longest 13–20, 15 µm long.

Thorax (Fig. 3C). Antepronotum with 0–1, 0 seta. Dorsocentrals 9–15, 11; acrostichals 6–13, 10, all decumbent, starting close to antepronotum; prealars 2–3, 2. Scutellum with 5–9, 6 setae, uniserial.

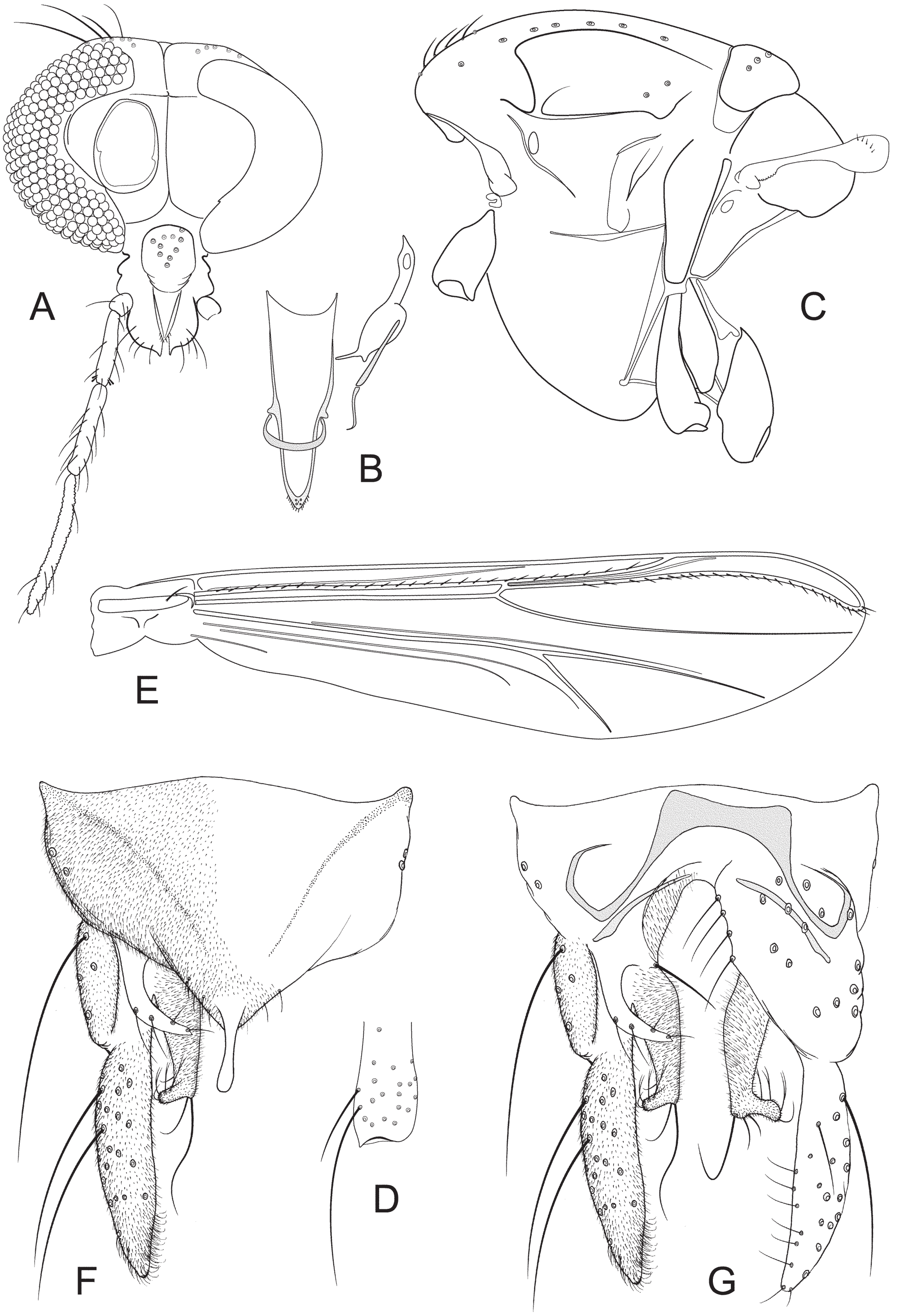

Figure 3. *Claudiotendipes froehlichi* n. sp., male. A, Head. B, Tentorium, stipes and cibarial pump. C, Thorax. D, Apex of fore tibia. E, Wing. F, Hypopygium, dorsal view. G, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right.

Wing (Fig. 3E). VR 1.16–1.24, 1.21. Brachiolum with 1–2, 1 setae; R 15–24, 19; R_1 with 6–11, 9; R_{4+5} with 23–36, 31 setae; other veins and cells bare. Squama bare.

Legs (Fig. 3D). Scale of fore tibia 6–9, 8 µm long; spur of mid tibia 41–52, 48 µm long; spur of hind tibia 47–66, 54 µm long. Comb on mid tibia 12–20, 18 µm long; comb on hind tibia 16–27, 22 µm long. Width at apex of fore tibia 49–59, 54 µm; of mid tibia 48–57, 52 µm; of hind tibia 52–64, 58 µm. Lengths and proportions of legs as in Table 1.

Hypopygium (Figs 3F, G). Tergite IX covered with microtrichia, with 3–6, 4 setae to each side of base of anal point; anal tergite bands ending slightly above base of anal point. Laterosternite IX with 0–3, 2 setae. Anal point weakly spatulate, 23–39, 28 µm long, 10–14, 13 µm wide at base, 4–6, 5 µm wide medially, 7–10, 8 µm wide near apex, Transverse sternapodeme 41–61, 53 (7) µm long, nearly straight. Phallapodeme 54–70, 67 (7) µm long. Gonocoxite 91–122, 106 (7) µm long. Superior volsella 38–48, 40 µm long, 11–15, 13 µm wide at base, 3–8, 4 wide at apex, with 2 dorsal and 2 lateral setae. Median volsella with 1–2, 1 setae. Inferior volsella 59–82, 64 µm long, 15–25, 19 µm wide at base, 6–9, 7 µm wide at apex, with 4–5, 5 simple setae, 1–2, 1 stout apical seta on main branch and 3–5, 4 apically split setae on side-branch. Gonostylus 96–120, 105 µm long. HR 0.85–1.13, 1.02 (7). HV 2.91–2.98 (3).

Female. Unknown.

Pupa (exuviae, n = 1). Total length 3.19 mm.

Coloration. Exuviae pale with brownish caudolateral spur.

Cephalothorax. Frontal apotome (Fig. 4A) subtriangular, 340 µm long, 296 µm wide. Frontal setae 144 µm long. Thorax (Fig. 4B) 695 µm long. Thoracic horn (Fig. 4C) with 4 branches. Dorsal antepronotal seta hair-like, about 53 µm long; lateral antepronotal hair-like, not measurable. Precorneals close together, hair-like, both about 30 µm long. Dc_1 31 µm long, Dc_2 35 µm long, Dc_3 33 µm long, Dc_4 21 µm long; Dc_1 1 µm in front of Dc_2, Dc_2 117 µm in front of Dc_3, Dc_3 29 µm in front of Dc_4. Wing sheath 1.07 mm long, without nose or pearl row.

Abdomen (Fig. 4D). Tergites I and IX bare; tergites II–IV with median field of fine shagreen and anterior band of slightly stronger shagreen, tergite IV with median field barely separated from anterior band; tergite V with continuous median, fine shagreen; tergite VI with widely separated anterior and posterior transverse fields of fine shagreen; tergites VII–VIII with anterolateral patches of fine shagreen. Sternites bare. Hook row on tergite II with 26 caudal hooklets in single, 148 µm wide row. Conjunctive IV/V with about 120 spinules in 4–5 rows. Pedes spurii B well developed posteriorly on segment II; pedes spurii A lacking. Segment VIII caudolateral spur (Fig. 4E) 160 µm long, with 3 strong apical teeth.

Abdominal setation. Segment I without L setae, segment II with 2–3 non-taeniate L setae, segments III–IV each with 3 non-taeniate L setae, segments V–VIII each with 4 taeniate LS setae. Dorsal and oral (‘O’) setae apparently present on segments II–VII, segments I and VIII apparently each with single pair of dorsal setae.

Anal lobe. Fringe with 18 taeniae. Male genital sac

Table 1. Lengths (in µm) and proportions of legs of *Claudiotendipes froehlichi* n. sp., male (n = 9).

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**	**ta_5**	**LR**	**BV**	**SV**	**BR**
p_1	746–1032, 912	663–847, 793	958–1160, 1104	451–566, 472	359–456, 389	270–350, 309	74–92, 85	1.33–1.44, 1.39	2.05–2.29, 2.16	1.47–1.61, 1.54	2.4–3.3, 2.7
p_2	847–1050, 963	682–903, 793	438–553, 505	175–249, 214	129–175, 157	83–111, 96	37–55, 45	0.57–0.69, 0.64	4.16–4.81, 4.43	3.42–3.70, 3.48	2.9–4.7, 3.6
p_3	903–1133, 1021	737–958, 843	626–774, 717	332–424, 363	258–295, 273	140–175, 158	46–64, 57	0.81–0.88, 0.85	2.76–3.22, 3.03	2.51–2.70, 2.60	4.4–7.0, 5.3

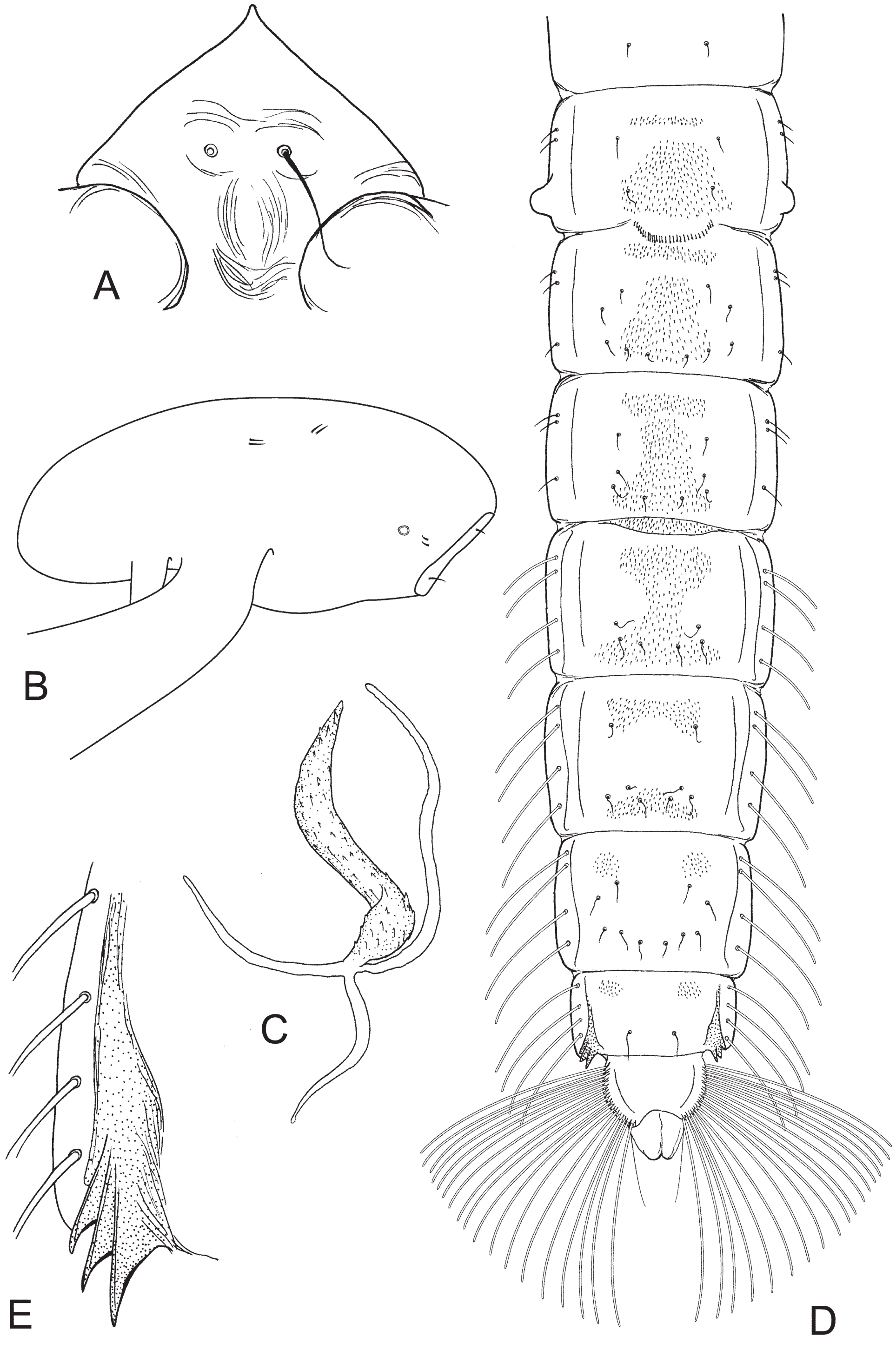

Figure 4. *Claudiotendipes froehlichi* n. sp., pupa. A, Frontal apotome. B, Thorax. C, Thoracic horn. D, Abdomen. E, Caudolateral spur.

overreaches anal lobe by 76 µm.

Larva (cast skin, n = 1). Total length not measurable. Head capsule 340 µm long. Postmentum 115 µm long.

Coloration. Head capsule yellowish brown with postoccipital margin, teeth of mandible and lateral teeth of mentum darker brown.

Head. Antenna as in Figure 5B. Antenna with 6 segments; AR 1.06; length of antennal segments (in µm): 113, 35, 37, 8, 17, 10; blade 73 µm long;

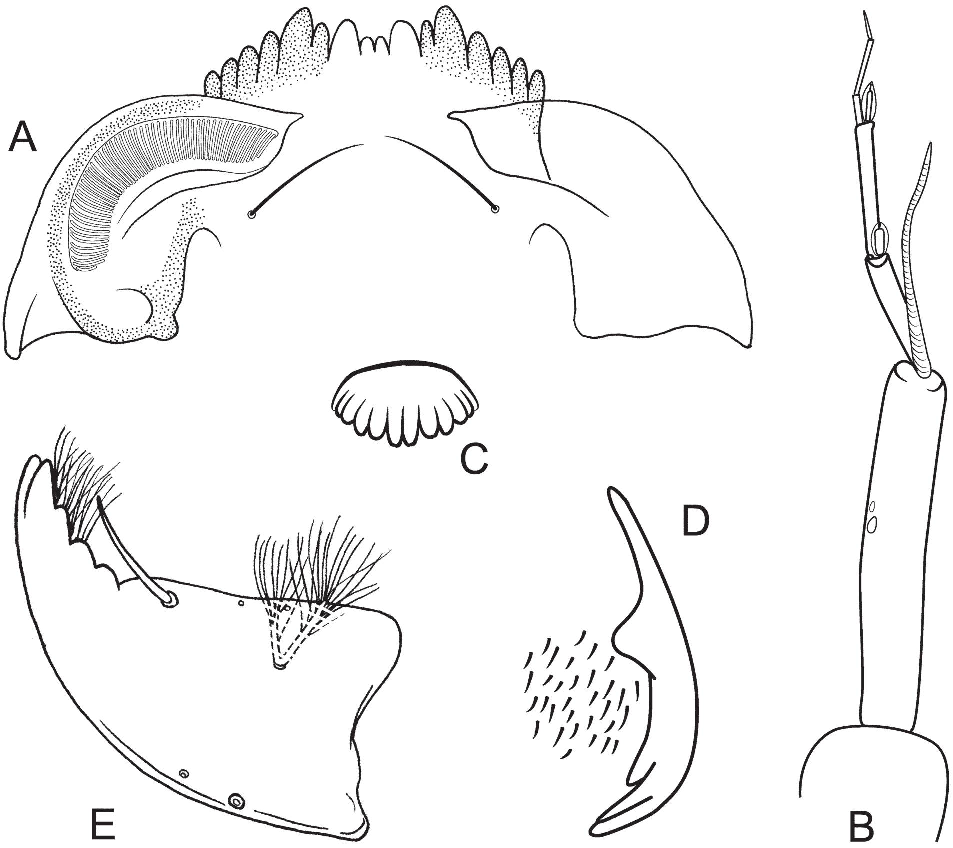

Figure 5. *Claudiotendipes froehlichi* n. sp., larva. A, Mentum and ventromental plates. B, Antenna. C, Pecten epipharyngis. D, Premandible. E, Mandible.

Lauterborn organs about 8 µm long; ring organ 58 µm from base in first segment. Pecten epipharyngis (Fig. 5C) consisting of single plate with 12 teeth; 17 µm wide. Premandible (Fig. 5D) 81 µm long. Mandible (Fig. 5E) 94 µm long with pale dorsal tooth and 3 inner teeth. Seta subdentalis well developed; seta interna with 2 main branches. Mentum (Fig. 5A) 83 µm wide, with 8 pairs of mental teeth; median pair short, pale yellowish brown; first lateral about twice as long, pale yellowish brown; second lateral about equally long, darker brown; third lateral about three times as long as median, darker brown; 4–7 lateral pairs gradually shorter, darker brown. Ventromental plate (Fig. 5A) curved, 66 µm wide, distance between plates 35 µm.

Abdomen. Procercus with 12 anal setae. Posterior parapods each with 13 claws.

Distribution and ecology

This species is apparently widely distributed along the Atlantic rainforest in Brazil, ranging from 220 m to over 1450 m altitude between latitudes 11° and 28°S. The larva was collected in leaf debris in a first order stream which flows through a forested area in Serra Bonita, Bahia State. Similar conditions occur at other localities where adults were collected in light or Malaise traps.

***Claudiotendipes epleri* new species**

http://zoobank.org/F16C55CF-A514-4379-B19D-50640527FE08

Type material: *Holotype:* COSTA RICA, Guanacaste, Guanacaste Conservation Area, Cerro Cacao, Rio San Josecito, 1000 m a.s.l., 4–5.v.1993, Malaise trap, T Andersen, 1 male (ZMBN).

Etymology: Named after Dr. John H. Epler for his many contributions to the taxonomy and ecology of chironomids from the New World.

Diagnostic characters: See key.

Description

Male (n = 1). Total length 1.73 mm. Wing length 1.02 mm. Total length / wing length 1.69. Wing length / length of profemur 1.91.

Coloration. Antenna and legs pale yellowish; head, thorax and abdomen whitish; wings hyaline without markings.

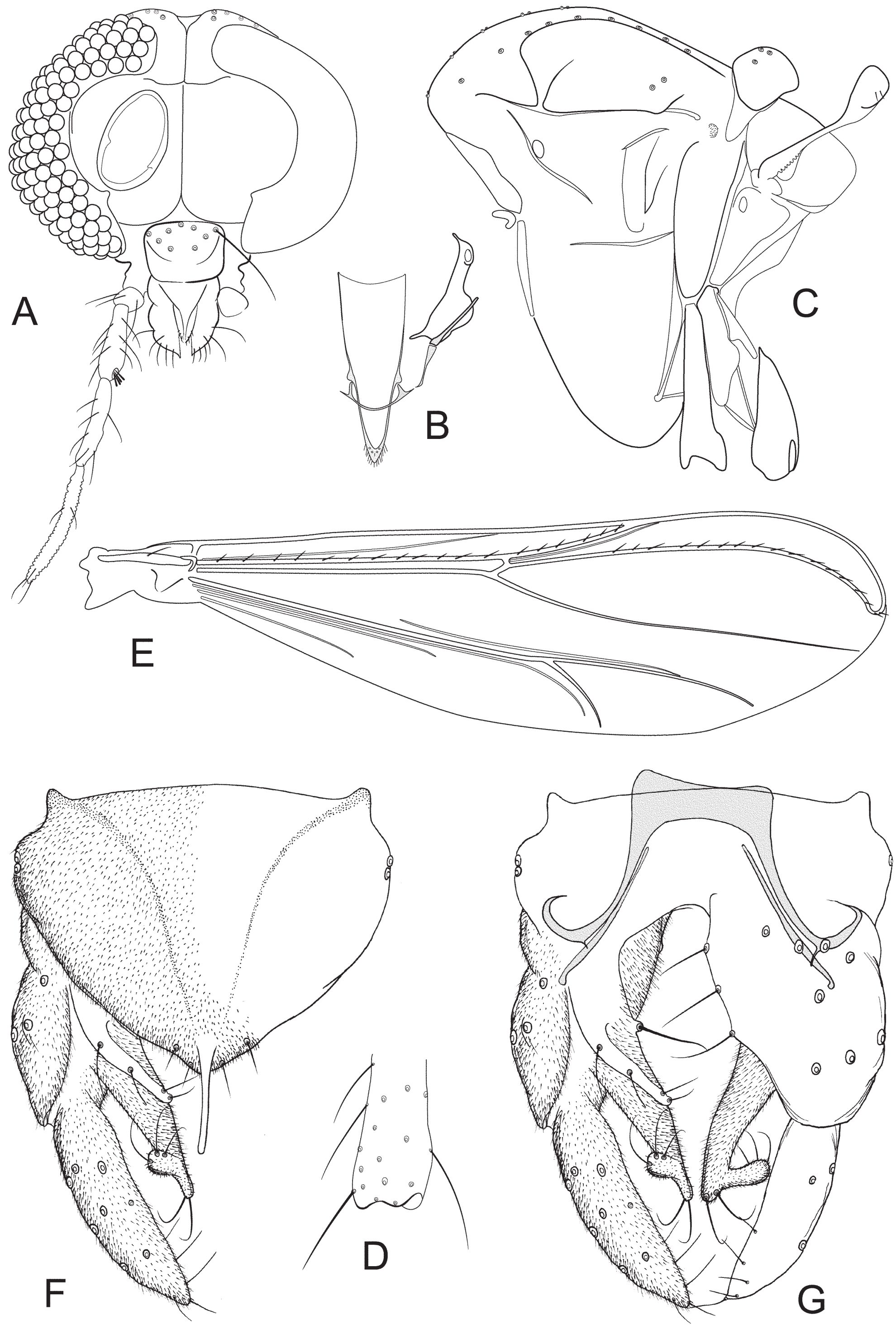

Figure 6. *Claudiotendipes epleri* n. sp., male. A, Head. B, Tentorium, stipes and cibarial pump. C, Thorax. D, Apex of fore tibia. E, Wing. F, Hypopygium, dorsal view. G, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right.

