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A peculiar new species of *Stilobezzia* Kieffer breeding in bamboo internodes in northeastern Argentina (Diptera: Ceratopogonidae)

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Stilobezzia enigma, a new Neotropical species, is described and illustrated from larvae, pupae and adults. Larvae and pupae were collected from internodes of two bamboo species, *Guadua chacoensis* (Rojas) and *Guadua trinii* (Ness) Ness ex Rupr (Bambuseae) in Misiones province, Argentina, and were examined in the laboratory with a scanning electron microscope. The generic placement of *S. enigma* is somewhat uncertain but we provide cladistic evidence indicating that it is probably an autapomorphic member of *Stilobezzia*.

Keywords: taxonomy; new species; Neotropical region

Introduction

For students of the diverse and species rich Ceratopogonidae, there continue to be surprising encounters with species that are difficult to place generically. Even with careful study, there is the question of whether they are autapomorphic members of recognised genera or whether they represent separate and distinct lineages. Herein, we describe a distinctive species of biting midge which we tentatively recognise as a member of *Stilobezzia* Kieffer, 1911. Our discussion describes evidence that it is an autapomorphic member of this genus.

Materials and methods

Larvae and pupae were collected from the internodes of two bamboo species, *Guadua chacoensis* (Rojas) and *Guadua trinii* (Ness) Ness ex Rupr (Bambuseae) in Misiones province, Argentina, using pipettes and transported to the laboratory in water taken from their natural environment. Larvae were separated by instar and reared and observed daily to record development. Larvae and pupae were examined with a scanning electron microscope JSM6360LV using the techniques of Ronderos et al. (2000, 2008) except that we used 30% glycolic acid to clean specimens and the exposure time of cleaning was increased to 8 minutes. For observation with a compound microscope, specimens were slide-mounted in Canada balsam using the technique described by Borkent and Spinelli (2007). Mounted larval exuviae were

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oriented ventral sides upwards to facilitate examination of the epipharyngeal combs within the head capsules and the alimentary channel, the latter of which is only visible when specimens are well cleared. Pupal exuviae were mounted dorsoventrally. Photomicrographs were taken with a Pentax Optico Power Shot S60 digital camera through a Leitz SM-Lux microscope at 10 × or 40 × magnification. Illustrations were prepared with the aid of a camera lucida.

For larval terminology see Ronderos et al. (2008), Borkent and Craig (2001) for the pupa, and the *Manual of Nearctic Diptera* by McAlpine et al. (1981) for adults. Terms for wing veins and cells follow the system of the *Manual of Nearctic Diptera*, with modifications of certain veins and cells as proposed by Szadziewski (1996). Type material is deposited in the Museo de La Plata, Argentina (MLPA), the Canadian National Collection of Insects, Canada (CNCI), as indicated, the US National Museum of Natural History, Washington, D.C., USA (USNM) and the Florida State Collection of Arthopods, Gainesville, Florida, USA (FSCA).

Abbreviations

Larva

Antennae (AN); collar (CO); dorsal comb (DC); epipharynx (EP); fossa mandibularis (MF); galeolacinia (GL); hypopharynx (HP); hypostoma (HY); labrum (LB); lacinial sclerites 1 and 2 (LC1 and LC2); lateral sclerite (LS); mandible (MD); maxillary palpus (MP); maxilla (MX); median sclerite (ms); median sclerite comb (msc); messors (MS); pharyngeal apparatus (PA); palatal bar (PB); palatum (PL); scopae (SC); sensilla campaniformia (SCa); sensilla maxilla (SM); sensilla styloconica (SS); sensilla trichoidea (ST); ventral comb (VC). Head capsule chaetotaxy is indicated by single letters.

Pupa

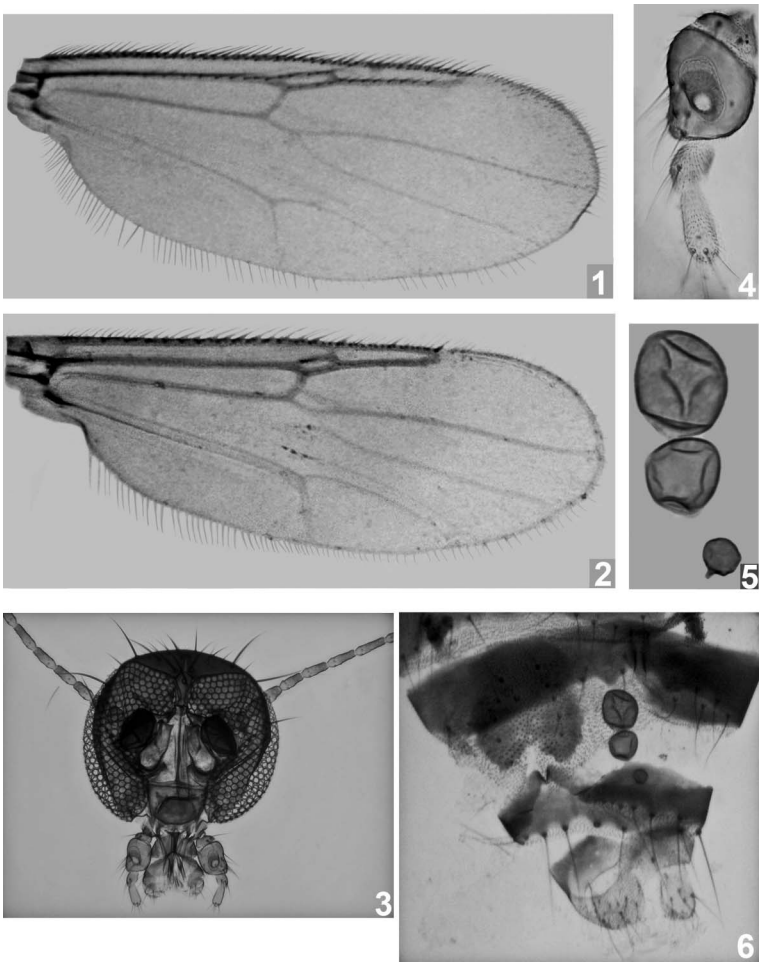
Anterodorsal setae (ad); anteromarginal setae (am); apicolateral processes (AP); dorsal setae (d); dorsolateral setae (dl); dorsomedial setae (dm); genital sac (GS); pedicel (P); pore at tubercle base (ps); respiratory organ (RO); ventrolateral setae (vl); ventromedian setae (vm). Fourth abdominal segment setae: dorsal posteromarginal setae (d.p.m.); dorsal anterosubmarginal setae (d.a.s.m.); lateral anterosubmarginal setae (l.a.s.m.); lateral posteromarginal setae (l.p.m.); ventral posteromarginal setae (v.p.m.).

Results

***Stilobezzia enigma* n. sp.** (Figures 1–53)

Diagnosis

Male, female adults. The only members of the genus with a very broad, stout third palpal segment bearing a deep sensory pit. In addition it is the only species of *Stilobezzia* in which the female has a vestigial, untoothed mandible and a wing length of 1.04–1.20 mm, and the only male in which the aedeagus has a well-developed basal arch.



Figures 1–6. *Stilobezzia enigma* n. sp., adult; (1, 3–6) female; (2) male. (1, 2) wing; (3) head, anterior view; (4) right palpus, anterior view; (5) spermathecae, partially collapsed; (6) tip of abdomen.