Antenna. AR 0.28. Ultimate flagellomere 96 µm long.

Head (Fig. 6A). Temporal setae 9 including 5 inner verticals, 2 outer verticals, and 2 postorbitals. Clypeus with 8 setae. Frontal tubercle barely indicated as pale area. Tentorium, stipes and cibarial pump as in Figure 6B. Tentorium 73 µm long, 18 µm wide. Stipes 61 µm long. Palp segment lengths (in µm): 16, 23, 64, 70, 107. Third palpomere with 6 sensilla, 4 of which in pit, longest 11 µm long.

Thorax (Fig. 6C). Antepronotum bare. Dorsocentrals 7; acrostichals 7, all decumbent, starting close to antepronotum; prealars 2. Scutellum with 6 setae, partly biserial.

Wing (Fig. 6E). VR 1.26. Brachiolum with 1 seta, R with 13, R_1 with 5, R_{4+5} with 19 setae, other veins and cells bare. Squama bare.

Legs (Fig. 6D). Scale of fore tibia 11 µm long; spur of mid tibia 32 µm long, of hind tibia 35 µm long. Comb on mid tibia 10 µm long, of hind tibia 12 µm long. Width at apex of fore tibia 30 µm, of mid tibia 34 µm, of hind tibia 36 µm. Lengths and proportions of legs as in Table 2.

Hypopygium (Figs 6F, G). Tergite IX covered with microtrichia, with 2 setae to each side of base of anal point; anal tergite bands reaching base of anal point. Laterosternite IX with 1 seta. Anal point nearly parallel-sided, 19 µm long, 3 µm wide. Transverse sternapodeme 29 µm long, nearly straight. Phallapodeme 38 µm long. Gonocoxite 59 µm long. Superior volsella 27 µm long, 6 µm wide at base, 4 µm wide at apex, with 2 dorsal and 2 lateral setae. Median volsella with 1 seta. Inferior volsella 39 µm long, 13 µm wide at base, 3 µm wide at apex, with 2 simple setae and 1 stout apical seta on main branch and 4 simple setae on side-branch. Gonostylus 54 µm long. HR 1.08. HV 3.21.

Female and immatures. Unknown.

Distribution and ecology

This species is only known from Guanacaste Conservation Area in northwestern Costa Rica where it was collected in a Malaise trap at a small, shallow river with stony bottom at 1000 m altitude.

Sigmoitendipes new genus

http://zoobank.org/E0F0B0ED-CC75-4162-9DDC-915166283F7B

Type species: *Sigmoitendipes susanae* n. sp.

Other included species: *Sigmoitendipes fittkaui* n. sp., *S. oliveirai* n. sp., *S. reissi* n. sp. and *S. spiesi* n. sp.

Etymology: The specific epithet refers to the shape of the superior volsella resembling the lower-case Greek letter sigma. The suffix *-tendipes* is a common ending among Chironominae genera. For the purposes of nomenclature, the gender of the genus name is masculine.

Diagnostic characters

The males can be separated from all other Chironomini by the combination of an antenna with 13 segments, antennal ratio about 2.0; squama with 5–11 setae; wings lacking markings; fore tibia with well-developed, bearded scale, comb of mid tibia with one spur, comb of hind tibia with two spurs; anal tergite bands weak, adjacent to anterior tergite margin, in some species with short median longitudinal extension; setae on tergite IX restricted to posterior margin and/or base of anal point; anal point strong, spatulate; superior volsella complex, composed of a median or ventral, rounded or subrectangular part densely covered with microtrichia and carrying a few setae in some species, and of a dorsal to apical part with several strong setae and in some species with sparse microtrichia, dorsal part with apex more or less hooked; inferior volsella near mid-length with bluntly triangular dorsal lobe.

The pupa shares with those of some other Chironomini the combination of long, robust frontal setae; well-developed, conical cephalic tubercles; strongly plumose thoracic horns; anterior, transverse bands of shagreen on tergites II–VI; robust, longitudinal anal combs; and no dorsal setae on the anal lobes. It can be separated by having only one median antepronotal seta, and 5 LS setae on segment VIII.

The larva has a six-segmented antenna with a single well-developed Lauterborn organ on the sec-

Table 2. Lengths (in µm) and proportions of legs of *Claudiotendipes epleri* n. sp., male (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
p_1	534	378	553	272	190	138	40	1.46	2.32	1.65	2.8
p_2	571	433	253	101	83	55	28	0.58	4.71	3.96	3.8
p_3	605	449	–	–	–	–	–	–	–	–	–

ond segment; the mandible has one dorsal, one apical and four inner teeth, with the seta subdentalis long, slender and straight; the mentum with 8 pairs of equally sclerotized teeth, the first lateral teeth larger than the median pair, the second lateral teeth smaller than both their immediate neighbours, and the further lateral teeth decreasing progressively to a minute seventh tooth; and the ventromental plates slightly wider than the mentum, and separated by the width of 4–5 mental teeth.

Generic description

Male. Medium sized species, wing length 1.9–2.2 mm.

Coloration. Body brown, wing translucent without dark markings.

Antenna. With 13 flagellomeres. AR 1.93–2.27.

Head. Eyes bare, with well-developed dorsomedial extension, with 6–7 ommatidia in first transverse row. Frontal tubercle indicated as paler area to small knob. Temporal setae 10–30, partly biserial, composed of 2–4 inner verticals, 5–15 outer verticals and 3–13 postorbitals. Clypeus with 10–25 strong setae. Palp with 5 segments, not shortened; third palpomere with 3–8 sensilla subapically, longest 22–27 µm long.

Thorax. Antepronotal lobes narrowly separated medially, each lobe narrowing dorsally. Scutum not overreaching antepronotum, tubercle absent or weak. Acrostichals 5–10, erect, starting some distance from antepronotum. Dorsocentrals 5–11, uniserial; prealars 2–5; supraalar absent. Scutellum with 7–13 setae, uniserial to partly biserial.

Wing. VR 1.07–1.13. Wing membrane bare, with fine punctation. Anal lobe well developed. Costa not extended, ending opposite to M_{1+2}; R_{2+3} ending in proximal 1/3 between apices of R_1 and R_{4+5}; FCu slightly distal to RM. Brachiolum, R, R_1 and R_{4+5} with setae, remaining veins bare. Squama with 5–11 setae.

Legs. Apex of fore tibia with rounded, unspurred scale, with long beard. Mid- and hind tibiae with fused combs, mid tibia with single spur, hind tibia with two spurs. With one pair of pseudospurs on ta_1–ta_3 on mid- and hind legs; sensilla chaetica apparently absent. Pulvilli simple, lobe-shaped, shorter than claw. LR_1 1.51–1.71.

Abdomen. Tergites and sternites moderately to densely setose with irregular setae. Segment VIII tapering slightly anteriorly.

Hypopygium. Anal tergite bands weak, following anterior margin of tergite. Tergite IX with posterior margin subtriangular, with few marginal setae to each side of anal point only. Anal point long, spatulate, with microtrichia at base only. Laterosternite IX with few setae. Transverse sternapodeme narrow, straight or sinuous, without oral projection. Phallapodeme normal. Superior volsella without setose base, complex; with median or ventral, rounded or subrectangular lobe densely covered with microtrichia, with or without a few apical setae; with dorsal to apical lobe, without or with sparse microtrichia, with several strong setae, apically hooked with apex projecting laterad. Median volsella absent. Inferior volsella long, with narrow base; medially with bluntly triangular, dorsal lobe; apical one-half widened, subrectangular to bluntly subtriangular, with several strong, orally directed setae, distally with one or two longer setae projecting caudally. Gonostylus long, rather stout, widest in apical one-half, subapically with row of short, strong setae along inner margin. HR 0.73–0.95.

Female. Unknown.

Pupa and larva. See description under *S. susanae*.

Remarks

Larvae of *Sigmoitendipes* n. gen. may represent the morphotype "*Endotribelos* sp. 2" (Trivinho-Strixino 2011), with which it appears to share the six-segmented antenna with well-developed Lauterborn organs on the second segment, the mentum with 16 dark teeth of alternating sizes, and the wide ventromental plates. However, the information available on larvae in *Sigmoitendipes* n. gen. are insufficient for reliable identification with that morphotype. Moreover, the pupal anal comb with about 30 minute teeth that is visible in a pre-pupa of "*Endotribelos* sp. 2" (S. Trivinho-Strixino, pers. comm.) appears to suggest distinct genera.

Key to males in *Sigmoitendipes* n. gen.

1. Dorsal lobe of superior volsella subtriangular, broad, with at most the very tip slightly curved. Mato Grosso State. …………….. *S. oliveirai* n. sp.

– Dorsal lobe of superior volsella narrow, strongly curved. …………….........………………….. 2

2. Superior volsella with median, rounded lobe with microtrichia, not reaching apex of volsella. 3

– Superior volsella with ventral, subrectangular lobe with microtrichia, reaching apex of volsella. 4

3. Apical, hooked part of superior volsella with sparse microtrichia and about 10 setae; hook moderately strong. São Paulo, Mato Grosso, Amazonas

and Pará States..…..................... *S. susanae* n. sp.

– Apical, hooked part of superior volsella without microtrichia, with 4 setae; hook very strong. São Paulo State. .... *S. reissi* n. sp.

4. Ventral, subrectangular lobe of superior volsella narrow, without seta. Mato Grosso and Pará States. .... *S. fittkaui* n. sp.

– Ventral, subrectangular lobe of superior volsella comparatively wide, with 2 apical setae. Mato Grosso State. .... *S. spiesi* n. sp.

Sigmoitendipes susanae **new species**

http://zoobank.org/5F79CF91-9117-4B94-8804-1D4D7DE88CAE

Type material: *Holotype:* BRAZIL, São Paulo, Ribeirão Preto, Lago Monte Alegre, 21°11’S 47°43’W, 9.v.2000, 500 m a.s.l., HF Mendes, 1 male with larval and pupal exuviae (reared) (MZUSP). *Paratypes:* BRAZIL, São Paulo, Parque Estadual da Serra do Mar, São Luís do Paraitinga, Núcleo Santa Virgínia, 14.iv.2009, light trap, R Mariano, LS Lecci, G Schulz, 1 male (UFSC). Amazonas: Coari (BOGPM), Urucu, igarapé Martha, 04°51’50”S 65°04’45”W, 05–06.xi.2007, Pensylvannia trap, SRM Couceiro, 9 males (MZUSP, ZMBN); as previous except: 16–21.xi.2007, 1 male (UFSC). Mato Grosso: Nova Xavantina area, igarapé Garapuat, expedition camp I, 24–27 viii.1965, Brundin net, EJ Fittkau, 1 male (ZSM - A565-4). Pará: near border to Suriname, Parque Indígena Tumucumaque, igarapé Okueima, 18.iv.1962, at light, EJ Fittkau, 1 male (ZSM - A371-1); as previous except: Parque Indígena Tumucumaque, “Kumadueni”, onça site, 19.iv.1962, 1 male (ZSM - A377); as previous except: Rio Paru, Malloca Apicó, 14.iv.1962, 1 male (ZSM - A366-1); as previous except: between Furo Maripanema and Rio Curuçá near the Atlantic Ocean, igarapé 7 km from Curuçá, 13.viii.1953, H Sioli, 1 male (ZSM).

Etymology: Named in honor of Professor Dr. Susana Trivinho-Strixino for her numerous contributions to the knowledge of Brazilian chironomids.

Diagnostic characters: See key.

Description

Male (n = 7–9, except when otherwise stated). Total length 4.44–5.15, 4.89 mm. Wing length 1.87–2.12, 2.00 mm. Total length / wing length 2.34–2.51, 2.44. Wing length / length of profemur 1.40–1.48, 1.44.

Coloration. Thorax brown, head and abdomen light brown, legs pale brown. Wing translucent.

Antenna. AR 2.13–2.27, 2.20. Ultimate flagellomere 882–972, 927 µm long.

Head (Fig. 7A). Temporal setae 20–29, 22; including 2–4, 3 inner verticals, 8–14, 10 outer verticals, partly biserial, and 8–13, 10 postorbitals. Clypeus with 14–25, 21 setae. Frontal tubercle indicated as paler area to small knob. Tentorium, stipes and cibarial pump as in Figure 7B. Tentorium 144–160, 152 µm long; 39–49, 44 µm wide. Stipes 129–160, 142 µm long; 6–10, 8 µm wide. Palp segment lengths (in µm): 37–41, 40; 45–57, 53; 82–90, 87; 92–113, 106; 123–148, 136. Third palpomere with 3–5, 4 sensilla, longest 23–27, 25 µm long.

Thorax (Fig. 7C). Antepronotum without setae. Dorsocentrals 5–7, 6; acrostichals apparently 6–10, 8, erect, starting some distance from antepronotum; prealars 3. Scutellar setae 10–13, 11, partly biserial.

Wing (Fig. 8A). VR 1.09–1.13, 1.10. Brachiolum with 2 setae; R 26–34, 29; R_1 with 21–26, 25; R_{4+5} with 30–37, 34 setae, other veins and cells bare. Squama with 6–11, 9 setae.

Legs (Figs 7D–F). Scale of fore tibia 47–55, 52 µm long; spur of mid tibia 57–62, 59 µm long including 29–36, 32 µm long comb; spurs of hind tibia 46–52, 50 µm and 40–46, 42 µm long including 29–33, 30 µm long comb. Width at apex of fore tibia 59–65, 63 µm; of mid tibia 61–68, 64 µm; of hind tibia 66–75, 70 µm. Lengths and proportions of legs as in Table 3.

Hypopygium (Figs 8B, C). Tergite IX covered with microtrichia; laterosternite IX with 2–5, 3 setae. Anal point 29–33, 31 µm long, 5–7, 6 µm wide at base, 11–14, 13 µm wide subapically, with 3–8, 6 setae to each side of the base, of which about half on the ventral side. Transverse sternapodeme 46–55, 50 µm long, concave. Phallapodeme 99–108, 103 µm long. Gonocoxite 164–178, 170 µm long. Superior volsella (Fig. 8D) boot-shaped, 94–108, 101 µm long, medially with partly double, rounded, setose lobe, apical part sparsely setose, with 10–14, 12 setae, apex recurved. Inferior volsella 131–149, 138 µm long; 23–26, 24 µm wide at base; 11–14, 12 µm wide at its narrowest; with 19–23, 21 µm long dorsal, bluntly triangular projection, starting 69–77, 73 µm from base; apically subrectangular, 18–21, 20 µm wide; with 14–19, 16 simple setae, longest apical seta 76–87, 80 µm long. Gonostylus 201–230, 219 µm long. HR 0.73–0.85, 0.78. HV 2.16–2.46, 2.28.

Female. Unknown.

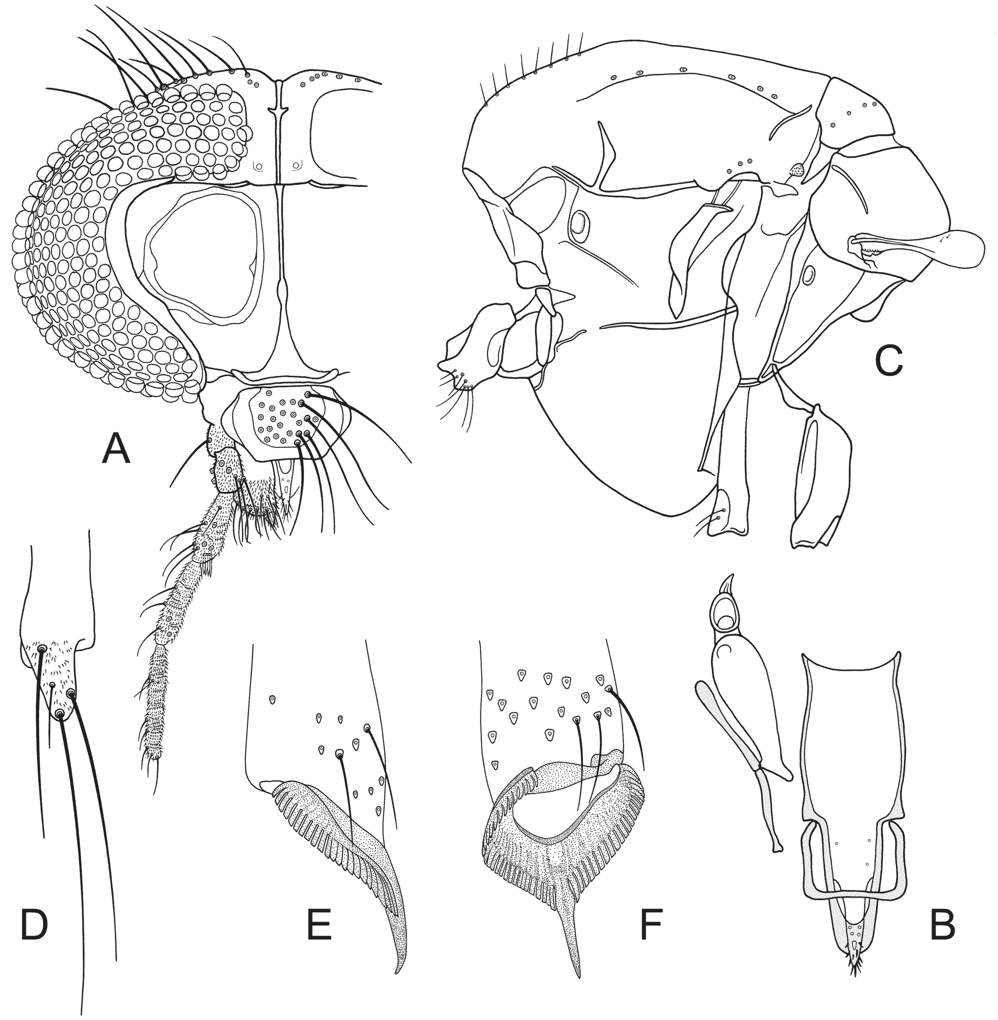

Figure 7. *Sigmoitendipes susanae* n. sp., male. A, Head. B, Tentorium, stipes and cibarial pump. C, Thorax. D, Scale of fore tibia. E, Comb of mid tibia, lateral view. F, Comb of mid tibia, posterior view.

Table 3. Lengths (in µm) and proportions of legs of *Sigmoitendipes susanae* n. sp., male (n = 5–7).

	fe	**ti**	**ta_1**	**ta_2**
p_1	1442–1504, 1467	1030–1112, 1071	1607–1710, 1664	845–886, 873
p_2	1257–1360, 1314	1071–1174, 1133	639–701, 676	412–433, 422
p_3	1463–1648, 1566	1092–1215, 1154	948–1030, 993	556–618, 593

	ta_3	**ta_4**	**ta_5**	**LR**
p_1	659–700, 676	597–639, 622	210–227, 221	1.51–1.60, 1.55
p_2	267–288, 284	144–165, 152	41–52, 45	0.58–0.61, 0.60
p_3	474–515, 503	247–268, 251	62–72, 67	0.85–0.87, 0.86

	BV	**SV**	**BR**
p_1	1.74–1.78, 1.76	1.49–1.55, 1.53	3.2–3.4, 3.3
p_2	3.33–3.56, 3.46	3.55–3.73, 3.62	3.8–4.6, 4.1
p_3	2.57–2.67, 2.62	2.70–2.78, 2.74	5.6–13.1, 8.4

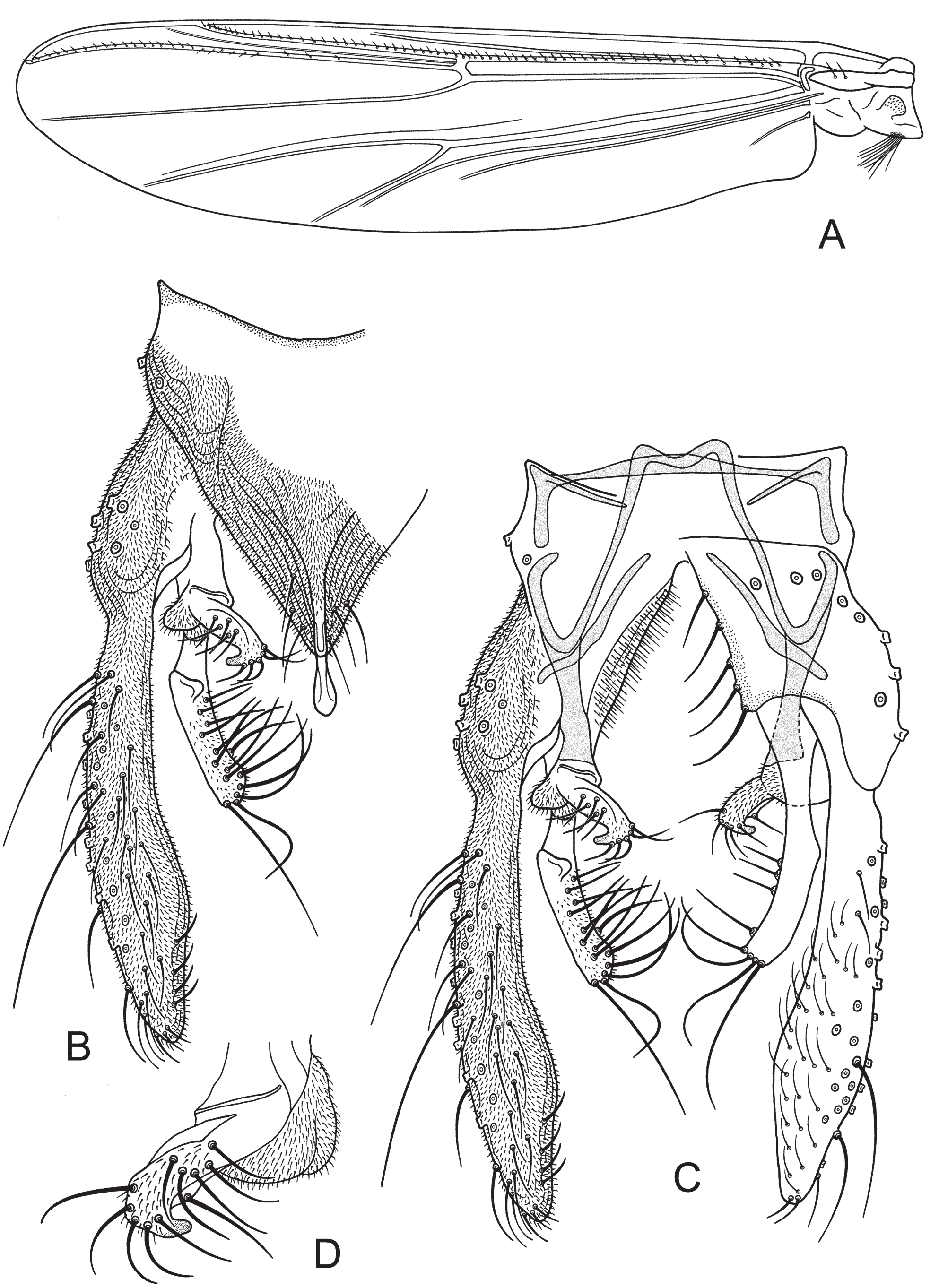

Figure 8. *Sigmoitendipes susanae* n. sp., male. A, Wing. B, Hypopygium, dorsal view. C, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right. D, Superior volsella.

Pupa (exuviae, n = 1). Total length 3.54 mm.

Coloration. Exuviae with light brown cephalothorax, wing sheath pale brown with slightly darker margins, abdominal segments VI–VII pale brown, caudolateral spur brown.

Cephalothorax (Fig. 9B). Frontal apotome (Fig. 9A) subtriangular, with wrinkles, 368 µm long, 332 µm wide. Cephalic tubercle conical, 36 µm long, frontal setae 65 µm long. Median antepronotal slightly taeniate, about 83 µm long. Precorneals close together, both slightly taeniate, anterior precorneal about 48 µm long, posterior precorneal about 83 µm long. Dc_1 62 µm long, Dc_2 76 µm long, Dc_3 73 µm long, Dc_4 78 µm long; Dc_1 54 µm in front of Dc_2, Dc_2 225 µm in front of Dc_3, Dc_3 39 µm in front of Dc_4. Wing sheath 1.44 mm long, without nose or pearl row.

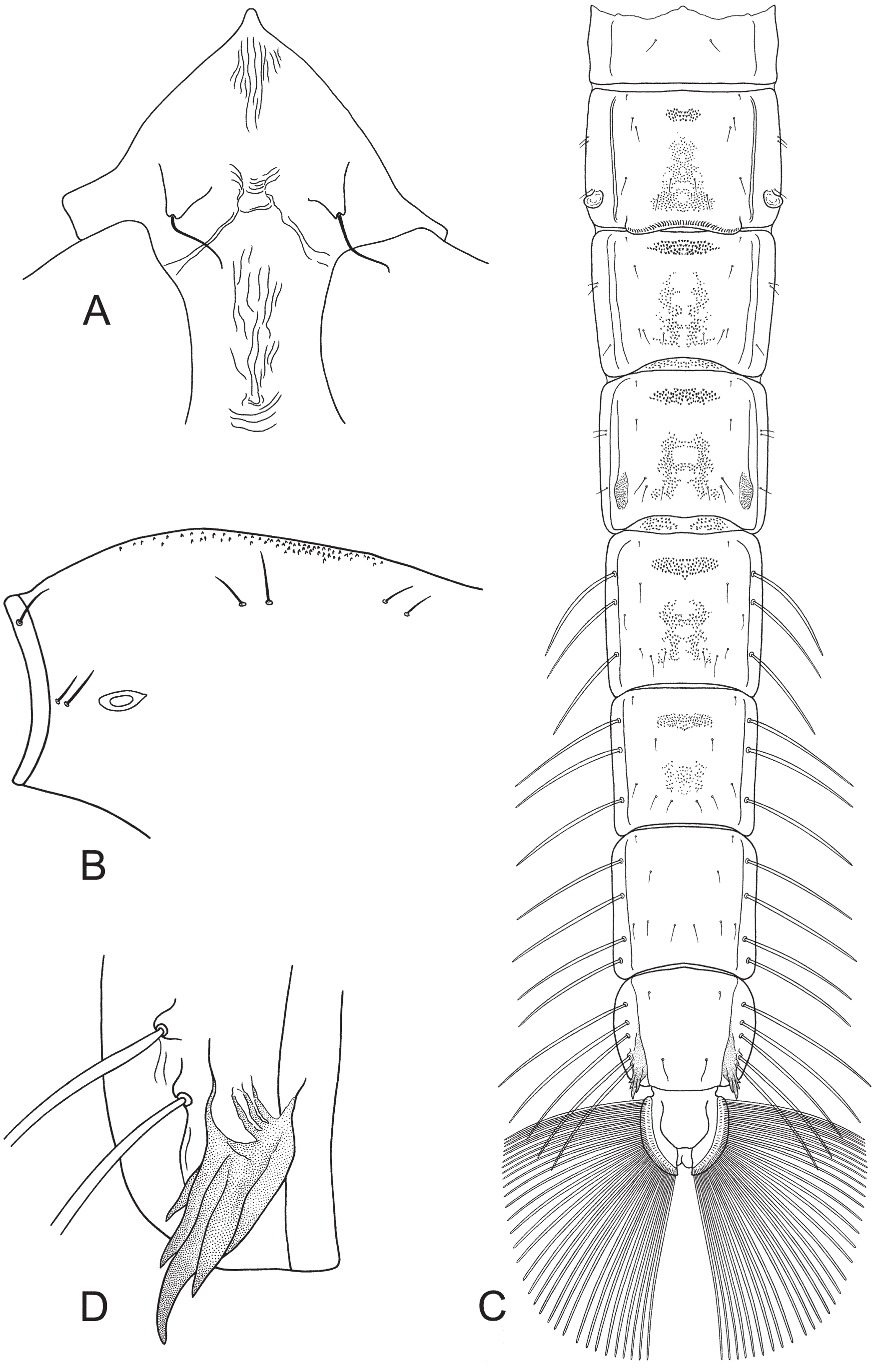

Figure 9. *Sigmoitendipes susanae* n. sp., pupa. A, Frontal apotome. B, Thorax. C, Abdomen. D, Caudolateral spur.

Abdomen (Fig. 9C). Tergite I bare, tergites II–VI each with 4 rows of strong spinules in anteriomedial band, tergites II–V each with medial anterior to posterior patches of finer shagreen, tergite VI with posterior patch of fine shagreen, tergites VII–VIII without spinules. Tergite II with 38 caudal hooklets in single, 388 µm wide row. Spinules on conjunctive III/IV in 3–4 rows; on conjunctive IV/V in 5–6 rows in separate, lateral patches. Pedes spurii A well-developed posterior on segment IV; indicated on segment V. Pedes spurii B well-developed posterior on segment II. Caudolateral spur (Fig. 9D) 145 µm long, with 1 strong apical tooth and 3 weaker teeth.

Abdominal setation. Segment I without lateral setae; segments II–IV each with 3 short, hair-like

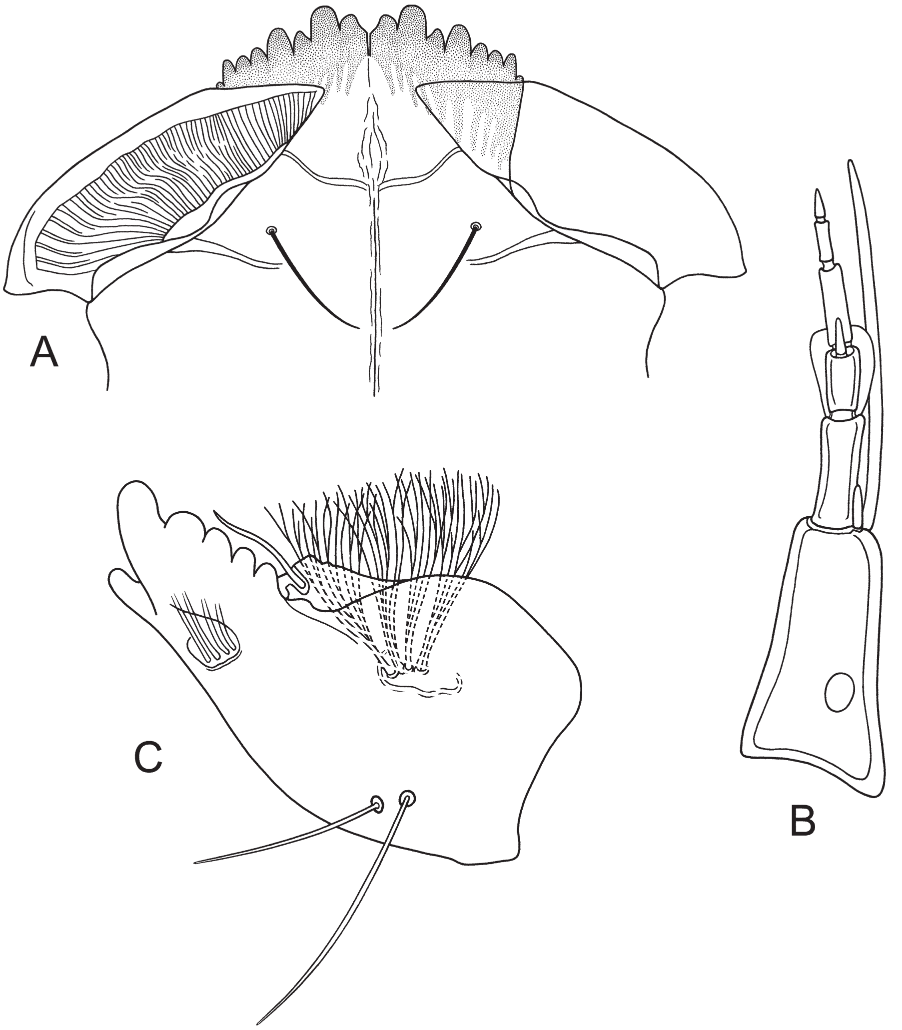

Figure 10. *Sigmoitendipes susanae* n. sp., larva. A, Antenna. B, Mandible. C, Mentum and ventromental plates.

lateral setae; segments V–VI each with 3 taeniae; segment VII with 4 taeniae; segment VIII with 5 taeniae.

Anal lobe. Anal lobe fringe with 47 taeniae. Male genital sac overreaches anal lobe by 82 µm.

Larva (exuviae, n = 1). Total length not measurable. Head capsule 420 µm long. Postmentum 180 µm long.

Coloration. Head capsule brown with teeth of mentum and mandible and postoccipital margin dark brown.

Head. AR 0.93; antennal (Fig. 10B) segment lengths (in µm): 57, 22, 10, 8, 11, 10. Basal antennal segment 30 µm wide; ring organ 18 µm from base; blade 67 µm long; accessory blade 7 µm long; Lauterborn organ 18 µm long. Pecten epipharyngis and premandible not discernible. Mandible (Fig. 10C) 135 µm long, with 1 dorsal, 1 apical and 4 inner teeth; seta subdentalis 36 µm long, slender and straight; setae interna prominent. Mentum (Fig. 10A) 124 µm wide, with 8 pairs of teeth; pair of median teeth 19 µm wide, first lateral tooth larger than median tooth, second lateral tooth smaller than first lateral tooth, third lateral tooth larger than second lateral tooth, fourth to sixth lateral teeth decrease progressively to a minute seventh tooth. Ventromental plate (Fig. 10A) 142 µm wide; distance between plates 39 µm; median apices of plates pointing towards each other. Ratio of ventromental plate / mentum 1.15. Setae submenti 55 µm long.

Abdomen. Not measurable.

Distribution and ecology

Found in São Paulo, Mato Grosso, Amazonas and Pará states. Fittkau collected the species using Brundin nets in shallow Amazonian forest streams of first, second or third order in evergreen tropical inundation forest. The streams were 1–2 m wide, shallow, flowing, and with bottoms of sand and leaves. During the flood season the water level in the streams rose up to approximately 4 m higher.

Sigmoitendipes fittkaui new species

http://zoobank.org/F054E059-AEC3-4B2F-B436-282BDD1EA14A

Type material: *Holotype:* BRAZIL, Pará, Rio Cururu, Missão São Francisco (Missão Cururu), 07°33’51”S, 57°44’23”W, 6.ii.1961, at light at the mission building, EJ Fittkau, 1 male (ZSM - A 88-11). *Paratype:* BRAZIL, Mato Grosso, Rio Tuatuari near Posto Leonardo, 12°11’55”S, 53°22’51”W, 3.ix.1965, at light, EJ Fittkau, 1 male (ZSM - A 573-5).

Etymology: Named in honor of the late Professor Dr. Ernst Josef Fittkau for his many contributions to the taxonomy and ecology of Neotropical chironomids.

Diagnostic characters: See key.

Description

Male (n = 1–2). Total length 4.47–4.57 mm. Wing length 1.98–2.04 mm. Total length / wing length 2.19–2.31. Wing length / length of profemur 1.48–1.55.

Coloration. Thorax brown; head, legs and abdomen paler brown. Wing translucent.

Antenna. AR 2.07–2.10. Ultimate flagellomere 890–931 µm long.

Head. Temporal setae 18–22, partly biserial, including 3–4 inner verticals, 8–9 outer verticals and apparently 6–8 postorbitals. Clypeus with 11–14 setae. Tentorium 144–154 µm long; 41–45 µm wide. Stipes 135–139 µm long; 12–14 µm wide. Palp segment lengths (in µm): 33–35, 49–51, 84–88, 105–113, 166–176. Third palpomere with 4–5 sensilla, longest 22–24 µm long.

Thorax. Antepronotum without setae. Dorsocentrals 6–7, acrostichals not discernible, prealars 2–3. Scutellum with 8–9 setae.

Wing (Fig. 11A). VR 1.12–1.13. Brachiolum with 1–2 setae, R 25–26, R_1 with 23–26, R_{4+5} with 21–27 setae, other veins and cells bare. Squama with 5–7 setae.

Legs. Scale of fore tibia 49–51 µm long, spur of mid tibia 54–57 µm long including 30–32 µm long comb, spurs of hind tibia 50–52 µm long and 44–46 µm long including 29–30 µm long comb. Width at apex of fore tibia 55–58 µm, of mid tibia 57–58 µm, of hind tibia 62–66 µm. Lengths and proportions of legs as in Table 4.

Hypopygium (Figs 11B, C). Tergite IX covered with microtrichia; laterosternite IX with 1–3 setae. Anal point spatulate, 48–55 µm long, 9–10 µm wide at base, 15–16 µm wide subapically, with 5–8 setae to each side of the base, of which about half on the ventral side. Transverse sternapodeme 39–46 µm long, straight. Phallapodeme 86–88 µm long. Gonocoxite 162–170 µm long. Superior volsella in dorsal view (Fig. 11D) straight, 91–95 µm long; ventral lobe subrectangular; dorsal lobe slightly shorter, with bare, hooked posterior projection and 4–5 lateral setae. Inferior volsella 133–148 µm long, 18–21 µm wide at base, 9–10 µm wide at its narrowest, with 17–19 µm long, rounded dorsal projection starting 66–77 µm from base; distal half tapering, 21–23 µm wide basally, 8–10 µm wide subapically, with 17–19 simple setae, longest apical seta 59–62 µm long. Gonostylus 193–197 µm long. HR 0.83–0.89. HV 2.32–2.35.

Female and immatures. Unknown.

Distribution and ecology

Collected in Mato Grosso and Pará States.

Sigmoitendipes oliveirai new species

http://zoobank.org/24D6FE7B-6AE9-43A1-952C-FB9EC05739A3

Type material: *Holotype:* BRAZIL, Mato Gros-

Table 4. Lengths (in µm) and proportions of legs of *Sigmoitendipes fittkaui* n. sp., male (n = 1–2).

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	1277–1339	927–989	1607	845	618	536
p_2	1133–1215	1009–1092	639–700	391–412	268–288	124–144
p_3	1545–1627	1112–1174	948	597	494	247

	ta_5	LR	BV	SV	BR
p_1	185	1.64	1.80	1.45	3.7
p_2	42–65	0.63–0.64	3.32–3.38	3.30–3.36	3.8–3.9
p_3	103	0.81	2.60	2.96	6.1

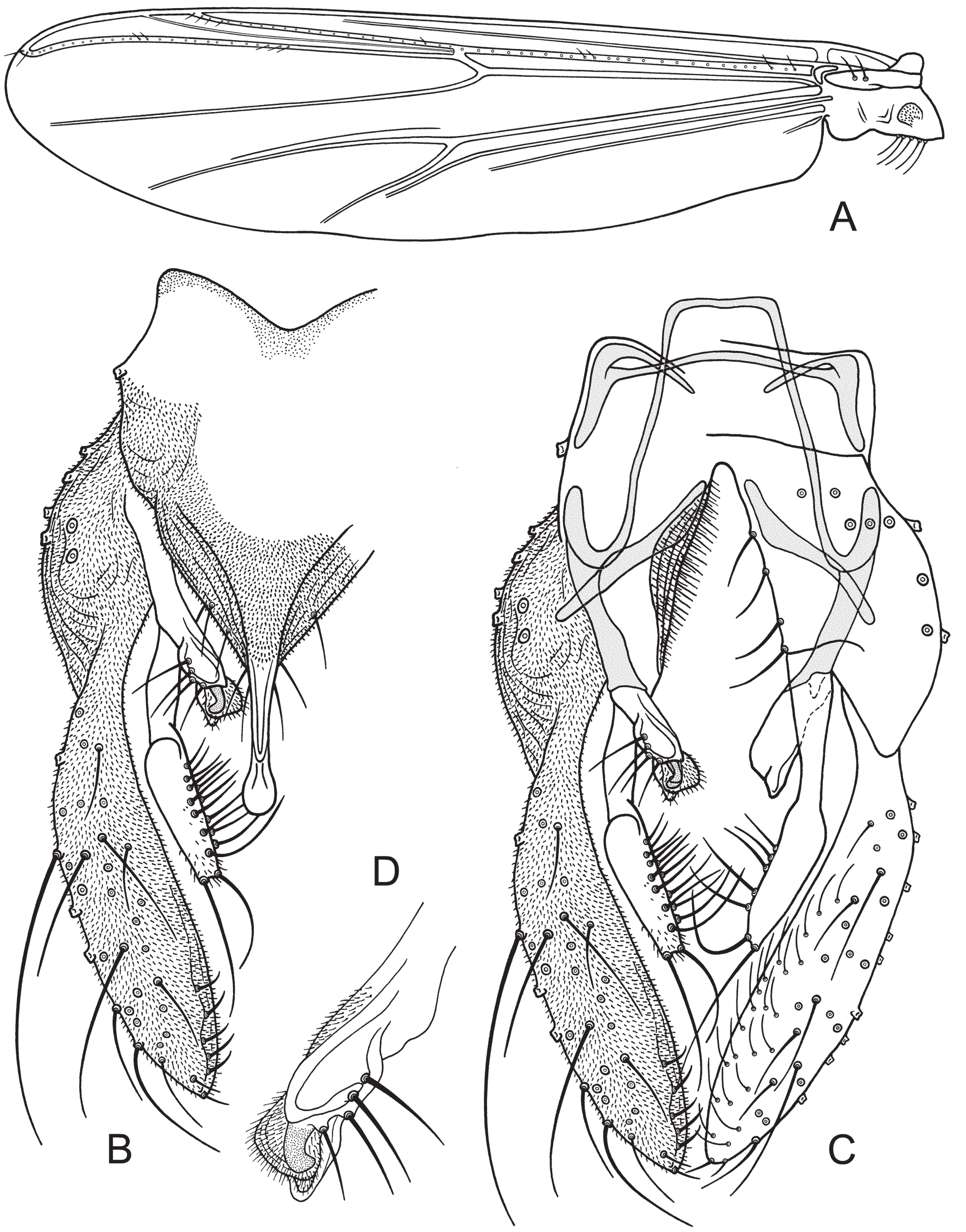

Figure 11. *Sigmoitendipes fittkaui* n. sp., male. A, Wing. B, Hypopygium, dorsal view. C, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right. D, Superior volsella.

so, Ribeirão Cascalheira, Fazenda Campina Grande, Rio Suiamissu, 12°48’35”S 52°06’56”W, 10.x.2007, light trap, LC Pinho et al., 1 male (MZUSP).

Etymology: Named after the late Dr. Sebastião José de Oliveira for his pioneering studies on the taxonomy of Brazilian chironomids.

Diagnostic characters: See key.

Description

Male (n = 1). Total length 4.53 mm. Wing length 1.97 mm. Total length / wing length 2.30. Wing length / length of profemur 1.43.

Coloration. Thorax brown; head, legs and abdomen paler brown. Wing translucent.

Antenna. AR 2.19. Ultimate flagellomere 946 µm long.

Head. Temporal setae 24 including 2 inner verticals, 11 outer verticals and 11 postorbitals. Clypeus with 14 setae. Frontal tubercle indicated as paler area. Tentorium 144 µm long, 35 µm wide.

Stipes 169 µm long, 12 µm wide. Palp segment lengths (in µm): 45, 47, 72, 100, 167. Third palpomere with 3 sensilla, longest 17 µm long.

Thorax. Antepronotum without setae. Dorsocentrals 7, acrostichals apparently absent, prealars 2. Scutellum with 8 setae in single row.

Wing (Fig. 12A). VR 1.10. Brachiolum with 1 seta, R with 27, R_1 with 23, R_{4+5} with 25 setae, other veins and cells bare. Squama with 5 setae.

Legs. Scale of fore tibia 55 µm long; spur of mid tibia 52 µm long including 20 µm long comb; spurs of hind tibia 50 µm and 45 µm long including 22 µm long comb. Width at apex of fore tibia 60 µm; of mid tibia 57 µm; of hind tibia 62 µm. Lengths and proportions of legs as in Table 5.