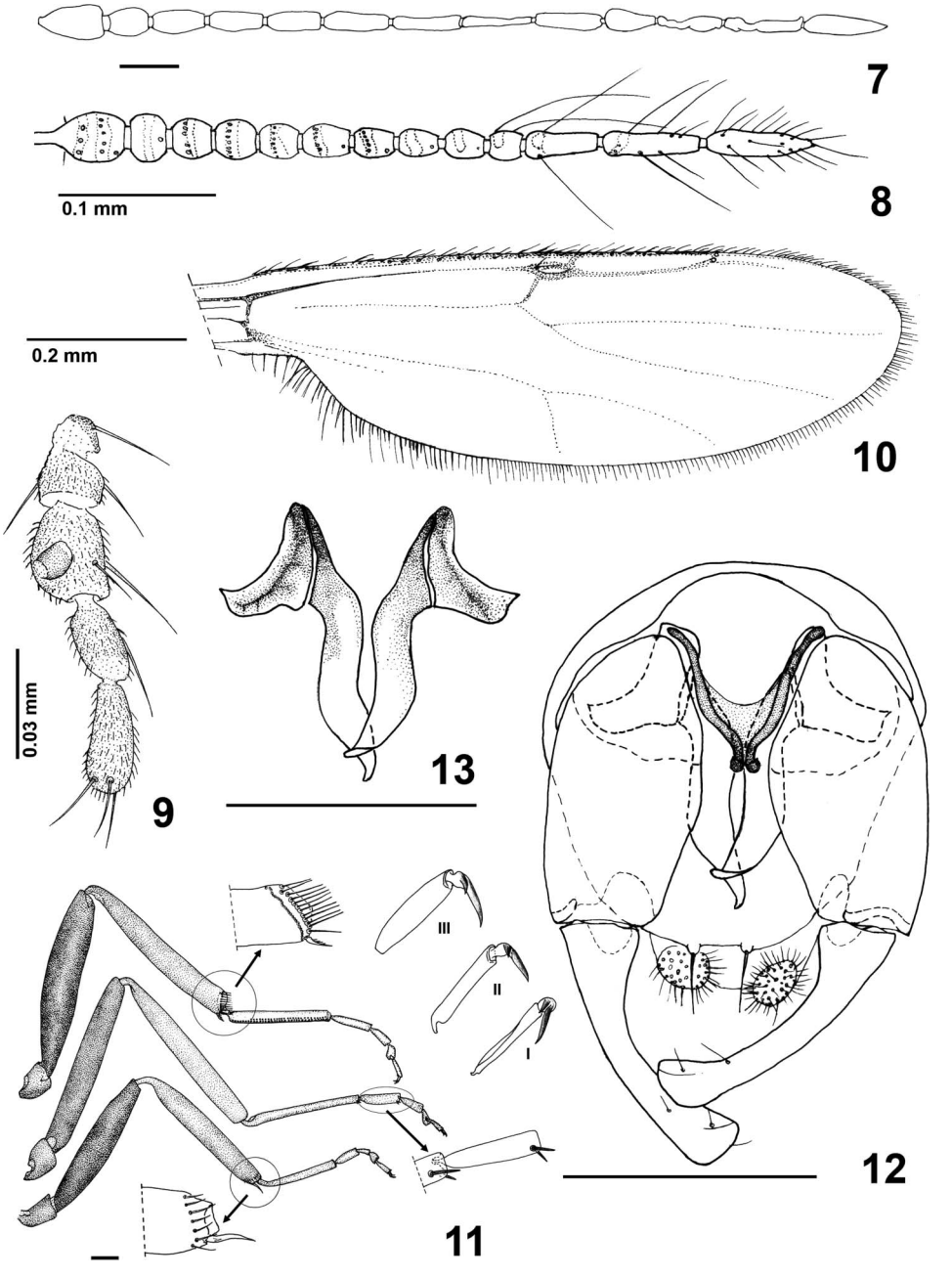
Pupa. Only member of the genus with elongate, slender apicolateral process and simple (non-bifurcating) abdominal setae.

Larva. Not diagnosable at the present time.

Description of male adult (Figures 2, 8–10, 12, 13).