Hypopygium (Figs 12B, C). Tergite IX covered with microtrichia; laterosternite IX with 1 seta. Anal point spatulate, 65 µm long, 20 µm wide at base, 17 µm wide subapically, with 4 setae to each side of the base of which 1 on the ventral side. Transverse sternapodeme 47 µm long, straight. Phallapodeme 87 µm long. Gonocoxite 147 µm long. Superior volsella (Fig. 12D) 62 µm long, with somewhat rounded, 45 µm long, 40 µm wide, setose lobe with 3 apical setae; with 62 µm long dorsal lobe with 4 outer and 1 inner setae, slightly hooked at apex. Inferior volsella 144 µm long; 25 µm wide at base; 10 µm wide at its narrowest; with 10 µm long, dorsal, rounded projection, starting 85 µm from base; apically subtriangular, 20 µm wide; with 16 simple setae, longest apical seta 57 µm long. Gonostylus 199 µm long. HR 0.74. HV 2.27.

Female and immatures. Unknown.

Distribution and ecology

Only known from Mato Grosso, where it was collected in a light trap at a fast flowing river with mostly rocky bottom sediments.

Sigmoitendipes reissi new species

http://zoobank.org/996D3689-1C37-4109-97FE-77C5FBF15817

Type material: *Holotype:* BRAZIL, São Paulo, São Simão, Ribeirão Águas Claras, ponte Tio Zito, 19.i.2003, light trap, T Andersen, HF Mendes, 1 male (MZUSP).

Etymology: Named in honor of the late Dr. Friedrich Reiss for his many contributions to the taxonomy and ecology of Neotropical chironomids.

Diagnostic characters: See key.

Description

Male (n = 1).Total length 5.08 mm. Wing length 2.20 mm. Total length / wing length 2.31. Wing length / length of profemur 1.70.

Coloration. All pale brown. Wing translucent.

Antenna. AR 2.07. Ultimate flagellomere 964 µm long.

Head. Temporal setae apparently 27, partly biserial, including 4 inner verticals, 15 outer verticals and 8 postorbitals. Clypeus with 15 setae. Frontal tubercle indicated as paler area. Tentorium 177 µm long, 47 µm wide. Stipes 159 µm long, 10 µm wide. Palp segment lengths (in µm): 48, 69, 80, 108, 137. Third palpomere with 8 sensilla, longest 23 µm long.

Thorax. Antepronotum without setae. Dorsocentrals 11, acrostichals apparently 5, prealars 4. Scutellum with 10 setae, partly biserial.

Wing (Fig. 13A). VR 1.07. Brachiolum with 2 setae, R with 29, R_1 with 28, R_{4+5} with 38 setae, other veins and cells bare. Squama with 7 setae.

Legs. Scale of fore tibia 55 µm long; spur of mid tibia 58 µm long including 29 µm long comb; spurs

Table 5. Lengths (in µm) and proportions of legs of *Sigmoitendipes oliveirai* n. sp., male (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
p_1	1379	946	1586	837	670	611	207	1.68	1.68	0.60	2.7
p_2	1162	1044	660	394	276	138	58	0.63	3.31	3.34	4.2
p_3	1527	1143	936	571	453	246	99	0.82	2.63	2.85	5.0

Table 6. Lengths (in µm) and proportions of legs of *Sigmoitendipes reissi* n. sp., male (n = 1).

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
p_1	1298	865	1483	845	659	577	165	1.71	1.62	1.46	3.6
p_2	1236	1071	721	433	288	124	62	0.67	3.34	3.20	3.3
p_3	1545	1133	–	–	–	–	–	–	–	–	–

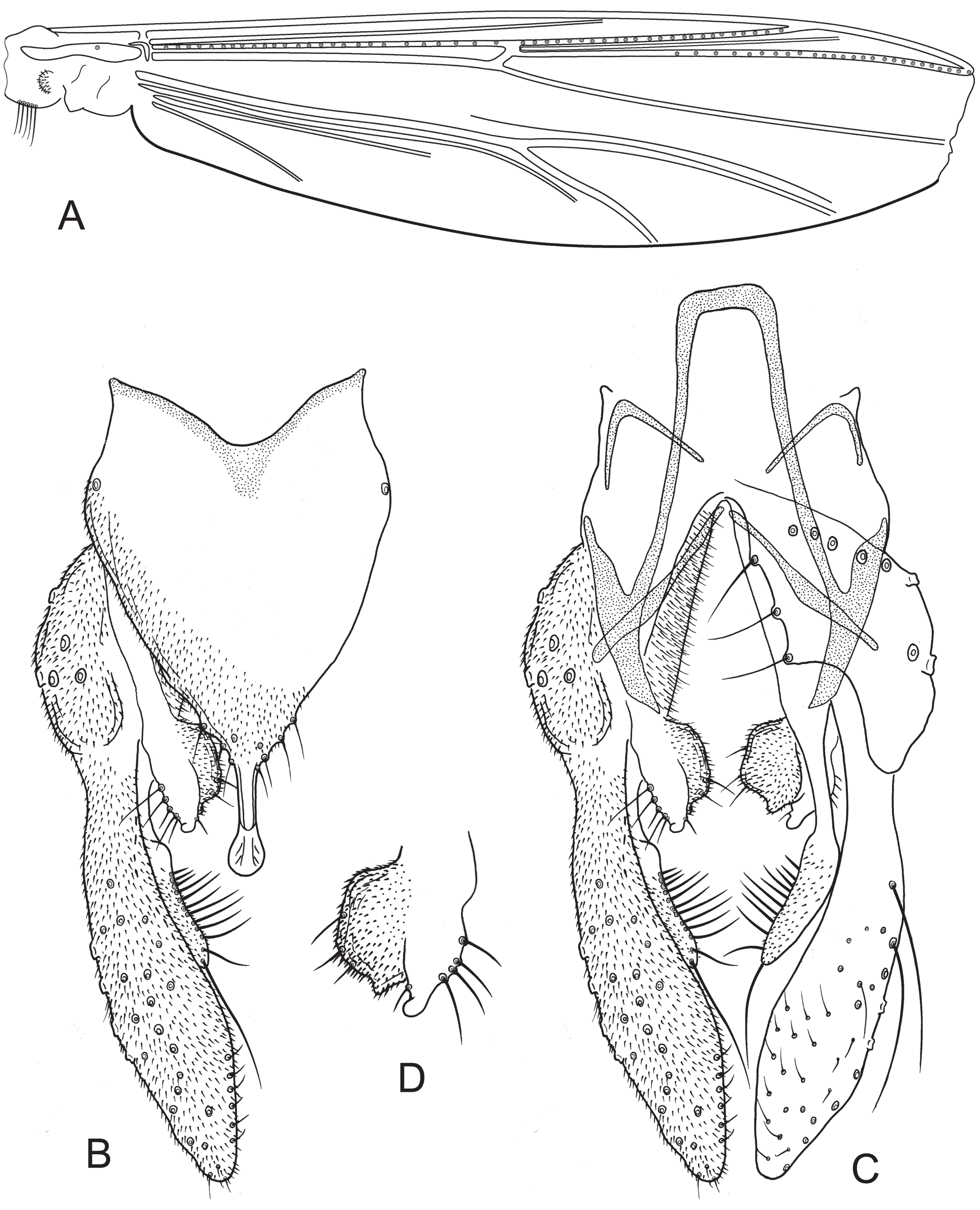

Figure 12. *Sigmoitendipes oliveirai* n. sp., male. A, Wing (tip missing). B, Hypopygium, dorsal view. C, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right. D, Superior volsella.

of hind tibia 55 µm long and broken, including 30 µm long comb. Width at apex of fore tibia 61 µm; of mid tibia 64 µm; of hind tibia 72 µm. Lengths and proportions of legs as in Table 6.

Hypopygium (Figs 13B, C). Tergite IX covered with microtrichia; laterosternite IX with 2 seta. Anal point spatulate, 68 µm long, 23 µm wide at base, 7 µm wide medially, 15 µm wide subapically, with 5 setae to each side of the base of which 2 on the ventral side. Transverse sternapodeme 55 µm long, concave. Phallapodeme 97 µm long. Gonocoxite 201 µm long. Superior volsella (Fig. 13D) 83 µm long, medially with rounded, setose lobe, apically with 30 µm long, hooked, bare projection with 4 strong setae. Inferior volsella 162 µm long; 19 µm wide at base; 10 µm wide at its narrowest; with 36 µm long, dorsal, bluntly triangular projection, starting 87 µm from base; apically subrectangular, 15 µm wide; with 17 simple setae, longest apical seta 110 µm long. Gonostylus 211 µm long. HR 0.95. HV 2.41.

Female and immatures. Unknown.

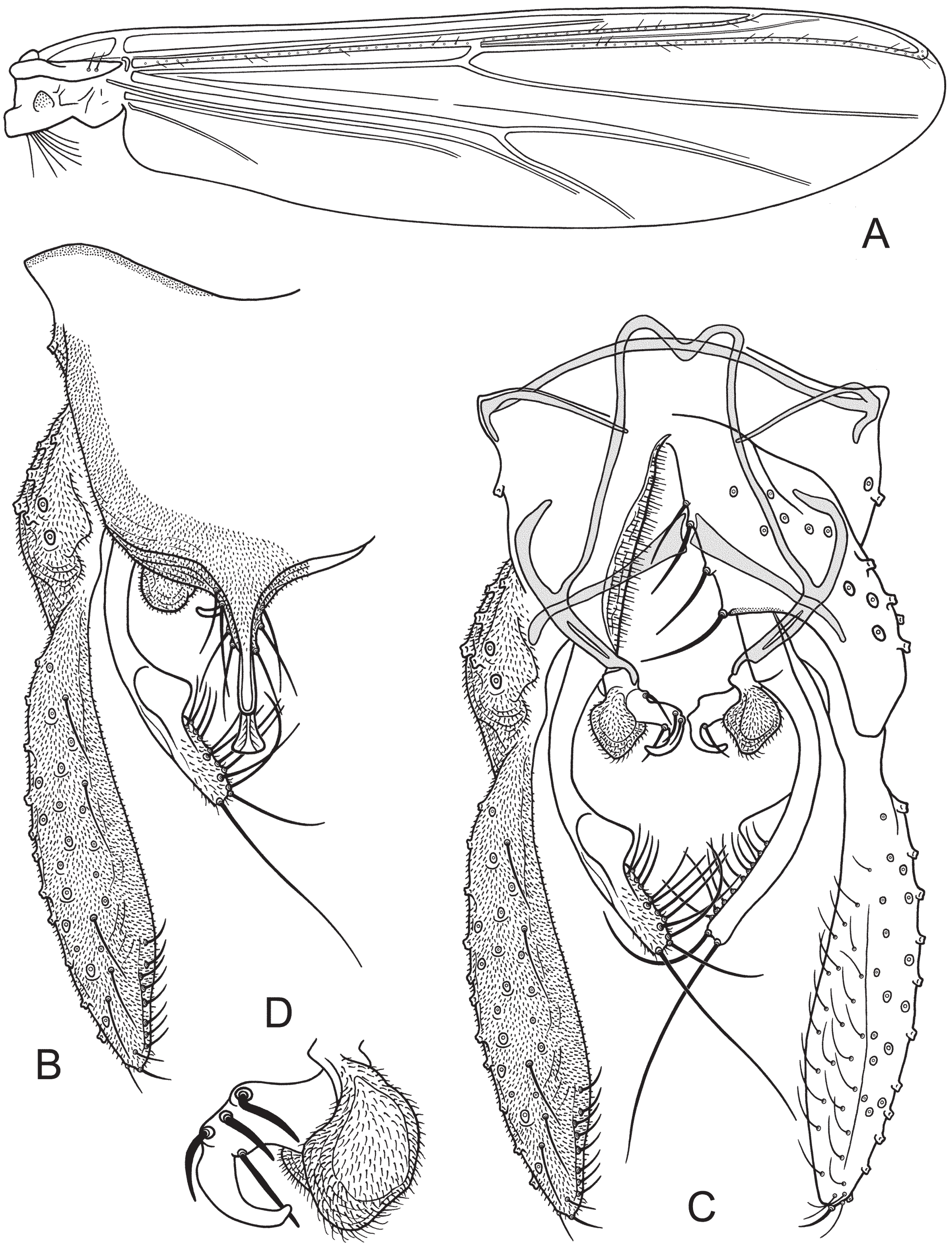

Figure 13. *Sigmoitendipes reissi* n. sp., male. A, Wing. B, Hypopygium, dorsal view. C, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right. D, Superior volsella.

Distribution and ecology

Only known from São Paulo State, where it was collected in a light trap at a small, rather slow flowing river with mostly fine bottom sediments.

Sigmoitendipes spiesi new species

http://zoobank.org/E46663A0-94FA-4039-BE72-224D1FD45D21

Type material: *Holotype:* BRAZIL, Mato Grosso, Nova Xavantina, Fazenda Sr. Queté, Córrego Cachoeira, 14°32.817'S 52°31.395'W, 16.x.2007, light trap, LC Pinho et al., 1 male (MZUSP). *Paratype:* BRAZIL, Mato Grosso, Nova Xavantina, Fazenda Sr. Queté, Córrego Voadeira, 14°41.577'S 52°27.203'W, 13.x.2007, 1 male (UFSC).

Etymology: Named after Martin Spies for his important contributions to the taxonomy and ecology of Neotropical chironomids.

Diagnostic characters: See key.

Description

Male (n = 1–2). Total length 4.64–5.52 mm. Wing length 2.00–2.12 mm. Total length / wing length 2.38–2.60. Wing length / length of profemur 1.74–1.78.

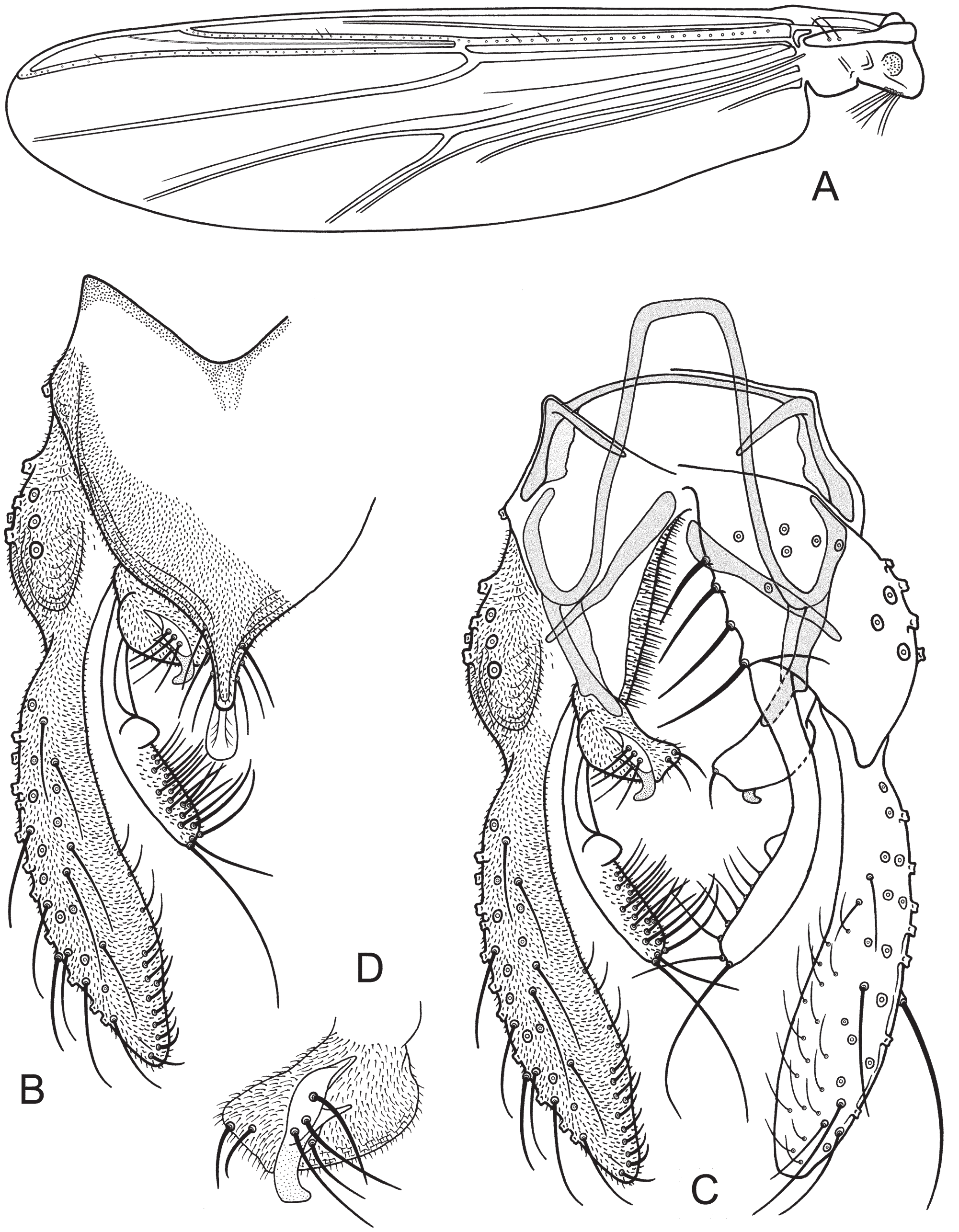

Figure 14. *Sigmoitendipes spiesi* n. sp., male. A, Wing. B, Hypopygium, dorsal view. C, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right. D, Superior volsella.

Coloration. Thorax brown; head, legs and abdomen paler brown. Wing translucent.

Antenna. AR 1.93–1.95. Ultimate flagellomere 867–915 µm long.

Head. Temporal setae 11–14 including 3 inner verticals, 5 outer verticals and 3–6 postorbitals. Clypeus with 15 setae. Frontal tubercle indicated as paler area. Tentorium 147–156 µm long, 35–37 µm wide. Stipes 155–180 µm long; 11–12 µm wide. Palp segment lengths (in µm): 41–45, 52–72, 87–96, 107–111, 172. Third palpomere with 5–6 sensilla, longest 25–27 µm long.

Thorax. Antepronotum without setae. Dorsocentrals 6–7, acrostichals apparently 6, prealars 3–5. Scutellum with 7–9 setae in single row.

Wing (Fig. 14A). VR 1.12. Brachiolum with 2 setae, R with 25–26, R_1 with 27–28, R_{4+5} with 30–31 setae, other veins and cells bare. Squama with 8 setae.

Legs. Scale of fore tibia 50–55 µm long; spur of mid tibia 55–65 µm long including 25–37 µm long

Table 7. Lengths (in µm) and proportions of legs of *Sigmoitendipes spiesi* n. sp., male (n = 1).

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**	**ta_5**	**LR**	**BV**	**SV**	**BR**
p_1	1298	927	–	–	–	–	–	–	–	–	–
p_2	1215	1051	701	412	288	144	41	0.67	3.35	3.24	3.3
p_3	1524	1092	968	556	433	227	82	0.89	2.76	2.70	–

comb; spurs of hind tibia 52–54 µm and 45–47 µm long including 25–29 µm long comb. Width at apex of fore tibia 55–57 µm; of mid tibia 60–62 µm; of hind tibia 65–70 µm. Lengths and proportions of legs as in Table 7.

Hypopygium (Figs 14B, C). Tergite IX covered with microtrichia; laterosternite IX with 2 seta. Anal point spatulate, 29–37 µm long, 10–11 µm wide at base, 15–17 µm wide subapically, with 7–8 setae to each side of the base of which 3–4 on the ventral side. Transverse sternapodeme 44–50 µm long, straight. Phallapodeme 100–106 µm long. Gonocoxite 169–189 µm long. Superior volsella (Fig. 14D) 89–109 µm long, with subrectangular, 47–55 µm long, 27–33 µm wide, setose lobe with 2–3 apical setae; with 40–47 µm long, apically hooked, dorsal lobe with 4 setae. Inferior volsella 144–159 µm long; 20–21 µm wide at base; 10–11 µm wide at its narrowest; with 25–27 µm long, dorsal, rounded projection, starting 82–88 µm from base; apically subrectangular, 15–18 µm wide; with 18–19 simple setae, longest apical seta 85–97 µm long. Gonostylus 199–219 µm long. HR 0.85–0.86. HV 2.33–2.52.

Female and immatures. Unknown.

Distribution and ecology

Only known from Mato Grosso State, where it was collected in a light trap at a small, rather slow flowing stream with mostly fine bottom sediments.

Acknowledgements

We are indebted to Dr. Claudio G. Froehlich, Dr. Susana Trivinho-Strixino and Dr. Ângela M. Sanseverino for comments on the manuscript. We are also indebted to Dr. Neusa Hamada for the loan of material of *Sigmoitendipes* n. gen. from the project “Insetos aquáticos: biodiversidade, ferramentas ambientais e a popularização da ciência para melhoria da qualidade de vida humana no estado do Amazonas” [supported by PRONEX–CNPq (MCT)–FAPEAM and INPA (MCT)], to Dr. Carlos J. E. Lamas, for the loan of material from the project “Limites geográficos e fatores causais de endemismo na Floresta Atlântica em Diptera” [supported by the BIOTA–FAPESP, proc. 03/12074–9], to FAPESC for financing the project “Insetos aquáticos do Parque Estadual da Serra Furada, SC” (proc. 11323/2012–9), to Martin Spies for the loan of material of *Sigmoitendipes* from the ZSM, and to Dr. Adolfo R. Calor, Dr. Sidnei Mateus, Dr. Rodolfo Mariano and Dr. Lucas S. Lecci for providing material and help during fieldwork. The authors also wish to thank the Willi Hennig Society for the free availability of the TNT program. Gladys Ramirez made most of the slide preparations.

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LIFE CYCLE OF NATURAL POPULATIONS OF *METRIOCNEMUS (INERMIPUPA) CARMENCITABERTARUM* LANGTON & COBO 1997 (DIPTERA: CHIRONOMIDAE) IN THE NETHERLANDS: INDICATIONS FOR A SOUTHERN ORIGIN?