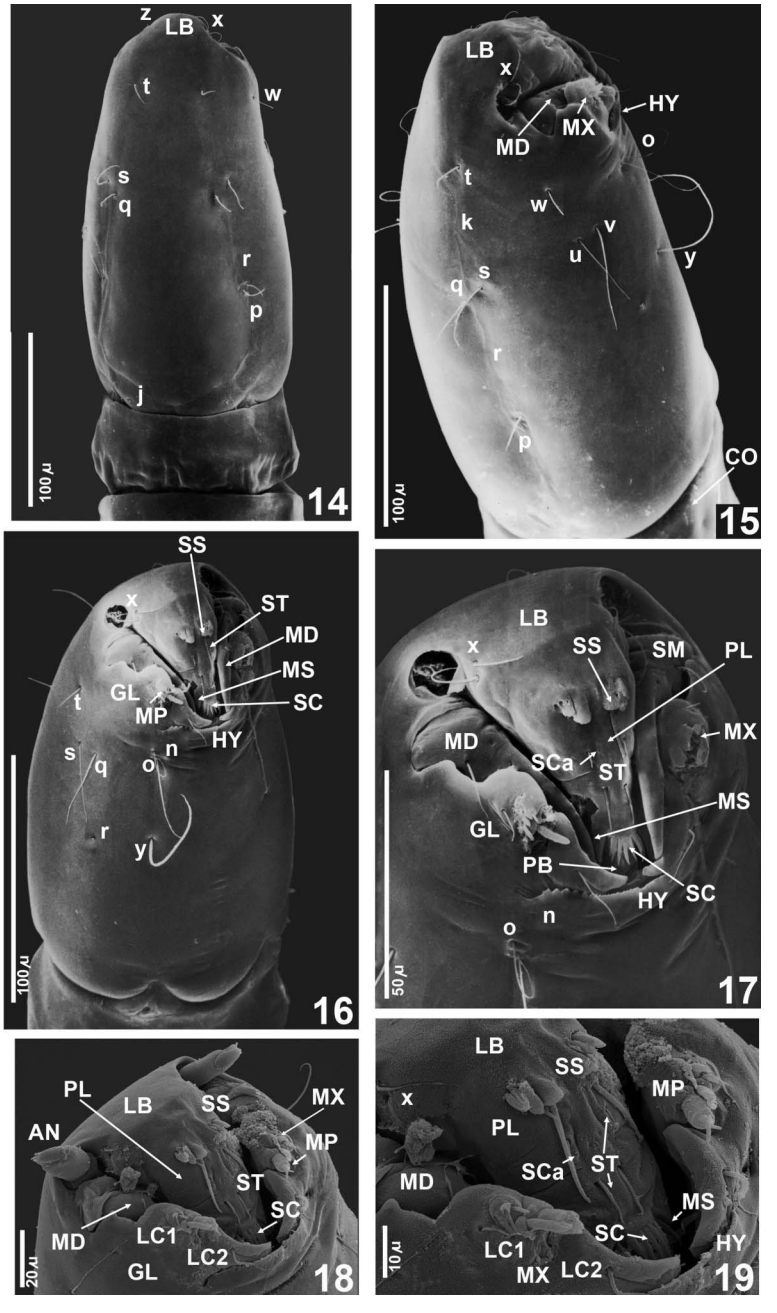
Similar to female with the usual sexual differences. Antennal flagellum (Figure 8) with flagellomeres 1–6 each partially fused to abutting flagellomeres; antennal ratio (11-13/1-10) 0.47–0.59 (0.53, $n = 11$). Palpus as in Figure 9. Wing (Figures 2, 10) length 0.77–1.01 (0.88, $n = 12$) mm; width 0.26–0.37 (0.32, $n = 12$) mm; CR 0.69–0.71 (0.69, $n = 12$); second radial cell three times longer than first (first nearly closed in one specimen); macrotrichia restricted to costa, R_1, one subapically on R_3 (Figure 2).

Genitalia (Figure 12). Tergite 9 extending just beyond distal level of gonocoxites, distal margin rounded, irregular, apicolateral processes very small, close together, each bearing slender, medium-sized seta, rounded; sternite 9 narrow, with broad, deep posteromedial excavation. Gonocoxite stout, twice as long as wide;