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Abstract

The life-history and behaviour of the chironomid *Metriocnemus* (*Inermipupa*) *carmencitabertarum* was studied for three years and a comparison was made between two populations in the Netherlands. Key life-history characteristics are reported, including the number of generations (2-5 generations) and duration of larval development (19-55 days). Life-history characteristics differed between the two populations and between generations within a population. These differences were likely related to differences in competitors (mainly *Chironomus riparius Meigen*) and thermal characteristics of the habitat from variations in water volume and ambient temperature. Results suggested a diapause in summer, when water levels dropped, and not in winter. A clear seasonal pattern in body size was found with pupal length increasing steeply at the beginning of the summer. The increase in body size was positively correlated with mean ambient temperature during larval development. This correlation is in contrast with the Temperature-Size Rule (TSR) in ectotherms, which postulates a negative correlation between temperature and adult body size. The chance of ectotherm predators being present in the habitat is low, which may explain this feature. Life history characteristics supported a recent northward range expansion for *M. (I.) carmencitabertarum* due to warmer winters. If correct, it is predicted that the species will be found in Scotland and northern Germany and/or southern Denmark in the near future.

Introduction

First described from specimens from the northwest of the Iberian peninsula (Langton and Cobo 1997), *M.* (*I.*) *carmencitabertarum* Langton & Cobo 1997 (Diptera: Chironomidae, subfamily Orthocladiinae) has since then been found on two islands from the Azores archipelago (Murray *et al*. 2004, Ramos et al. 2010), in England in 2010 (Langton and Wilson 2012), in The Netherlands in 2011 (Kuper & Moller Pillot 2012), in Ireland in 2012 (Murray 2012) and in Northern Ireland in 2013 (Langton, 2014; Murray et al. 2014). More northern reports of *M.* (*I.*) *carmencitabertarum* for Poland and Estonia (Spies and Sæther 2013) and Spitsbergen (Langton and Visser 2003) are probably incorrect, as there are no published records (Murray 2012, eElurikkus 2015) or are not cited in recent lists (Coulson 2014).

Larvae of *M.* (*I.*) *carmencitabertarum* were first found in granite rock holes filled with rain water in northwestern Spain (Langton and Cobo 1997). Since then larvae have been reported from similar, yet artificial, water-filled habitats such as animal drinking troughs, rain-filled water butts and buckets in urban surroundings (Murray et al. 2004, Ramos et al. 2010, Langton and Wilson 2012, Kuper & Moller Pillot 2012, Murray 2012, Murray 2013, Murray 2016). Besides this, little is known about the ecology and life-history. Murray (2013) gives some notes on larval behaviour. Kuper (2015) reported biometric measurements to distinguish the four larval stages and also presented biometric data for pupal exuviae (skins), which revealed a larger average size for females. This sexual size difference was probably already present in fourth instar larvae. The finding of new populations of *M.* (*I.*) *carmencitabertarum* in The Netherlands gave the opportunity to collect empirical life history data from two populations during 2012-2014. Here, for the first time, the species' life cycle characteristics are presented and indications for a southern origin of the Dutch populations of *M.* (*I.*) *carmencitabertarum* are discussed.

Material and Methods

Study sites

Two populations of *M.* (*I.*) *carmencitabertarum* were studied in The Netherlands in 2012 and 2013 to elucidate the species' life cycle. One population was living in a garden bucket in Appingedam (53°19'16.71" N, 6°51'41.49" E, Fig. 1). The second population was living in a garden water butt in Nijmegen (51°50'19.73" N, 5°50'33.58" E, Fig. 1). The bucket (capacity of 15 litres) and the water

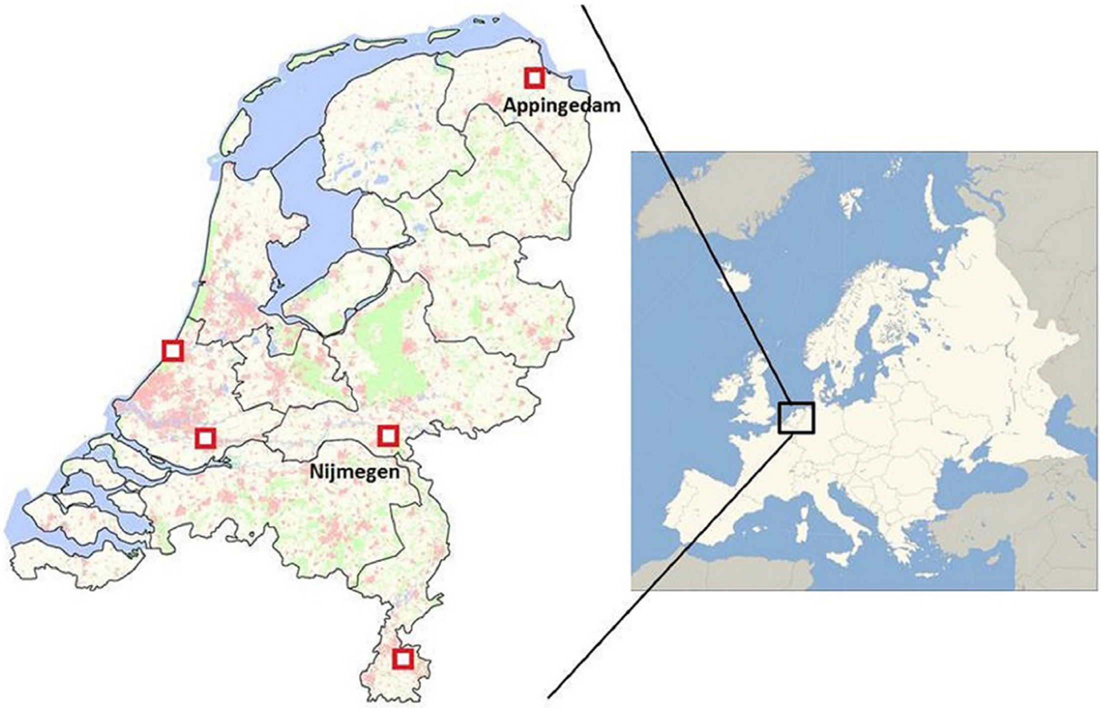

Figure 1. Distribution of *Metriocnemus* (*I.*) *carmencitabertarum* in the Netherlands and the location of the two research sites Appingedam and Nijmegen.

butt (capacity of 200 litres in 2012 and 100 litres in 2013) were located under a roof gutter. During rain showers, rainwater was washed into the bucket and the water butt from the roof. When rain showers were prolonged or heavy water overflowed from the bucket and the water butts. Algae grew on the bottom and along the sides of the bucket and the butts. Much organic debris was present in the bucket. The bucket and the butts never dried up, although water volume and level in the butt declined significantly at the end of August 2012 (at least 75-80% reduction in water volume) and in July 2013 (at least 60% reduction), when water was used for gardening purposes. In late spring and summer, the bucket and the butts were sun-exposed until ca. 0900 hrs (CET). During ten days in February 2012, when outdoor maximum temperatures were below 0^oC, the bucket was placed indoors at a temperature of $6-7^oC$. During the winter of 2012-2013 the bucket was left outdoors and the water was possibly completely frozen for some time. During the winters of 2011-2012 and 2012-2013, the 200-liter water butt in Nijmegen was partly frozen for some time, with probably a central core of water running down to the base. In April 2013 the 200 litres butt was replaced by a 100 litres butt after the former had burst due to ice formation.

Wintering stages

To study the wintering stages of *M.* (*I.*) *carmencitabertarum* an outdoor rearing experiment began in June 2013 in Appingedam in a glass vase (capacity of six litres). The vase was closed at the top by a plastic lid with four small holes for air circulation. The lid was placed to keep adults inside for continuous reproduction and to exclude other fauna. The distance between the water layer and the lid was 20-25 cm's, depending on the water level. Some organic debris was added. On 7 July, 2013 algal growth was observed along the sides of the vase and on the organic debris. Then, one egg string was collected from the bucket and placed in the vase. On 9 February, 2014 the lid was removed from the vase to give emerging adults the opportunity to escape.

In late spring and summer, the vase was sun-exposed until ca. 0900 hrs (CET). During the last one and a half weeks of January until 1 February, 2014 an ice layer 10-15 cm thick formed on top and along the sides. A central core of water remained down to the algae and the larvae. Ice-formation most probably did not reach the larvae. In this period a layer of snow approximately eight centimetres thick was deposited on top of the lid. The vase was placed in a water-filled bucket to prevent it from bursting. On 2 February 2014, the vase was taken out of the bucket, when temperatures stayed above 0 °C. Larvae were sampled with a sieve by up-whirling the debris and algae on the bottom on 29 December 2013 and on 9 February 2014. In December, larvae were sub-sampled and the abundance of the different larval stages was estimated while in February larvae were (probably) completely sampled and counted. After counting, the larvae were replaced in the vase. Sixteen larvae of various sizes were collected and preserved in 70% ethanol to verify larval stages. Larvae were assigned a larval stage by comparison with the collected individuals. Head width (HW) and head length (HL) of the collected individuals were measured to the nearest 4 µm and 10 µm, depending on the magnification, with a Zeiss microscope at 312.5x and 125x magnification. To assign larval stages, results were plotted in a HW-HL graph of 1st to 4th instar larvae, obtained from Kuper (2015).

On 21 December 2013, the Appingedam bucket was also examined for wintering larvae that were collected and scanned under a Wild M3B stereomicroscope at 64x magnification. Following examination all larvae, the original water and the debris were replaced in the bucket.

Life cycle data collection

Observation period. In Appingedam, the population living in the bucket was observed from 24 March 2012 until 1 December 2013. In 2012 observations were interrupted from 29 May until 15 June and from 30 June until 14 July. In 2013 no observations were made from 21 July until 9 August and from 15 September until 26 September.

The population in Nijmegen was studied from 22 May 2012 (when it was discovered) until 20 September 2013, when the water butt was removed and observations had to be stopped. Observations were interrupted in 2012 from 23 May until 17 June, from 1 July until 16 July, and from 21 September until 29 September. In 2013 no observations were made between 22 July and 10 August.

Daily observations

Data collection at Appingedam and Nijmegen in 2012 consisted of establishing the daily number of adults and number of egg masses, whereas in 2013 pupal exuviae were also collected. In 2013 larval activity was also observed in Nijmegen.

In Appingedam, midge activity was primarily observed on weekend days. During observation days, the bucket was regularly visited, starting at ca. 0800-0900 hrs (CET) until sunset. In 2012 observations were made on average during 3.1 days per week. In 2013, observations were made during 5.2 days per week on average until the second week of June. Then observation frequency decreased to 2.7 days per week. In Nijmegen, observations were mostly carried out during working days, once per day after ca. 1800 hrs (CET). In 2012 observations were made on average during 2.3 days per week and during 4.9 days per week in 2013.

Adults. At Appingedam the largest number of adults that were seen within a radius of ca. 4 metres of the bucket during one observation period represented daily number of adult midges. At Nijmegen the daily number of adults was established once after 1800 hrs (CET), by counting the number of adults inside the water butt and on the wall of the adjacent shed. A flash light was used when it was dark. The observed number of adults probably did not reflect rate of emergence perfectly as individuals could fly away to and arrive from the environment. Therefore pupal exuviae were collected in 2013.

Egg strings. The number of egg strings in Appingedam and Nijmegen were counted around sunset or later. A flash light was used when it was dark.

Larvae. In Nijmegen larvae were counted well after sunset. A flashlight was used to shine at the inner sides of the water butt to reveal larval activity. Larvae could be observed down to ca. 50 cm's beneath the water surface. Larvae were either judged to be "large" or "small". Large larvae were presumed to be fourth (possibly third) instar, small larvae were presumed to be younger than fourth instar. Larvae were not collected to verify the categories.

Pupal exuviae. In the Appingedam bucket, pupal exuviae were sampled from the beginning of July in 2013. In Nijmegen exuviae were collected throughout the adult activity period in 2013. The exuviae were collected around sunset or later, or before 1000 hrs (CET) the next day by sieving the water surface with a small net. Exuviae that were attached to the sides of the butt or bucket were removed by tweezers. Pupal exuviae from the wintering population in the Appingedam vase were sampled daily in April and May in 2014. Exuviae were stored in 70% alcohol for counting, sexing and measuring the length to the nearest 0.1 mm as the distance from the top of the frontal apotome to the tip of the anal lobe, at 10x magnification (Nikon SMZ645 stereo-microscope).

Generation development time

The emergence period of a generation was defined by the observed peak in adult activity (number of adults, number of egg strings laid, number of pupal exuviae) between periods of lower activity. Generation development time was considered to be the number of days between two consecutive peaks of adult numbers or, if peaks were in a period without observations, by the number of days between date of first adult activity at the start of an activity period and the date of first adult activity at the start of the next activity period.

Temperature data

If not stated otherwise, all ambient temperature data for 2012-2014 were obtained from AccuWeather.com (2012-2014). Daily temperatures only differed in detail between Appingedam and Nijmegen in 2012 and 2013.

Statistics

To test if ambient temperature and pupal exuviae size were correlated it was assumed that development time for each exuviae of a generation was the same as was calculated for the generation it belonged to. Then, for each exuviae the mean ambient temperature during development was calculated by averaging daily temperatures of the number of development days prior to the date of emergence. Daily temperature was taken as the mean of minimum and maximum temperature of that day. Correlation between mean ambient temperatures during development and skin size, was tested with the model 'mean ambient temperature during development * skin size with sexes distinguished' in an ANOVA, with mean temperature as a second order polynomial. Analyses were performed in R statistics version 3.0.1 (R-Core-Team, 2013) with standard packages.

Results

Wintering population

On 29 December 2013, 40 to 50 larvae were sampled from the Appingedam glass vase and assigned larval stages. Approximately 40-50% were 3rd and approx. 50-60% were 4th instar. On 9 February, 34 larvae (32.1%) were in 3rd and 72 larvae (67.9%) were in 4th larval stage (Fig. 2). Larvae were free living. Larvae of other species were not found. From the beginning of April until the end of May, adults from the wintering population in the vase emerged over a period of almost two months (Fig. 3). The emergence pattern followed a 'normally' distributed curve over time.

Larvae of *M .(I.) carmencitabertartum* were not found in the bucket in Appingedam in December

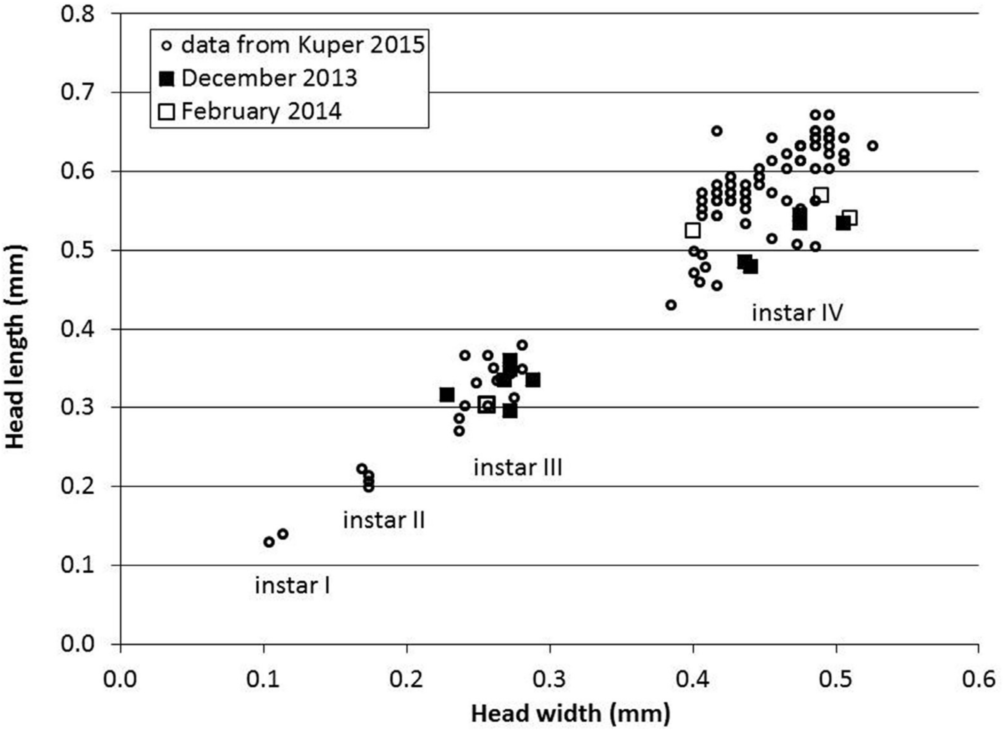

Figure 2. Head width and head length of wintering larvae collected in the Appingedam vase in December 2013 and February 2014. Results are projected in a HW-HL graph of the four *M.* (*I.*) *carmencitabertarum* instars obtained from Kuper (2015) revealing wintering in 3rd and 4th larval stage in the Appingedam vase.

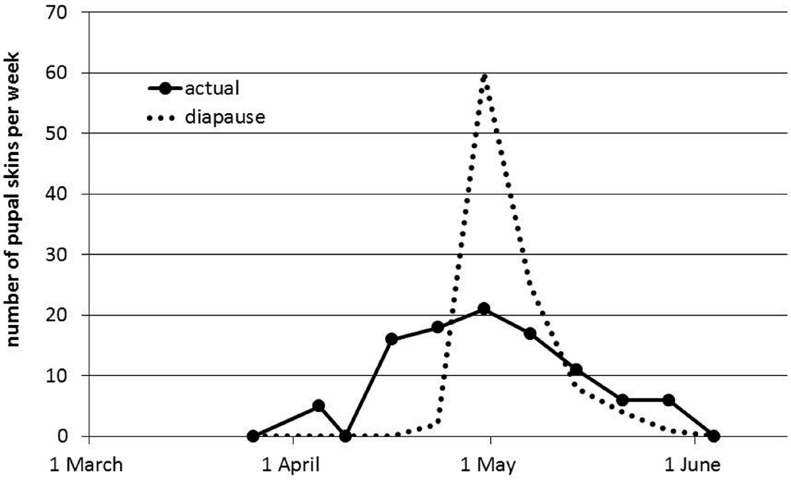

Figure 3. Emergence pattern of the wintering generation from the Appingedam vase in spring 2014 (black dots and solid line) and hypothetical emergence pattern when larval development would have been interrupted by a diapause (dotted line). Number of pupal exuviae are added up per week.

2013. On the other hand, 121 4th instar and 6 3rd and/or 2nd instar larvae of *Chironomus riparius* (Chironomidae, subfamily Chironominae) were counted, as well as 12 4th instar larvae of *Zavrelimyia* sp. (Chironomidae, subfamily Tanypodinae). Larvae of *M. carmencitabertarum* were also not found in an earlier study in December 2011 when larvae of *C. riparius* and *Zavrelimyia sp.* were also present (Kuper & Moller Pillot 2012). From May until October 2013, at least three generations of *C. riparius* emerged. A total of 413 pupal exuviae were collected, exceeding the 39 *M.* (*I.*) *carmencitabertarum* pupal exuviae by far.

Phenology

Data on adult activity in 2012 and 2013 in Appingedam and Nijmegen and of larval activity in 2013 in Nijmegen are presented in Fig. 4. The initial and final records of adult activity differed between years and between sites. The number of adult activity peaks, representing emergence of different generations, differed between sites, giving two or perhaps three generations per year in Appingedam (Fig. 4 A, B) and five generations in Nijmegen (Fig. 4 C, D), including the wintering generations that emerged in spring.

In Appingedam the first adult activity in 2012 was observed on 28 April, whereas in the following year it was three weeks later, on 18 May 2013. The final record of adult activity in 2012 was on 17 November, the same date as in 2011 (Kuper & Moller Pillot 2012). In 2013 however the final record of adult activity was on 7 September, more than two months earlier than other years. Records of adult *M.* (*I.*) *carmencitabertarum* in the spring of both years, followed by a period of no activity, probably depicts the emergence of the wintering generation. These individuals must have originated from the neighbourhood, as larvae did not winter in the bucket. After that, no well-defined peaks of adult activity were seen in 2012, which made it impossible to distinguish generations. In 2013 a continuous period of emerging adults (by means of the presence of pupal exuviae) was evident from the beginning of July up and until the first week of September. This might reflect a long period of adults emerging from the eggs that were deposited in spring. Then two generations would have been present in 2013. Yet, within the three weeks observation gap around the beginning of August, a period of no emergence might have occurred, which then would suggest the presence of an additional generation in August. Then three generations, including the wintering generation, emerged.

In Nijmegen the first adult activity in 2012 was observed on 22 May, when the wintering generation was presumably at peak of emergence, indicated by the finding of 30 adults, 20 egg strings, one pupal skin and one pupa. The final adult activity in 2012 was recorded on 2 October, one and a half months earlier than in Appingedam. This could not be linked with ambient temperature differences between the two sites. In 2013, the first observation of adult activity was on 10 June, when the first summer generation emerged. No wintering larvae were present in the butt as it was newly placed in

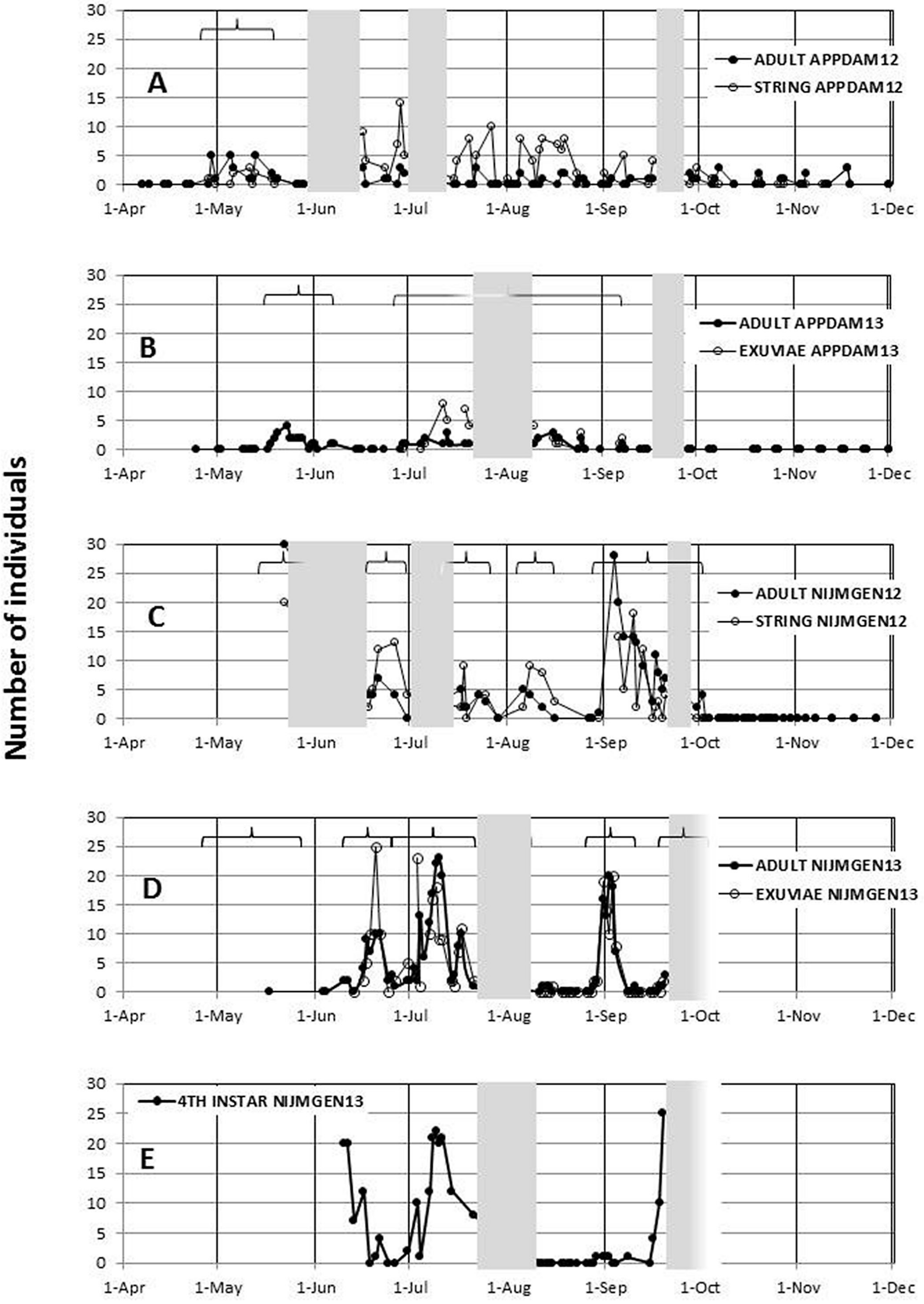

Figure 4 A-E. Numbers of adults, egg strings and pupal exuviae of *Metriocnemus* (*Inermipupa*) *carmencitabetarum* recorded between April and December in 2012 and 2013 at Appingdam and Nijmegen and of 4th instar larvae at Nijmegen in 2013: 4A number of adults and egg strings at Appingedam, 2012; 4B number of adults and pupal exuviae at Appingedam 2013; 4C number of adults and egg strings at Nijmegen 2012; 4D number of adults and pupal exuviae at Nijmegen 2013; 4E numbers of 4th instar larvae recorded in the upper 50 cm of the water butt at Nijmegen, 2013. Horizontal brackets indicate periods of adult activity of one generation. Periods with no observations are shaded.

April. Egg deposition by the wintering generation (which must have emerged elsewhere in the neighbourhood), presumably in May, was missed. The final observation was on 20 September, after which the butt was removed and observations had to be ended. No other chironomid species were found in Nijmegen, except from the middle of September 2013 when *C. riparius* started to emerge.

The population in Nijmegen showed well-defined peaks of activity in 2012 and 2013, indicating emergences of consecutive generations. The start of the last emergence bout on 19 and 20 September 2013 was abruptly interrupted by the removal of the butt. This emergence bout was accompanied by a steep increase of fourth instar larvae moving up to the top of the butt as was also seen during the emergence of adults in June and July 2013 (Fig. 4 E). Thus, in both years five generations (including the wintering generation) emerged from May until September. From 11 August 2013 onwards, a two and a half week period with no larval and hardly any adult activity was observed. In the second half of July up to and including the first week of August, 2013, water volume in the butt decreased by at least 60% as water was used for gardening purposes. Observations re-commenced on 11 August when the butt was filled again with rain water. At the end of August the adults of this generation emerged in a time frame of seven to ten days, with the majority emerging in a period of just four days. This emergence was not accompanied by 4th instar larval activity along the sides of the butt. In June, July and September 4th instar larval activity was at its peak during or just before emergence.

Generation development time

At Appingedam the development time of the (first) summer generation was between 31 and 49 days in 2012. An exact number of days cannot be given because the first activity of the summer generation probably took place in a period when no observations were made and a clear activity peak was not observed (Table 1, Fig. 4 A). In 2013 the development time of the (first) summer generation was 42 days (Table 1, Fig. 4 B). At Nijmegen in 2012, the development time of summer generations ranged from 21 days for the third summer generation in August to ca. 30 days for the first summer generation in June (Table 1, Fig. 4 C). In 2013 the development time ranged from 19 days for the second summer generation in July up to 55 days for the third summer generation at the end of August (Table 1, Fig. 4 D). The latter included a diapause of at least two and a half weeks (see Phenology). The overall, mean development time for a generation in the summer period in Nijmegen (excluding the generation that probably had a diapause) was 24.2 ± 4.4 days.

Seasonal length differences in pupal exuviae

A seasonal difference in length of male and female pupal exuviae was observed in the Nijmegen population during the summer of 2013 (Fig. 5), with a sharp increase of pupal skin size at the beginning of the summer. The model 'mean ambient temperature during development * skin size with sexes distinguished' tested highly significant (ANOVA, $R^2 = 0.74$, $p<0.001$). Pupal skin size of males increased clearly less markedly with increasing mean temperature during development compared to those of females (Fig. 6).

Discussion

Phenology

In Appingedam, over-wintering larvae were absent and were not found in two thorough searches in December 2011 and 2013. Adults from the neighbourhood recolonised the bucket each spring. The

Table 1. Generation development time in days. The generation concerned is denoted after the arrow under 'generation'. DT is development time of a generation. For Appingedam 2012 only a minimum and maximum for DT could be given.

Site and year	Generation	Measuring point	Period	DT (days)
Appingedam 2012	winter → 1st	first adults	28 Apr - 29 May	31 (min)
	winter → 1st	first adults	28 Apr - 16 Jun	49 (max)
Appingedam 2013	winter → 1st	first adults	18 May - 29 Jun	42
Nijmegen 2012	winter → 1st	peak adults	~22 May - 21 Jun	~30
	1st → 2nd	peak adults	21 Jun - 18 Jul	27
	2nd → 3rd	peak adults	18 Jul - 8 Aug	21
	3rd → 4th	peak adults	8 Aug - 4 Sep	27
Nijmegen 2013	1st → 2nd	peak adults	20 Jun - 9 Jul	19
	2nd → 3rd	peak adults	9 Jul - 2 Sep	55 (diapause)
	3rd → 4th	first adults	28 Aug - 18 Sep	21

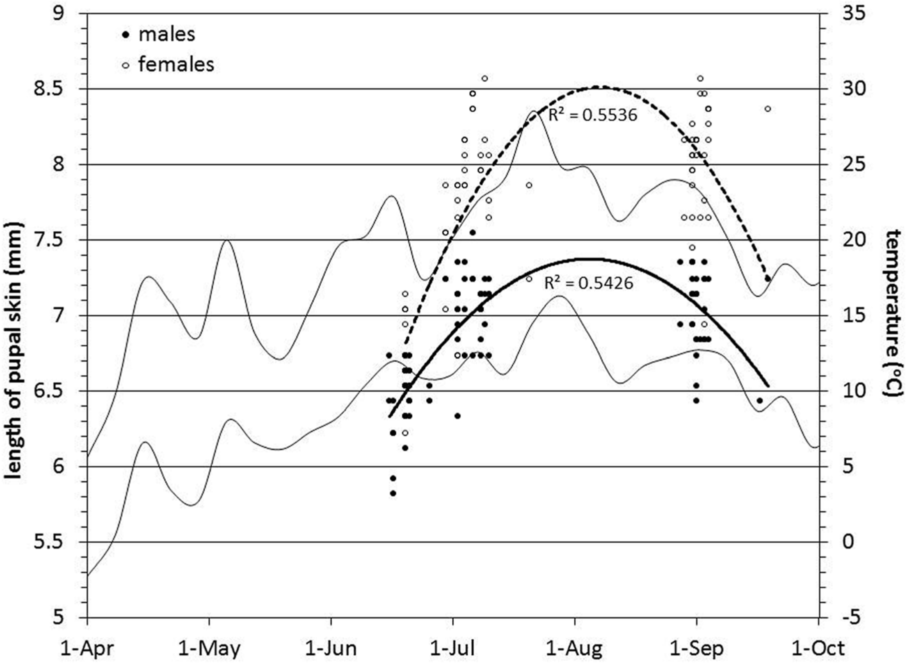

Figure 5. Seasonal length differences in male and female pupal exuviae in Nijmegen in 2013, with a binomial fit (left y-axis). The two wavy lines represent mean weekly minimum and maximum temperatures from April to October (right y-axis).

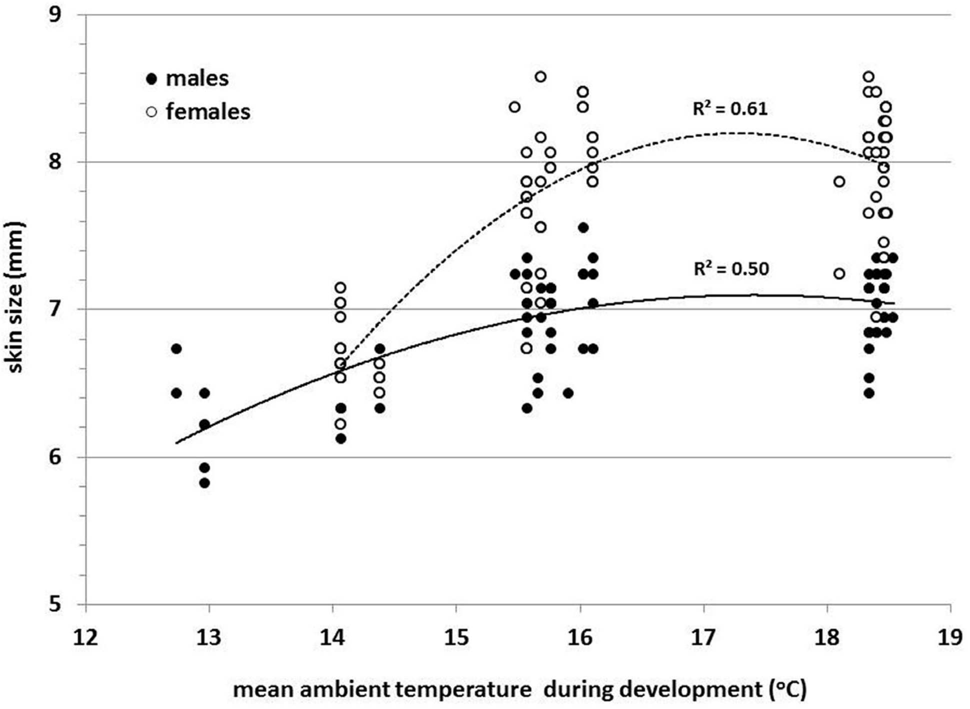

Figure 6. Correlation between mean ambient temperature during development and skin size for males and females from the Nijmegen water butt in 2013.

first observations of adult activity in 2012 were made at the end of April and three weeks later in 2013, in the middle of May. This three week difference is most probably caused by a colder period from the midst of February until the end of March in 2013 compared to 2012. The first observations of emergence of the wintering generation in 2012 in Nijmegen were made on May 22, which was presumably a peak in emergence following the onset of emergences around the middle of May. This was about two to three weeks later compared to first observations of adults in Appingedam in 2012. As the volume of the water butt was large (200 litres) it is possible that temperature in the butt was increasing more slowly than in the unknown habitat of the wintering population(s) in Appingedam in late winter and spring. The rate of development of chironomid larvae generally increases with increasing ambient temperatures (e.g. Mackey 1977, Pinder 1986). Differences in winter/spring temperatures and differences in the warming up rate of the larval habitat might thus explain observed differences in timing of emergence of the wintering populations between years and between habitats.

The final records of adult activity differed markedly between years in Appingedam and between Appingedam and Nijmegen. As autumn temperatures did not differ much between years and between both sites, factors other than temperature determined the end of adult activity.

In Ireland, *M.* (*I.*) *carmencitabertarum* adults were active from the end of March until the middle of November in 2012 and from the middle of April until at least the end of October in 2013 (Murray 2013). The earlier onset of emergence compared to The Netherlands is probably due to the more temperate winter in Ireland compared to The Netherlands, as minimum temperatures rarely reach values below zero degrees Celsius, in contrast to the Netherlands. The earlier start of the adult activity period in 2012 was probably influenced by a warmer winter compared to 2013, as was the case in the Netherlands.

Diapause

Results suggested that larvae of *M.* (*I.*) *carmencitabertarum* did not have a winter diapause. Spring emergence of adults from the wintering population was not synchronized but had a Gaussian distribution shape (Fig. 3). Emergence took place over a period of almost two months. For a winter diapause, a shorter and skewed period of emergence, as depicted in Fig. 3, would have been expected, because most (or all) larvae would be in a distinct developmental stage during winter (Goddeeris 1983, Goddeeris 1986). Also, the presence of only 3rd and 4th instar larvae, with the latter in a small majority in December 2013, suggested that larvae were not in diapause. Many species have a diapause in the 2nd to 4th instar (Grodhaus 1980, Goddeeris 1983). In several *Psectrocladius* spp. an accumulation of 2nd and 3rd instars with almost no 4th instars was found in autumn while one *Psectrocladius* sp. was found in early 4th instar only. These finds suggested a diapause triggered by the shortening of days in autumn (Goddeeris 1983, Goddeeris 1986, Moller Pillot 2013). Goddeeris in Moller Pillot (2013) states that the 3rd larval stage would be the most appropriate for a winter diapause.

A long period of inactivity in August 2013 in the Nijmegen butt, followed by a sudden and short high-peaked emergence of adults, suggested a summer diapause. Emergence peaked in a period of only four days. No (4th instar) larvae were observed just before or simultaneously with the emergence period which was in contrast with emergence periods in June, July and September. This diapause might have been triggered by a large decrease in water volume in the butt at the end of July, possibly in combination with high temperatures. To avoid disturbance, no butt bottom samples were taken. Therefore, although diapause was not actually confirmed, it seems the most probable explanation for these observations.

Number of generations and generation development time

At least two generations were noted in Appingedam in 2012 and 2013. In Nijmegen five generations per year were recorded in 2012 and 2013. Exact information about number of generations for other *Metriocnemus* species in Western Europe is not available but some species can be found all year round, implying that several generations per year will develop (Moller Pillot 2013). Some similar sized Orthocladiinae of stagnant waters, e.g. *Psectrocladius* spp. and *Cricotopus* spp. (Moller Pillot 1984), have two to seven generations per year (Moller Pillot 2013).

In Appingedam the development time of a generation in the summer was about 42 days. Without taking the diapause generation into account, average development time per generation in Nijmegen was 24.2 days (19-ca. 30 days) during the summer. For *Psectrocladius* spp. and *Cricotopus* spp., development time of a generation is one to four months (Moller Pillot 2013). In *P. limbatellus*, mass emergence was found 18-20 days after colonisation of sand filter beds (Wotton et al. 1992). Thus in Nijmegen the summer generations of *M.* (*I.*) *carmencitabertarum* developed faster than has generally been found in other Orthocladiinae species of similar size in natural habitats, and is only little longer than was found for the fastest development in *P. limbatellus*, which probably had close to optimal growing conditions.

The longer development time in Appingedam with only one or two generations in the summer, was probably caused by competition from *Chironomus riparius*. Larvae of this species were present year-round in the Appingedam bucket, by far exceeding the number of *M.* (*I.*) *carmencitabertarum* larvae. In the Nijmegen butt a generation of *C. riparius* larvae was only present in September 2013, but this did not slow down development time of *M.* (*I.*) *carmencitabertarum* larvae (table 1). As the Nijmegen butt was much larger than the Appingedam bucket, interspecific competition in the butt was probably low. In general, high (interspecific) competition pressure slows down development rate, thus time for development increases (e.g. Vallenduuk and Moller Pillot 2007, Moller Pillot 2013).

Temperature effect on pupal skin length and the Temperature-Size Rule

A temperature dependent correlation was found for pupal skin length and average temperature during development. According to the temperature-size rule (TSR) body size in ectotherms decreases with increasing, yet not stressful, temperatures, although exceptions exist (Atkinson 1994, Atkinson 1995). The TSR also applies to chironomids (Eggermont and Heiri 2011), as was shown in several laboratory experiments (e.g. Frouz et al. 2002, Dettinger-Klemm 2003, McKie and Cranston 2005, Baek et al. 2012). A decrease in length with increasing temperatures is the outcome of a more accelerated development rate compared to growth rate (e.g. Mackey 1977).

M. (*I.*) *carmencitabertarum* seems to be one of the species to which the TSR does not apply. Atkinson (1995) poses four possible mechanisms to explain exceptions to the TSR. One that seems applicable here, is that if ectotherm predators are present (in aquatic habitats), it would be advantageous for prey species to be small when temperatures increase, because feeding rates of ectotherm predators generally increase with increasing temperatures (Atkinson 1995). In this study ectotherm predators were only present in the Appingedam bucket in the winter as 4th instar *Zavrelimyia* larvae. Whether these individuals were responsible for *M.* (*I.*) *carmencitabertarum* larvae not being present may be questioned. Larvae of *Zavrelimyia* probably feed on small and less mobile prey species (see Vallenduuk & Moller Pillot 2007). Up to third instar larvae of *M.* (*I.*) *carmencitabertarum* are probably small enough but are quite mobile. Thus M. (I.) carmencitabertarum larvae may not be the preferred prey type for *Zavrelimyia* larvae. In the small-sized, possibly temporary, habitats of *M.* (*I.*) *carmencitabertarum* the chance of ectotherm predators being present, is probably low. Thus for *M.* (*I.*) *carmencitabertarum* there is a low pressure to grow small when temperatures are high to decrease chance of predation.

Northward bound?

Murray (2013) suggested that *M.* (*I.*) *carmencitabertarum* had recently arrived in Ireland as the species was not present in his extensive 50-years collection from Ireland. Moreover, because the pupal exuviae are very characteristic and easy to identify (see also Murray 2016) and the species is found in anthropogenic habitats, the chance that the species has been overlooked until recently seems very unlikely. Therefore, the recent finds of *M.* (*I.*) *carmencitabertarum* in England and Ireland and in The Netherlands in a short time frame indeed suggest an expansion of the species´ range. Could this expansion be from southern origin? There are some indications that this might be the case. More northern reports of *M.* (*I.*) *carmencitabertarum* were not confirmed, indicating that the species is probably not present in Poland and Estonia and certainly not in Spitsbergen. Thus, it does not seem likely that *M.* (*I.*) *carmencitabertarum* is migrating from northern or northeastern directions. The species does not have a diapause in winter to overcome freezing conditions nor do the larvae build cocoons when temperatures are near freezing point (see e.g. Oliver 1971, Frouz et al. 2003, Specziár 2008). To the south of The Netherlands winters are milder. For example, winter temperatures in north-western Spain almost never reach sub-zero values, with no freezing up of *M.* (*I.*) *carmencitabertarum* habitats. Here, adjustments to overcome low temperatures are therefore not needed. According to Hellmann's formula to describe winter temperatures, twelve winters were 'mild', 'very mild' or 'extraordinary mild' while seven winters were 'average' after 1997 until 2017 in The Netherlands (KNMI 2017). Although frost periods were present, winters in general were apparently mild enough for *M.* (*I.*) *carmencitabertarum* populations to survive since its first discovery in August 2011.

If the conclusion is justified that *M.* (*I.*) *carmen-*

citabertarum has recently shifted its range northwards, then it is predicted that the species will be found in Scotland and in the north of Germany and/or in the south of Denmark in the near future.

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A REDESCRIPTION OF *ZAVRELIA BRAGREMIA* GUO & WANG, 2007 (DIPTERA: CHIRONOMIDAE)

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Abstract

Examination of the holotype of *Zavrelia bragremia* Guo & Wang, 2007, revealed a few mistakes in the original description. Based on the type material and additional adult males from Oriental China, *Z. bragremia* is here redescribed and figured. An updated identification key to the males of *Zavrelia* is given.

Introduction

The genus *Zavrelia* was erected by Kieffer, Thienemann and Bause in Bause (1913) with *Z. pentatoma* as the type species. The genus belongs to the subtribe Zavreliina within tribe Tanytarsini, subfamily Chironominae and was reviewed by Ekrem and Stur (2009). At present, there are eleven valid species: *Z. pentatoma* Kieffer & Bause in Bause, 1913, *Z. simantoneoa* (Sasa, Suzuki & Sakai, 1998), *Z. tusimatijea* (Sasa & Suzuki, 1999), *Z. clinovolsella* Guo & Wang, 2004, *Z. bragremia* Guo & Wang, 2007, *Z. elenae* Zorina, 2008, *Z. pseudopentatoma* Zorina, 2008, *Z. aristata* Ekrem & Stur, 2009, *Z. casasi* Ekrem & Stur, 2009, *Z. hudsoni* Ekrem & Stur, 2009 and *Z. sinica* Ekrem & Stur, 2009 (Ekrem and Stur 2009, Kobayashi 2014). Only four species of the genus *Zavrelia* have previously been recorded in China: *Z. clinovolsella*, *Z. bragremia*, *Z. pentatoma* and *Z. sinica* (Ekrem and Stur 2009). The known immature stages of most *Zavrelia* species occur in streams and rivers; except *Z. pentatoma* which is found in standing waters.

Here, we redescribe *Z. bragremia* based on the holotype and additional recently collected specimens, and give an updated identification key to the adult males of *Zavrelia*.

Material and Methods

Field work was conducted in China to supplement material for *Z. bragremia*. Studied specimens were slide-mounted in Euparal, following the procedures outlined by Sæther (1969). Morphological terminology follows Sæther (1980). Colouration is based on slide-mounted specimens. Measurements are given as ranges followed by the mean, when four or more specimens are measured, followed by the number of specimens measured (n) in parentheses. Digital photographs were taken with a resolution of 300 dpi using a Leica DFC420 camera mounted on a Leica DM6000 B compound microscope using bright field setting and the software Leica Application Suite 4.8. The holotype is deposited in the College of Life Sciences, Nankai University, Tianjin, China (BDN); several examined specimens are deposited in the NTNU University Museum (NTNU-VM).