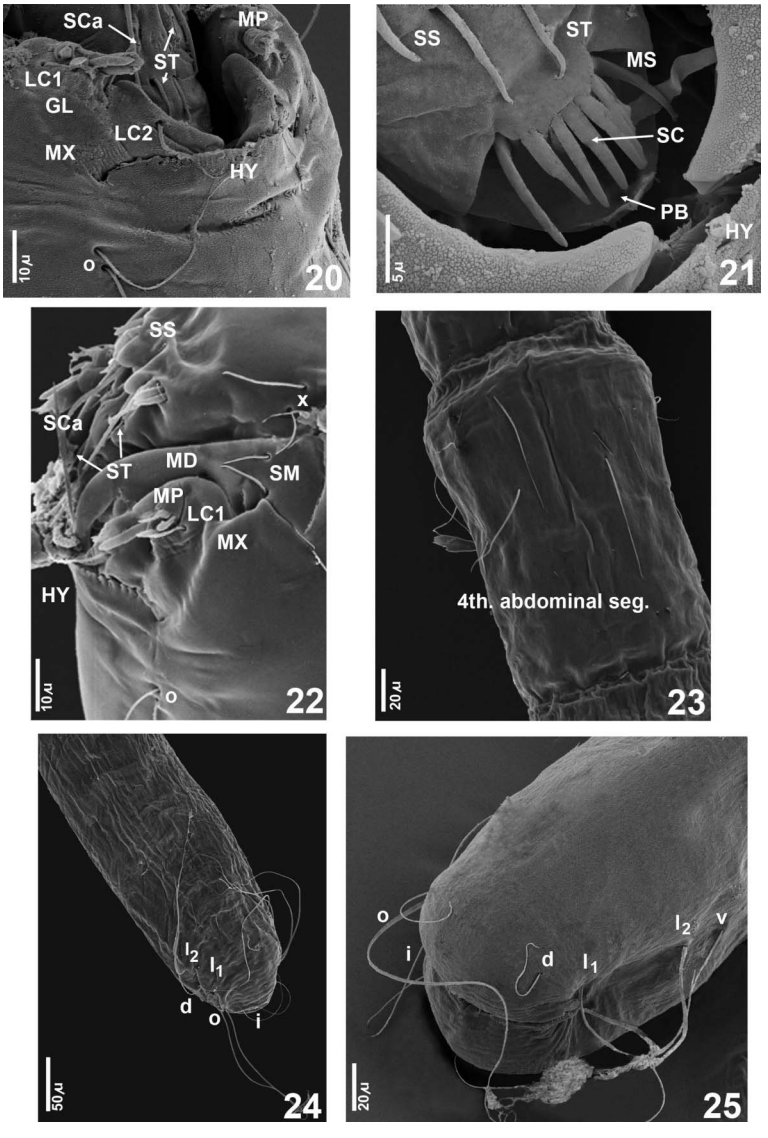
Figures 7–13. *Stilobezzia enigma* n. sp., adult; (7, 11) female; (8–10, 12, 13) male. (7, 8) flagellum; (9) left palpus; (10) wing; (11) legs (top to bottom, hind-mid-fore), with detail of apex of fore and hind tibiae, apex of midtibia and tarsomere 1, and tarsomeres 5 and claws; (12) genitalia, ventral view; (13) parameres.

gonostylus as long as gonocoxite, yellowish, moderately curved, tip broad, spoon-shaped. Parameres (Figure 13) separate; basal arm heavily sclerotised, bilobed; midportion stout, nearly straight, tapering to narrow, recurved, pointed apex that



Figures 14–19. *Stilobezzia enigma* n. sp., fourth instar larva. (14) head capsule, dorsal view (chaetotaxy); (15) head capsule, ventrolateral view (chaetotaxy); (16) mouthparts and chaetotaxy (antenna broken), anteroventral view; (17) detail of mouthparts (antenna broken), anteroventral view; (18) detail of mouthparts, anterolateral view; (19) detail of labrum-palatum.