Zavrelia bragremia Guo & Wang, 2007

(Figs 1–10)

Zavrelia bragremia Guo & Wang, 2007: 318, figs 1–3. Holotype ♂ (BDN: 5486), China, Sichuan Province, Emeishan City, 29.60°N, 103.48°E, 17.v.1986, XH Wang.

Additional examined specimens. 8♂♂ (BDN: G5A49, G5A53–56, G5A69; NTNU-VM: G5A4, G5A7), China, Zhejiang Province, Jinhua City, Pan'an County, Dapanshan National Nature Reserve, Huaxi Village, near a stream, 28.792°N, 120.525°E, 800 m a.s.l., 17–21.vii.2012, light trap, XL Lin.

Diagnosis

The adult male of *Zavrelia bragremia* can be distinguished from known species of *Zavrelia* by the following combination of characters: wing length 0.88–1.04 mm; AR 0.91–1.08; frontal tubercle minute; LR_1 about 2.09; laterosternite without seta; anal point with small spinulae between the anal crests at base, but without microtrichia; setiger of superior volsella without conspicuous constriction in apical 1/3.

Description

Adult male (n = 5, unless otherwise stated). Total length 1.24–1.34, 1.29 mm. Wing length 0.88–1.04, 0.95 mm. Total length/wing length 1.31–1.52, 1.38.

Colouration. Head capsule and mouthparts light brown, antenna and eyes dark brown to black. Thorax ground colour brown with dark brown stripes anteriorly on scutum, laterally under parapsidal suture, postnotum and on preepisternum, scutellum paler; legs and abdomen brown.

Head (Figs 1–2). Antenna (Fig. 1) with 10 flagellomeres, ultimate flagellomere 260–295, 278 µm long. AR 0.91–1.08, 0.98. Frontal tubercles minute, 3–4, 3 µm long, 3–4, 3 µm wide at base. Eye (Fig. 2) hairy, reniform, without dorsomedial extension. Temporal setae 5–6, 5. Clypeus with 8–10, 9 setae. Tentorium 88–98, 93 µm long, 8–15, 12 µm wide. Palpomere lengths (in µm): 20–27, 23; 25–30, 27; 65–75, 68; 78–83, 81; 110–120, 115. Third palpomere with one sensillum clavatum distally.

Thorax chaetotaxy (Fig. 3). Acrostichals 6 (n = 4); dorsocentrals 5–6, 6; humerals 0; prealars 1–2, 1; scuttellars 4; haltere with 3–6, 4 setae.

Wing (Fig. 4). VR 1.21–1.35, 1.28. Brachiolum with one seta, Sc bare, R with 17–25, 20 setae, R_1 with 16–20, 17 setae, R_{4+5} with 15–20, 17 setae, M_{1+2} with 40–53, 47 setae, M_{3+4} with 14–18, 16 setae, false vein with 78–86, 81 setae, Cu with 14–17, 16 setae, Cu_1 with 10–12, 11 setae, PCu with 36–43, 40 setae, An with 17–18, 18 setae, remaining veins bare. Cell r_{4+5} with c. 190–210, 201 setae, m with 3–8, 5 setae, m_{1+2} with c. 180–200, 190 setae, m_{3+4} with 62–80, 71 setae, cu+an with 85–100, 91 setae, remaining cells bare. Anal lobe strongly reduced.

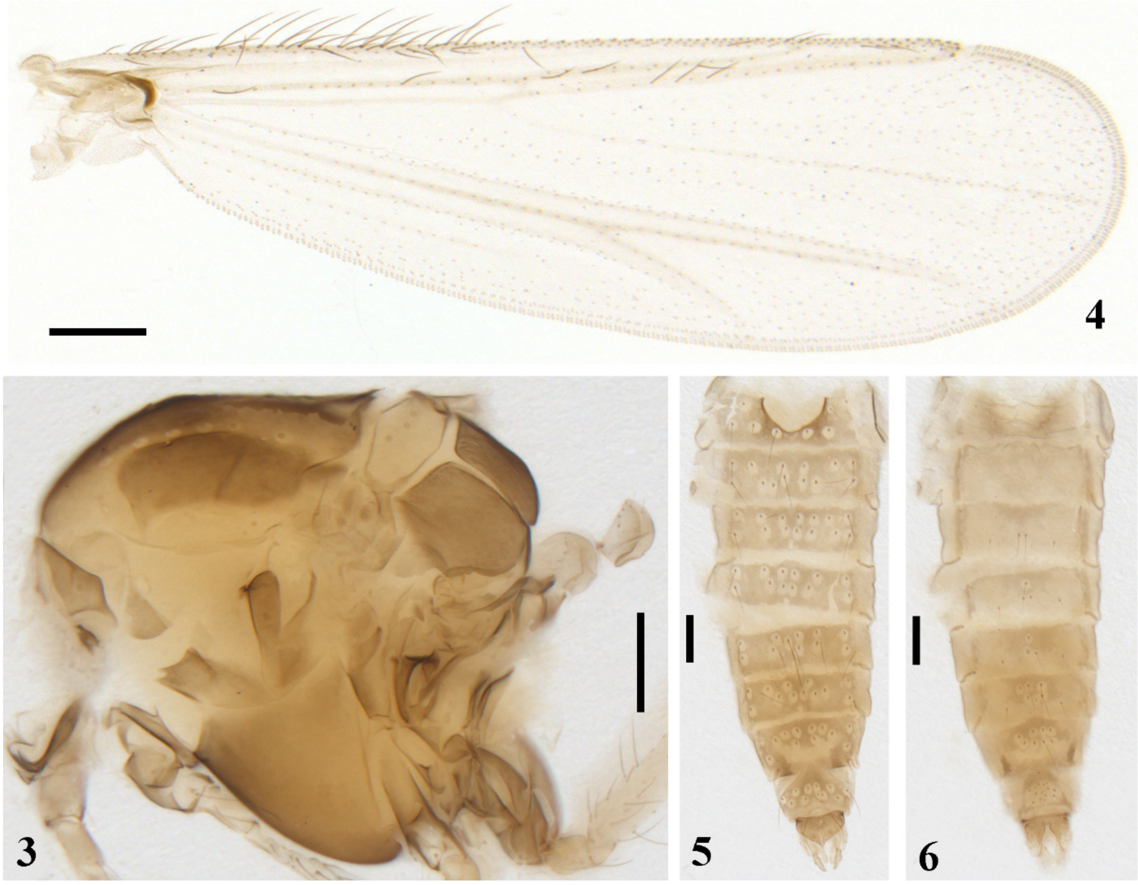
Legs. Fore leg bearing single tibial spur, 10–18, 14 µm long. Combs of mid tibia 13–18, 15 µm wide with 13–15, 14 µm long spur, and 11–13, 12 µm

Table 1. Lengths (in µm) and proportions of legs for *Zavrelia bragremia* Guo & Wang, adult male.

	fe	**ti**	**ta$_1$**	**ta$_2$**	**ta$_3$**	**ta$_4$**
p_1	309–490, 435	220–250, 235	460 (n = 1)	240 (n = 1)	190 (n = 1)	150 (n = 1)
p_2	410–480, 458	320–360, 340	220–240, 225	85–100, 90	50–70, 57	30–50, 45
p_3	470–560, 512	360–400, 388	240–300, 270	145–170, 150	120–150, 136	70–90, 80

	ta$_5$	**LR**	**BV**	**SV**	**BR**
p_1	70 (n = 1)	2.09 (n = 1)	1.65 (n = 1)	1.33 (n = 1)	3.76 (n = 1)
p_2	25–43, 34	0.64–0.70, 0.67	4.13–5.18, 4.52	3.24–3.70, 3.51	2.67–5.40, 3.92
p_3	50 (n = 4)	0.66–0.72, 0.69	2.77–2.83, 2.79	3.25–3.46, 3.32	3.86–5.33, 4.45

Figures 1–2. *Zavrelia bragremia* Guo & Wang, 2007, male. 1, head and antenna, scale = 100 µm; 2, eye, scale = 50 µm.



Figures 3–6. *Zavrelia bragremia* Guo & Wang, 2007, male. 3, thorax; 4, wing; 5, abdomen, dorsal view; 6, abdomen, ventral view. Scales = 100 µm.

wide with 10–13, 12 µm long spur; combs of hind tibia 15–20, 17 µm wide with 15–20, 18 µm long spur, 15–18, 17 µm wide with 10–15, 13 µm long spur. Sensilla chaetica on basitarsus of mid leg absent. Lengths (in µm) and proportions of legs as in Table 1.

Abdomen (Figs 5–6). Setation see Figs 5–6.

Hypopygium (Figs 7–10). Laterosternite IX without seta. Anal tergite 56–68, 62 µm long, with 3–7, 5 median setae, 16–20, 18 apical setae. Anal point well developed, 17–20, 19 µm long, with 17–30, 23 small spinules scattered between anal crests at base, apex rounded; no microtrichia free area around base of anal point. Transverse sternapodeme 30–41, 37 µm long, without oral projections. Phallapodeme 45–75, 59 µm long. Superior volsella broadly digitiform with pointed apex, bearing two anteromedian and 3–5, 4 dorsal setae; microtrichia apparently absent. Median volsella (Fig. 10) short, knob-shaped, with 3–4, 4 medially directed simple and subulate lamellae. Inferior volsella slightly curved, 45–55, 51 µm long, bearing 6–9, 7 setae in distal part. Gonocoxite 65–73, 70 µm long. Gonostylus 38–45, 41 µm long, curved inwards, broadest at middle, tapering to narrowly rounded apex. HR 1.55–1.83, 1.67, HV 2.62–3.35, 3.03.

Immature stages and adult females. Unknown.

Identification key to adult males of *Zavrelia*

1. Anal point densely covered with strong spines …... *Z. pentatoma*

– Anal point bare, with microtrichia or short spinules only …... 2

2. Setiger of superior volsella with conspicuous constriction in apical 1/3 ….................................. 3

– Setiger of superior volsella without conspicuous constriction in apical 1/3 ….................................. 5

3. Setiger of superior volsella with pointed apex .. ..… *Z. clinovolsella*

– Setiger of superior volsella with somewhat rectangular apex …... 4

4. Anal point with only few microtrichia in between crests; distinct microtrichia-free areas on anal tergite around base of anal point …... *Z. sinica*

– Anal point with numerous microtrichia in between crests; microtrichia present all around base of anal point .... *Z. pseudopentatoma*

5. Anal point with small spinules in between crests 6

– Anal point bare, or with microtrichia in between crests at most 7

6. AR 1.23; wing length about 1.50 mm; LR_1 about 1.45; laterosternite with one seta; anal point with small spinules scattered between entire length of anal crests .... *Z. tusimatijea*

– AR 0.91–1.08; wing length 0.88–1.04 mm; LR_1 about 2.09; laterosternite without seta; anal point with small spinules scattered between anal crests at anal point base only .... *Z. bragremia*

7. Anal point bare 8

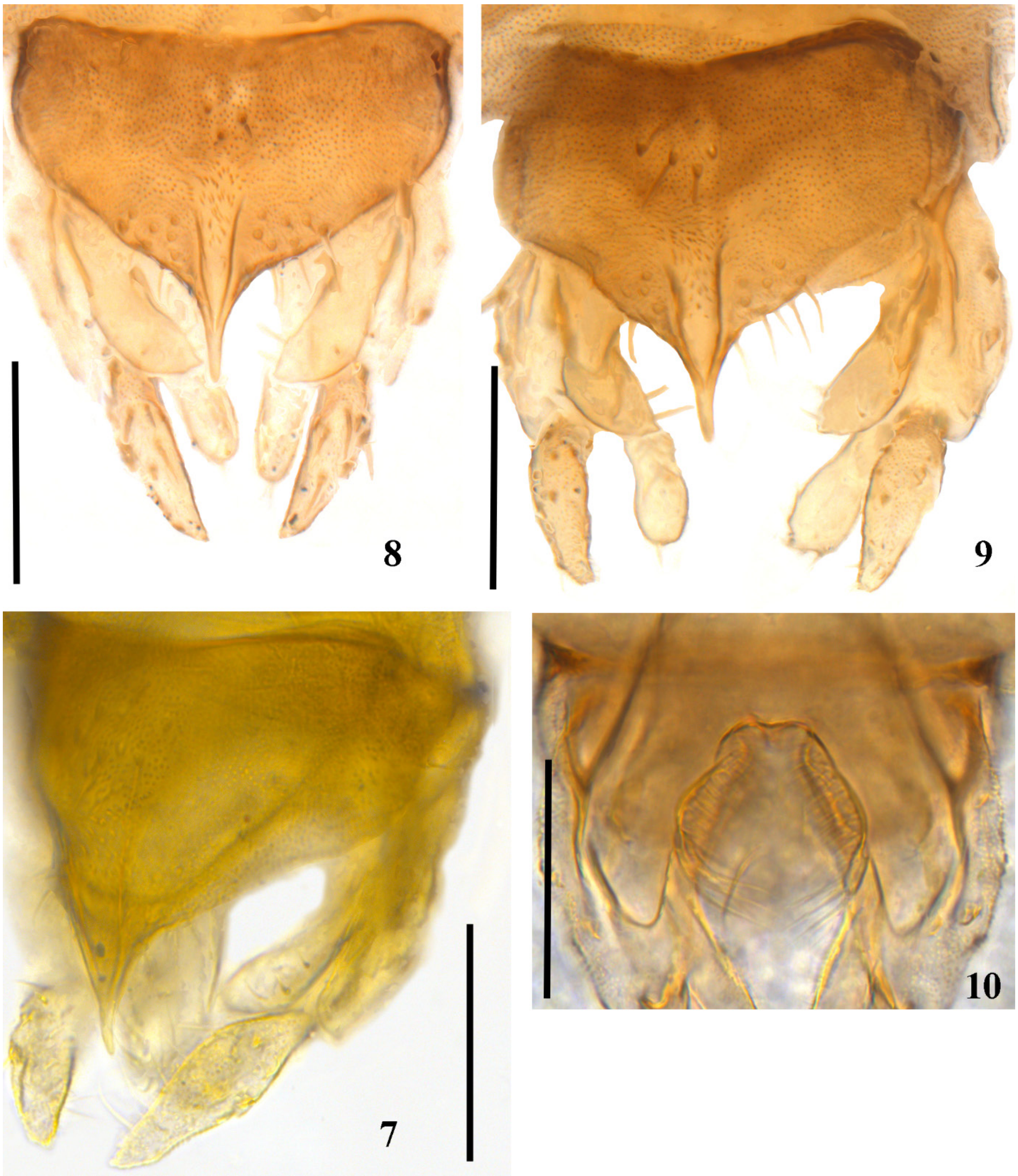
– Anal point with microtrichia in between crests . 9

8. AR 1.00–1.18; LR_1 1.36–1.46; superior volsella with pointed apex .... *Z. elenae*

– AR 0.45; LR_1 1.96; superior volsella with rounded apex .... *Z. simantoneoa*

9. Wing length c. 1.40 mm; AR c. 0.75.. *Z. hudsoni*

– Wing length c. 1.00 mm; AR c. 0.90... *Z. aristata*



Figures 7–10. *Zavrelia bragremia* Guo & Wang, 2007, male. 7, holotype hypopygium, dorsal view; 8, hypopygium (BDN: G5A69), dorsal view; 9, hypopygium (BDN: G5A53), dorsal view; 10, median volsellae. Scales = 50 µm.

Discussion

Zavrelia bragremia was described based on a single specimen from Sichuan, China by Guo and Wang (2007). By reexamining the holotype, we found that some diagnostic characters in the original description require emendation. For instance, the anal tergite bears three to seven median setae; the anal point does not have two spines (an artifact resulting from slide preparation might have been interpreted as spines, Fig. 7); the anal point has scattered spinules between anal crests at anal point base only. The only complete recently collected specimen of *Z. bragremia* from Oriental China has also a higher LR_1 value (2.09) – the highest within all known *Zavrelia*. This was previously unknown since the fore tarsi are missing in the holotype.

Due to slide preparation methods and a distinct variability of *Zavrelia* species, it is extremely difficult to observe and define key characters to accurately separate species on the basis of their morphology. An intraspecific variability of the type species *Zavrelia pentatoma* was detected by Giłka (2008). On the other hand, some morphological variable characters of *Z. pentatoma* have been found as diagnostic in other species like the presence of an obvious constriction in the apical 1/3 of the setiger of the superior volsella (see identification key). Cryptic species, particularly within some of the more widely distributed *Zavrelia* are thus expected to be revealed. A complete DNA barcode reference library of *Zavrelia* would be beneficial to explore the species boundaries within this genus, since DNA barcoding (Hebert et al. 2003a, b) has proven effective in biodiversity assessments and taxonomic revisions (e.g. Anderson et al. 2013, Lin et al. 2017). At present (August 2017), however, there is only one species, *Zavrelia pentatoma*, with DNA barcodes in the Barcode of Life Data Systems (BOLD, http://www.boldsystems.org/).

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Impact of the bird-manure caused eutrophication on the abundance and diversity of chironomid larvae (Diptera; Chironomidae) in lakes of the Bolshoy Aynov Island (Russia, Barents Sea)

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Abstract

We report on the perceived impact of bird faeces as an agent of organic matter transfer on the density and species richness of the Chironomidae in the lakes of the Bolshoy Aynov island (Barents Sea, Northern Russia). In total we recorded 15 species of Chironomidae. Highest species richness and specimen numbers were recorded in Lake Severnoe, closest to the large bird colony.

Introduction

Sea bird manure is an important source of nutrient transfer from aquatic ecosystems to terrestrial (Ellis et al. 2006). It is well known that removal of phosphorus and nitrogen from marine environments to terrestrial contributes to soil formation on the isles. Influx of nutrients with bird manure is crucial to maintain productivity of the northern aquatic systems, which are normally oligotrophic or dystrophic. Bird manure is also important for the formation of ornithogenic soils and waters (Tatur 2002). In some areas, like the maritime Antarctic, high Arctic and northern Europe, such ornithogenic landscapes are common and important for the functioning of the coastal and inland freshwater ecosystems (Tatur 2002). In this short communication, we will examine the Chironomidae communities in several small lakes of the Bolshoi Ainu Island in the secluded Ainu archipelago in the Barents Sea, close to Murmansk, Russia. This island is an important sea bird sanctuary, inhabited by large colonies of puffins, cormorants and little gulls (Khleboslov et al. 2005); thus, we hypothesized that this site would be a suitable place to study possible bird colonies impact on freshwater Chironomidae species richness and density.

Materials and Methods

Bolshoy Aynov Island is in the eastern part of Varangerfjord, Barents Sea (Russia). The island is about two kilometers long and about one and a half kilometers wide. The landscape is mainly flat (Fig 1). The vegetation is typical for the southern tundra. The climate of the Island is warmer than the climate of the adjacent coast of the Kola Peninsula because it is heated by the North Atlantic Current. The Bolshoy Aynov Island is protected and included in the Kandalaksha State Nature Reserve. The island has five small lakes (about 5-20 meters length) and one relatively large lake (more than 100 meters in length).

The material was collected on seven sites from four small lakes of Bolshoy Aynov Island. All the samples were collected by using a hand-net with mesh size 1000 µm during mollusc collection, thus, due to the inappropriate mesh-size, smaller specimens probably were lost. Samples were preserved in formaldehyde. The samples were sorted by hand using an MBS-10 dissecting microscope with x56 magnification. The site descriptions are given in table 1.

Table 1. Characteristic of the collection sites.

Lake name	Substratum	Depth, cm
Maloe (Site 1)	Silt with dead vegetation	40-50
Maloe (Site 2)	Dead organic matter, living *Fontinalis* sp. and algae	50
Maloe (Site 3)	*Fontinalis* sp.	60-70
Srednee	Dead organic matter	70
Severnoe (Site 1)	Silt, dead organic matter	15
Severnoe (Site 2)	Silt, dead organic matter	120
Zapadnoe (Site 1)	Silt, dead organic matter, sand	40

Figure 1. Landscape of the Greater Ainu Island.

The morphological terms are according to Sæther (1980). Larvae were identified using the following keys: Pankratova 1970, 1977, 1983 and Epler et al. 2013.

Results

Seven sampled sites yielded 15 species of Chironomidae larvae, belonging to three subfamilies (Table 2). Among them two species of Tanypodinae, nine species of Chironominae and four species of Orthocladiinae.

Highest species richness of eleven species was recorded for Lake Severnoe, while other lakes have richness of one-five species. Species of the subfamily Orthocladiinae were only recorded on the near-shore site of Lake Severnoe. Highest specimen density was recorded on the second site of Lake Severnoe, with the water depth 1.2 meters. This site was characterized by high numbers of *Glyptotendipes paripes* and *Procladius sp.*

Discussion

Among all the lakes sampled, Lake Severnoe was remarkably different in terms of chironomid species richness and diversity. This difference was probably caused by the proximity of a colony of marine birds, mainly puffin (*Fratercula arctica* (Linnaeus, 1758)) and cormorant (*Phalacrocorax carbo* (Linnaeus, 1758)). Nutrients from the faeces deposited in this colony are often washed away by the rains into the nearby Severnoe lake. In this lake a number of the species are traditionally associated with high nutrient load (*Procladius* sp*.*, *Chironomus* sp.); there were also numerous phytophagous species (*Cricotopus* sp*.*, *Glyptotendipes paripes* (Edwards, 1929)*, Psectrocladius obvius* (Walker, 1856)) (Epler et al. 2013). We hypothesize that the higher species richness and specimen numbers of the Chironomidae larvae in Lake Severnoe were promoted by increased organic enrichment leached from bird faeces. The other lakes were further away from bird colonies, resulting in a lower influx of ornithogenic nutrients. Further research into the matter is required in order to assess the impact of the organic matter transfer by birds from the sea into the freshwater ecosystems.

Table 2. List of chironomid larvae collected (by sites).

Lake name	Maloe (Site 1)	Maloe (Site 2)	Maloe (Site 3)	Srednee	Severnoe (Site 1)	Severnoe (Site 2)	Zapadnoe (Site 1)
Subfamily Tanypodinae							
Krenopelopia sp.	1	2	2				
Procladius sp.						49	
Subfamily Chironominae							
Paratanytarsus sp.		1	2		5	5	
Cladotanytarsus gr. *vanderwulpi*				1	1		
Tanytarsus cf. *usmaensis*		1					
Tanytarsus cf. *verralli*						14	
Chironomus sp.	4	1	1		16		1
Endochironomus dispar gr.	1		3	6	12	8	
Polypedilum (*Ursipedilum*) *sp.*	1						
Polypedilum gr. convictum				1			
Glyptotendipes paripes (Edwards, 1929)						73	
Subfamily Orthocladinae							
Acricotopus sp.					2		
Cricotopus sp.					8		
Eukiefferiella sp.					1		
Psectrocladius obvius (Walker, 1856)					2		

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New range extensions for the Canadian Chironomidae fauna from two urban streams

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Abstract

We report on 2 first records of Chironomidae for Ontario, *Rheosmittia spinicornis* (Brundin, 1956) and *Sublettea coffmani* (Roback 1975), and confirm a previous record of *Odontomesa fulva* (Kieffer 1919) from Ontario. Specimens of *S. coffmani* have been only reported from New Brunswick whereas *R. spinicornis* has records from northern, western and central provinces and territories. Reports of *O. fulva* were not confirmed in Ontario. Here we report its occurrence for the second time in south central Ontario, confirming its occurrence within the province.

Introduction

The range of a species is the fundamental unit of biogeography. New records give us insights into how changing land use and climate alters the distribution of species. The taxa from urban and suburban streams are typically well-studied and well-known. As a result, these areas are often overlooked as potential sites to look for new range records. In this paper, we use data obtained during the Central Lake Ontario Conservation Authority's biomonitoring program in 2017 from urban and suburban streams. We report on new range records of Chironomidae, including 2 first provincial records for the province of Ontario.

Study area and methods

Specimens were collected from Bowmanville Creek in Bowmanville and Lynde Creek in Whitby, Ontario, Canada as part of Central Lakes Ontario Conservation Authority's biomonitoring program from March-August 2017. Bowmanville Creek, Lynde Creek and their tributaries are located within the Regional Municipality of Durham. The watershed of Lynde Creek passes through the Township of Uxbridge, Township of Scugog, City of Pickering, Town of Ajax, and the Town of Whitby in Ontario, Canada. The urban area of the Town of Bowmanville occupies the south part of the Bowmanville watershed and the mid-to-northern part of the watershed are in agricultural lands. Lynde Creek and its tributaries drain an area of approximately 130 km^2. The Bowmanville/Soper Creek Watershed drain an area of approximately 170 km^2. The headwaters of both waters originate in the Oak Ridges Moraine, an ecologically important area in southcentral Ontario. Lynde Creek drains into Lake Ontario through Lynde Creek Marsh and Bowmanville drains into Lake Ontario through the Bowmanville Coastal Wetland Complex, a provincially significant wetland (Central Lake Ontario Conservation 2012, 2013).

Samples were collected, sorted, and stored using Ontario Benthos Biomonitoring Network standard methods (Jones et al. 2007). Specimens of immatures and adults were mounted following methods described by Epler (2001) and Pinder (1978) and images were obtained using an OMAX A3550U Camera mounted on AMScope compound microscope. All abbreviations follow Sæther (1980). Voucher specimens were submitted to the Canadian National Collection of Insects, Arachnids and Nematodes (CNC) in Ottawa, Canada.

Results

Odontomesa fulva (Kieffer, 1919)

Larva (n=2). Figs. 1a-g.

Description. Postmentum L=184.3-202.5 µm (194.3 µm). Antenna 4 segmented (Fig. 1a), AR=2.0-2.4 (2.2). Labrum with S IV A and premandibles (Fig. 1b). Mentum (Fig. 1c). Posterior margin of ventromental plates more or less straight and parallel with anterior margin (Fig. 1d). Mandible with seta interna split into 3 segments (Fig. 1e); basal external seta of mandible splits into 2-8 setae from the base (Fig. 1e-f). Procercus well-sclerotized with 8 apical setae. 2 long anal setae are present (Fig. 1g). Posterior parapods 3X longer than wide (Fig. 1g). Four cone shaped anal tubules are present (Fig. 1g).

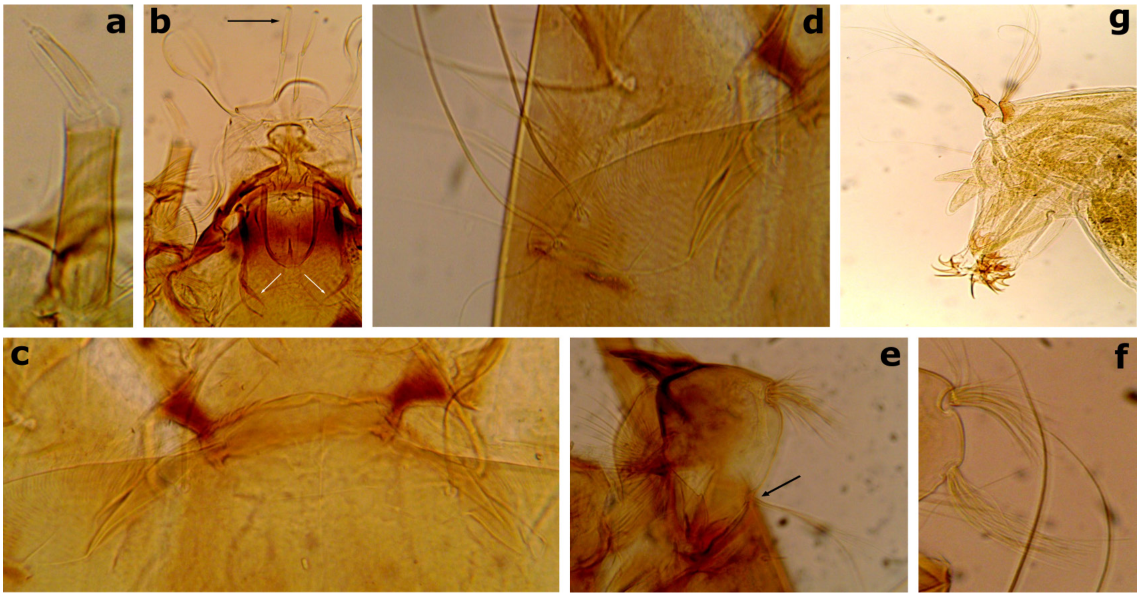

Figure 1. *Odontomesa fulva* (Kieffer, 1919) larva. a) Antenna, b) Labrum, SIV A (black arrow) and premandibles (white arrows), c) Mentum, d) Ventromental plate, e) Mandible, arrow points to basal external seta with 2 branches at the base, f) Variation of basal external seta with several branches, g) Posterior portion of the larva.

Notes. Larval stages of *O. fulva* and *O. ferringtoni* (Sæther 1986) were revised by Liu et al. (2016). A major distinguishing character for the larvae is the shape of the ventromental plates. As compared to *O. ferringtoni,* the posterior margin of the ventromental plate of *O. fulva* is straight and practically parallel with the anterior margin (Liu et al. 2016). This contrasts Sæther's (1986) use of basal external setae characteristics to separate the two species. Additionally, Chinese specimens of *O. fulva* examined by Liu et al (2016) have a postmentum length of 180-200 µm, extending the range of 131-158 µm presented by Sæther (1986); notably, the specimens discussed here have a postmentum length of 184.3-202.5 µm. The seta interna of the mandible splits into 3 segments in our specimens, corresponding with characteristics of larval *O. fulva nearctica* Sæther, 1986; however, basal external setae of the mandible split into 2-8 branches as opposed to 12-23 branches for *O. fulva nearctica* reported by Liu et al. (2016).

Study sites. Bowmanville Creek, Bowmanville, Ontario; 43° 54' 22.90" N 78° 41' 12.8" W.

Ecology and habitats. Larvae inhabit shallow pools of rivers and streams, especially cold-water streams. This species is univoltine in its emergence phenology (Phillips et al. 2013).

Nearctic distribution: Canada (Ontario, Saskatchewan, Yukon Territory?); USA (Florida, Georgia, Illinois, Maine, Michigan, Minnesota, Mississippi, Montana, New Mexico, North Carolina, Pennsylvania, South Carolina, Wisconsin).

Notes: According to Phillips et al. (2013), there are debates regarding the occurrence of this species in Canada. The CNC collected specimens by D.R. Oliver in 1990 and record *O. fulva* from Frankford, Kintor, and Trout creeks in southern Ontario. Additionally, a single specimen was collected in 1976 by K. Dance near Waterloo, Ontario (Phillips et al. 2013). Report of the species from Yukon Territory is also not confirmed, although, Philips et al. (2013) report the species from this territory. Finding this species in Bowmanville Creek confirms the species' occurrence in Ontario and extends its range from southern to more central regions of the province. The species has also a Palearctic distribution, as it is widespread in Europe and has been reported in Xinjiang Uygur Autonomous Region of the Palearctic China (Liu et al. 2016).

Rheosmittia spinicornis **(Brundin, 1956)**

Pharate male (n=3). Fig. 2a-b.

Diagnosis. Antenna with 11 flagellomeres, AR=0.47. Thorax with prominent scutal tubercle (Fig. 2a). Inferior volsella digitiform and directed posteriorly (Fig. 2b).

Pupa (n=3). Fig. 2c, d.

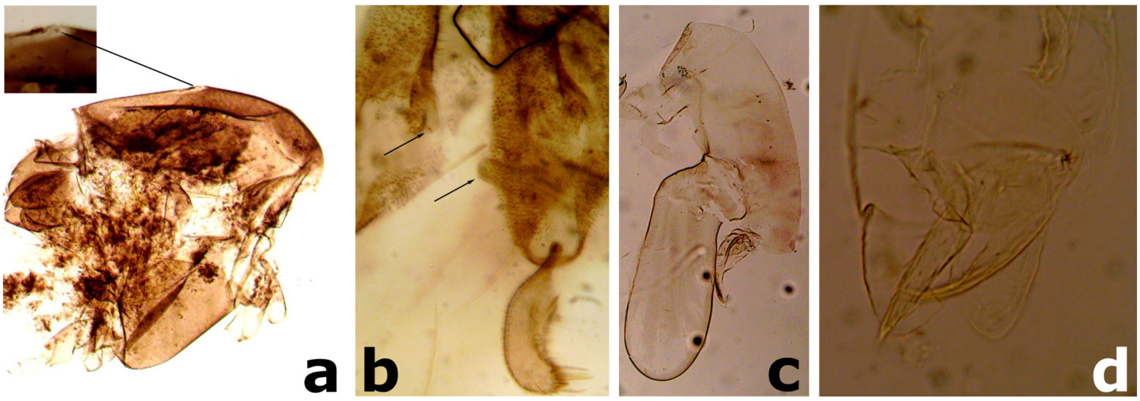

Figure 2. *Rheosmittia spinicornis* (Brundin, 1956) pharate male a-b, pupa c-d. a) Thorax, arrow indicates the location of scutal tubercle, b) Hypopygium, arrows indicate the inferior volsella, c) Cephalothorax, d) Anal lobes and genital sacs.

Description. Exuviae hyaline (Fig. 2c). Frontal setae absent. Precorneal seta 1-3 Ls =43.3, 51.0, 33.1 µm long. Dorsocentral setae about 38.8-47.8 µm long. Anal lobe and genital sac (Fig. 2d).

Notes. A detailed description of species is given by Cranston and Sæther (1986).

Study sites. Bowmanville Creek, Bowmanville, Ontario; 43° 54' 22.90" N 78° 41' 12.8" W. Lynde Creek, Whitby, Ontario; 43° 54' 42.27" N 78° 57' 52.4" W.

Ecology and habitats. Larvae of *Rheosmittia* are psammophile (Cranston and Sæther 1986).

Nearctic distribution: Canada (British Colombia, Northwest Territories, 1st record Ontario, Saskatchewan, Yukon Territory).

Notes. Originally, this species was reported only from the Palearctic region (Ashe and O'Connor 2012); however, according to entries in the Barcode of Life Data Systems (Ratnasingham and Hebert 2007), this species also occurs in British Columbia, Northwest Territories, Saskatchewan and Yukon Territory. Considering our new record in Ontario, this species is probably widespread across the northern, western and central region of Canada.

Sublettea coffmani (Roback, 1975)

Pupa (n=2). Figs. 3a-e.

Description. Thoracic horn with long apical filaments (Fig. 3a). Robust patches of spines present on segments II-V (Fig. 3b), spine patches on segment IV and V with longer lateral spines (Fig. 3c). Paired spine patches are located in the anterior corners of the ventral intersegmental membrane of sternite VIII. (Fig. 3d). Anal lobe and genital sac as in Fig. 3e; anal lobe with robust patches of spines and long setae (Fig. 3e).

Notes. A detailed description of this species is given by Roback (1975). This is the only species of *Sublettea* described from the Nearctic.

Study sites. Lynde Creek, Whitby, Ontario; 43° 54' 42.27" N 78° 57' 52.4" W.

Ecology and habitats. Larvae of *Sublettea* inhabit lotic habitats with a preference for cool, clear and fast flowing streams (Ashe and O'Connor 1994). Larvae of *S. coffmani* construct soft, non-transportable cases of fine sediments, which they attach to stream substrates (Roback 1975).

Nearctic distribution: Canada (New Brunswick, 1st record Ontario); USA (Alabama, Georgia, Kentucky, Mississippi, North Carolina, Pennsylvania, South Carolina, Tennessee, Maryland, Virginia, and West Virginia).

Acknowledgments

We are grateful to the Central Lake Ontario Conservation staff especially Dan Moore for providing us with the specimens for this paper.

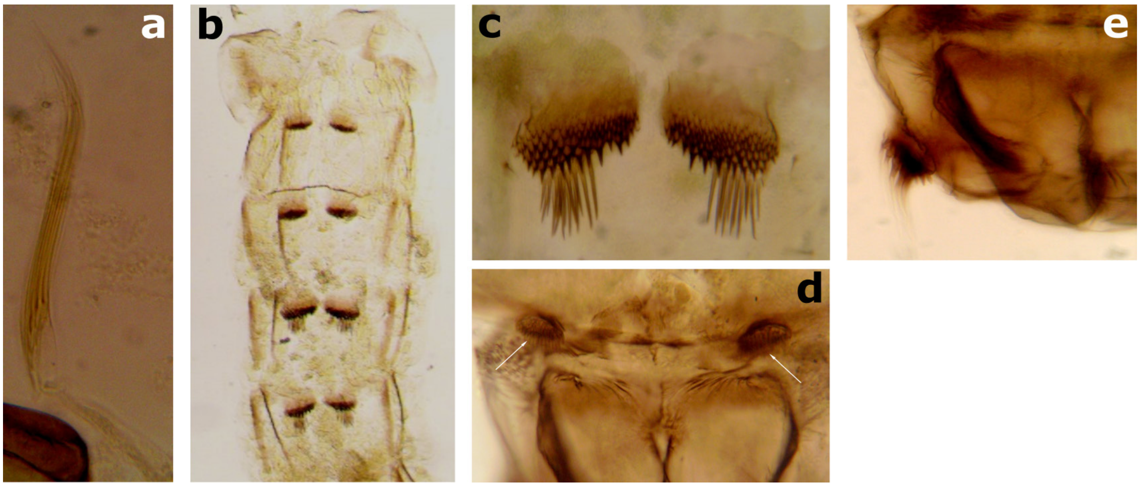

Figure 3. *Sublettea coffmani* (Roback, 1975) pupa. a) Thoracic horn, b) Abdominal tergite I-V, c) Spine patches on segment IV, d) Spine patches on ventral side of sternite VIII (arrows), e) Anal lobe and genital sac.

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CHIRONOMUS Journal of Chironomidae Research, No. 30, 2017: 81. News for the Chironomidae Community.

The 2nd Chinese Symposium on Chironomidae

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The 2nd Chinese Symposium on Chironomidae was held at the College of Life Sciences, Tianjin Normal University, Tianjin, China, on October 20-22, 2017. The symposium was co-organized by Tianjin Normal University, Tianjin Agricultural University and Tianjin Zoological Society.

Fourty-five participants from 18 institutions attended the symposium, representing Nankai University, Chinese Academy of Sciences, The Chinese Center for Disease Control and Prevention, Jinan University, Shanghai Ocean University, Qinghai University, Tianjin Normal University, Tianjin University of Science & Technology, Tianjin Agricultural University, Chongqing Normal University, Shenyang Normal University, Taizhou University, Hubei University for Nationalities, South China Institute of Environmental Science, MEP, Haihe Water Conservancy Commission of Ministry of Water Resources, Chinese Research Academy of Environmental Sciences, Liaoning Environmental Monitoring Center and The Geological Museum of China.

The representatives shared recent research progress on Chironomidae in China. Prof. Dr. Xinhua Wang, Nankai University, delivered a keynote speech entitled “The present and prospect of Chironomids research in China”. Altogether 22 oral presentations and 7 posters were presented during the symposium. The scientific communication included many aspects of Chironomidae science: morphology, taxonomy, faunistics, DNA barcoding, molecular systematics, zoogeography, ecology and environmental monitoring. We believe the Symposium will enhance cooperation among Chinese workers and promote future development of this discipline in China.

The next (3rd) symposium is scheduled to be held in 2019 in Xianju National Park, Zhejiang Province, and will be organized by Taizhou University.

Participants at the 2nd Chinese Symposium on Chironomidae.

CHIRONOMUS Journal of Chironomidae Research, No. 30, 2017: 82-83. In memoriam.

In Memoriam: Prof. Dr. Wolfgang Friedrich Wülker

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Prof. Wolfgang Wülker and his long-time research assistant Fr. Renate Rössler seek larvae of the phylogenetically basal species *Chironomus holomelas* in a raised bog (Hochmoor) in Jura, Switzerland, May 1981. Photo: Malcolm G. Butler.

With great regret and our sincerest sympathies we announce that our best friend and colleague Prof. Dr. Wolfgang Wülker passed away on January 12, 2017 at the age of 91. We lost a scientist who has made valuable contributions to morphological and karyological systematics of the Chironomidae. As an early leader in bringing cytological evidence to bear on the systematics of Northern *Chironomus,* he combined banding patterns of salivary gland chromosomes with detailed morphological analyses of larvae, pupae, and male imagos. Prof. Wülker authored or participated in many descriptions and revisions of *Chironomus* species, including extending the Standard chromosome maps of H.-G.Keyl to cover the rather difficult chromosome arms B, C, and D. He also made important contributions concerning the karyotypes, morphology, ecology and distribution of the genera *Sergentia* and *Diamesa.* Besides the dozens of precisely written papers with elaborate chironomid chromosome pictures, Dr. Wülker also published many seminal articles on chromosome evolution and phylogeny (Martin and Hoffrichter 2010)[1]. Prof. Wülker worked not only with karyosystematics of European chironomids, but also with Chironomidae in America and Africa. Working with Siberian specialists, he found endemic African banding patterns in the polytene chomosomes of African *Chironomus*, while documenting that "basic" banding patterns of polytene chromosomes occur in *Chironomus* species from different continents.

The following example illustrates the broad range of Prof. Wülker's interests in the natural sciences, as well as the potential of any scientific advance to have unanticipated ramifications. Influenced partly by his father's brief career in parasitology, Prof. Wülker conducted numerous investigations on chironomid parasites, particularly the effects of mermithid nematodes on adult chironomid morphology. To understand how parasites altered larval development, Prof. Wülker recognized the need to better understanding the developmental process in normal larvae. His collaboration with P. Götz produced a detailed documentation of imaginal disk development in *C. riparius*, as pupal and imaginal structures take form during the final instar. The nine "developmental phases" defined by Wülker & Götz over the course of the 4th instar in *Chironomus* provided a much more precise tool for the investigation of chironomid larval development. The Wülker & Götz developmental scheme has been used extensively in diverse studies involving Chironomidae, ranging from genetics and physiology, to life history analysis and biomonitoring of environmental pollution.

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[1] One additional paper was published after 2010: Wülker, W.F., Kiknadze, I.I., Istomina, A.G. 2011. Karyotypes of *Chironomus* Meigen (Diptera: Chironomidae) species from Africa. - *Comp. Cytogen* 5: 23-46. doi: https://doi.org/10.3897/compcytogen.v5i1.975

Throughout his long research career Prof. W. Wülker developed fruitful collaborations with many specialists in countries throughout the world (Australia - Prof. J. Martin; USA- Prof. M. Butler, Prof. J. Sublette and his wife M. Sublette; Russia- Prof. I. I. Kiknadze and her team ("the Russian ladies" as he liked to refer to them); Hungary – Prof. G. Devai; Bulgaria- Prof. P. Michailova; Switzerland – Profs. H. M. Ryser and A. Scholl). Publications with these and many other international colleagues produced important contributions to our understanding of the genetics of natural chironomid populations and evolution within the important genus *Chironomus*, long before the current age of molecular sequencing techniques. Throughout most of the 20th Century, cytogenetic analysis of polytene chromosomes was not only a valuable taxonomic tool for the important genus *Chironomus*, it also provided great insight into broader topics like genetic differentiation of isolated populations and the role of chromosomal rearrangements in population adaptation, divergence, and speciation. Prof. W. Wülker's work contributed directly to these themes, and greatly facilitated advances made by many others.

Prof. Dr. Wolfgang Wülker has been one of the most active participants in the International Chironomid Symposia. Present at the First Symposium held in Plön, Germany in 1964, Prof. Wülker hosted the 13th Symposium at Freiburg in September 1997. Though unable to attend the 2003 Symposium in person, he presented - by video recording - the Thienemann honorary lecture at the 15th International Chironomid Symposium in Minneapolis, USA. His address on "The Role of Chromosomes in Chironomid Systematics, Ecology and Phylogeny" was an insightful overview of the many ways in which cytogenetic studies have enhanced our knowledge of chironomid taxonomy, genetics, ecology, evolution, and biogeography.

We express our deepest grief on the death of our dear colleague and collaborator. We will miss his friendship, competence and advice.

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Pepi Michailova, Jon Martin and Malcolm Butler