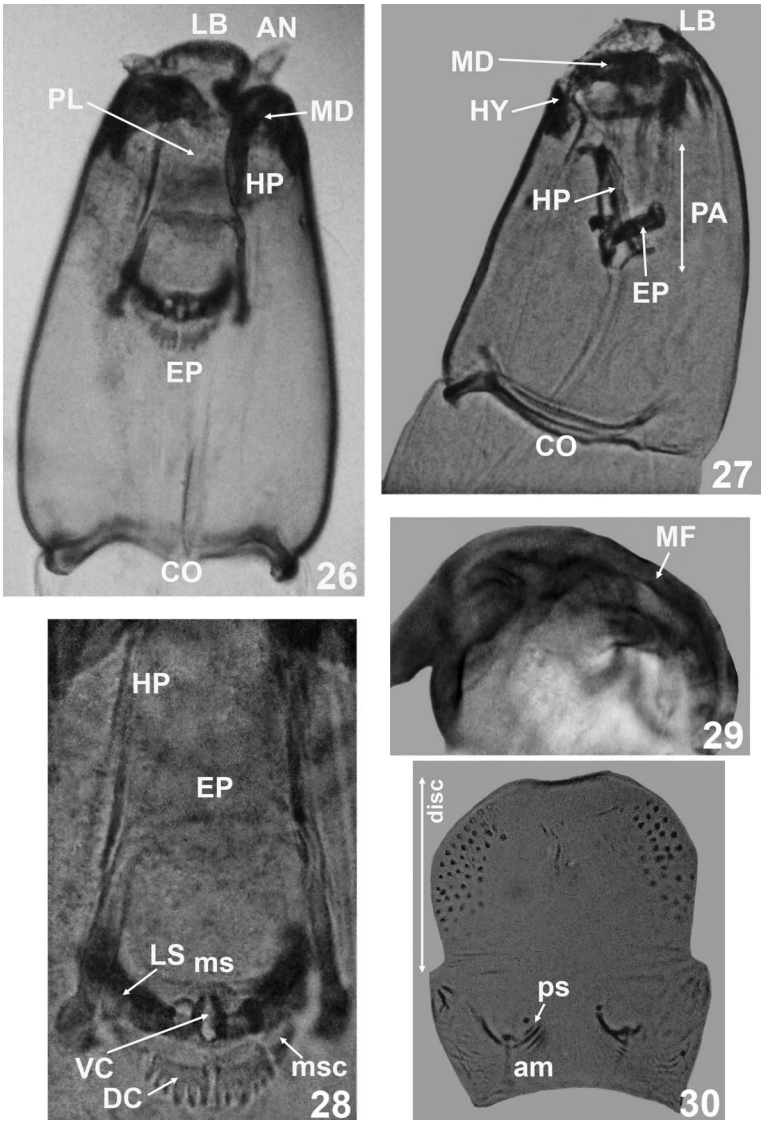
overlaps apex of opposite side. Aedeagus triangular; basal arch extending to 0.5 of total length; lateral arms heavily sclerotised, slightly curved sclerites with recurved tips. Base of cercus posteroventral to apex of tergite 9.



Figures 20–25. *Stilobezzia enigma* n. sp., fourth instar larva; (20) mouthparts, anteroventral view; (21) detail mouthparts, anteroventral view; (22) palatum and mouthparts, lateral view; (23) fourth abdominal segment, ventral view; (24) caudal segment, dorsal view; (25) caudal segment, lateroventral view.

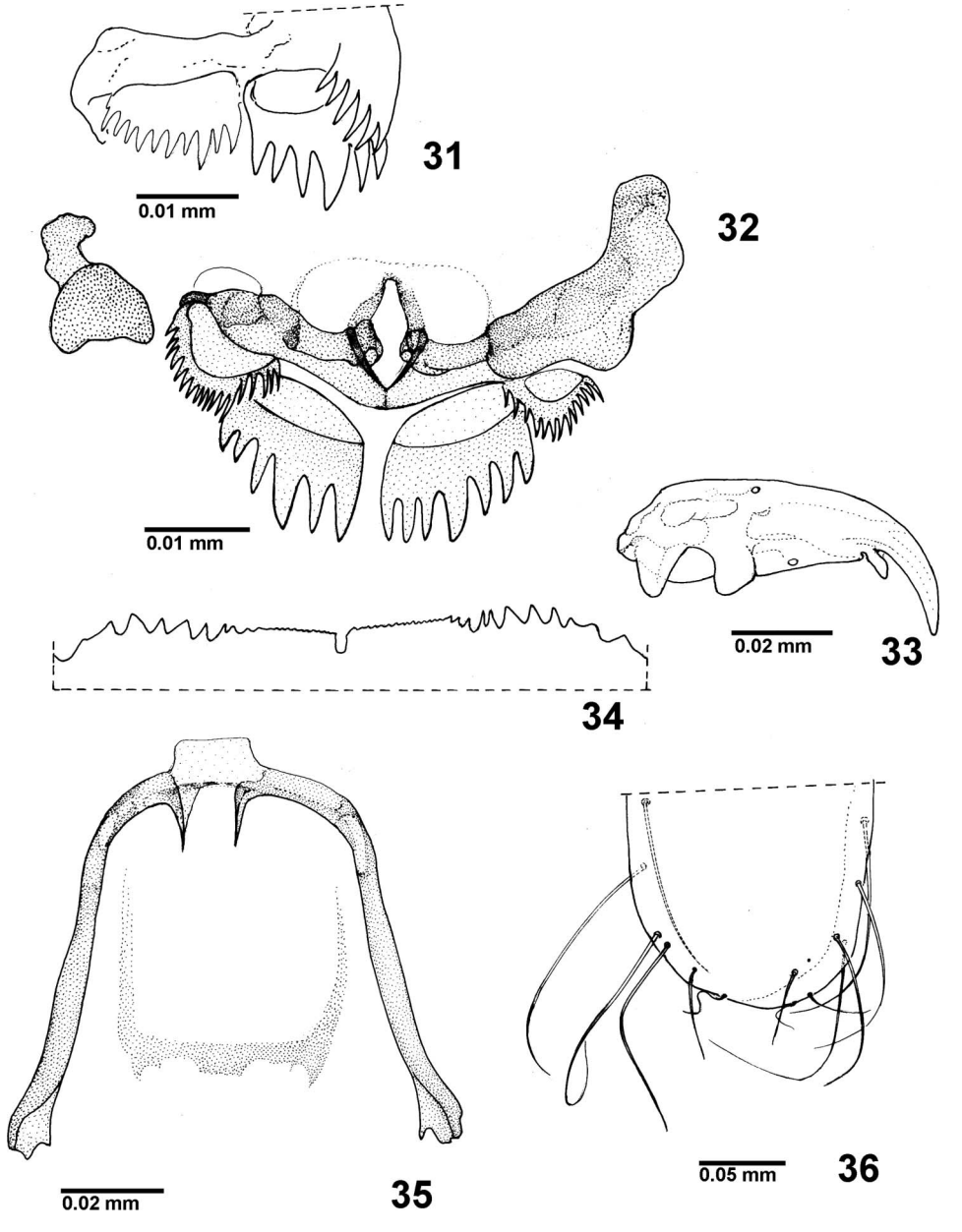
Female adult (Figures 1, 3–7, 11).

Head (Figure 3). Dark brown. Eyes with numerous elongate interommatidial spicules, separated medially by width of two to three ommatidia. Antennal flagellum (Figure 7) uniformly dark brown; flagellomeres vasiform, elongate; AR 0.73–0.82 (0.78, $n = 2$). Palpus (Figure 4) dark brown; segment 1 small, very short; segment 2 swollen, broadly abutting base of segment 3; segment 3 greatly swollen with very deep, conspicuous sensory organ opening to narrow, rounded subapical pore; PR 1.23–1.30 (1.265, $n = 2$). Proboscis short, 0.3 times width of head. Mandible vestigial, without teeth.



Figures 26–30. *Stilobezzia enigma* n. sp.; (26–29) fourth instar larva; (30) female pupa; (26) head capsule, ventral view; (27) head capsule, lateral view; (28) pharyngeal apparatus (epipharynx and hypopharynx); (29) left mandible, lateral view; (30) operculum.

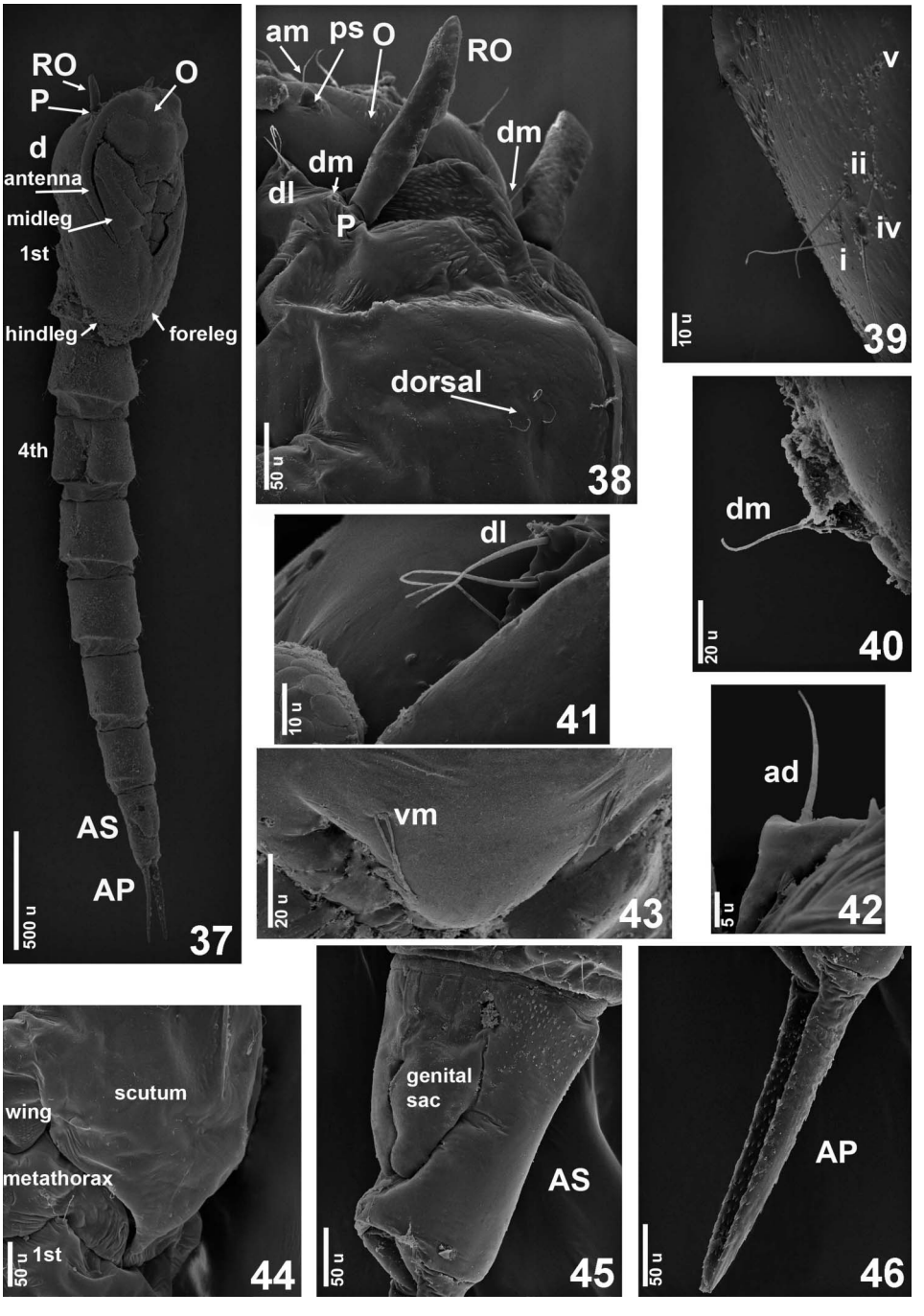
Thorax. Uniformly dark brown, scutum with longitudinal rows of moderately stout setae; scutellum with six larger setae, four smaller ones. Legs (Figure 11) dark brown, stout, pilose; fore tibia with slender, curved, apical spur; hind tibial comb with 8–12 slender spines, spur very short; hindtarsomere 1 with two rows of palisade setae (the inner one incomplete); hind tarsal ratio 2.60–2.80 (2.68, $n = 3$); fourth tarsomere of each leg cordiform; tarsal claws of each leg a single, small, mostly straight claw, each with a short, stout basal tooth (Figure 11). Wing (Figure 1), length 1.04–1.20 (1.10, $n = 3$) mm; width 0.41–0.45 (0.43, $n = 3$) mm; CR 0.74–0.75 (0.75, $n = 3$); membrane very slightly infuscated; second radial cell 2.2 times longer



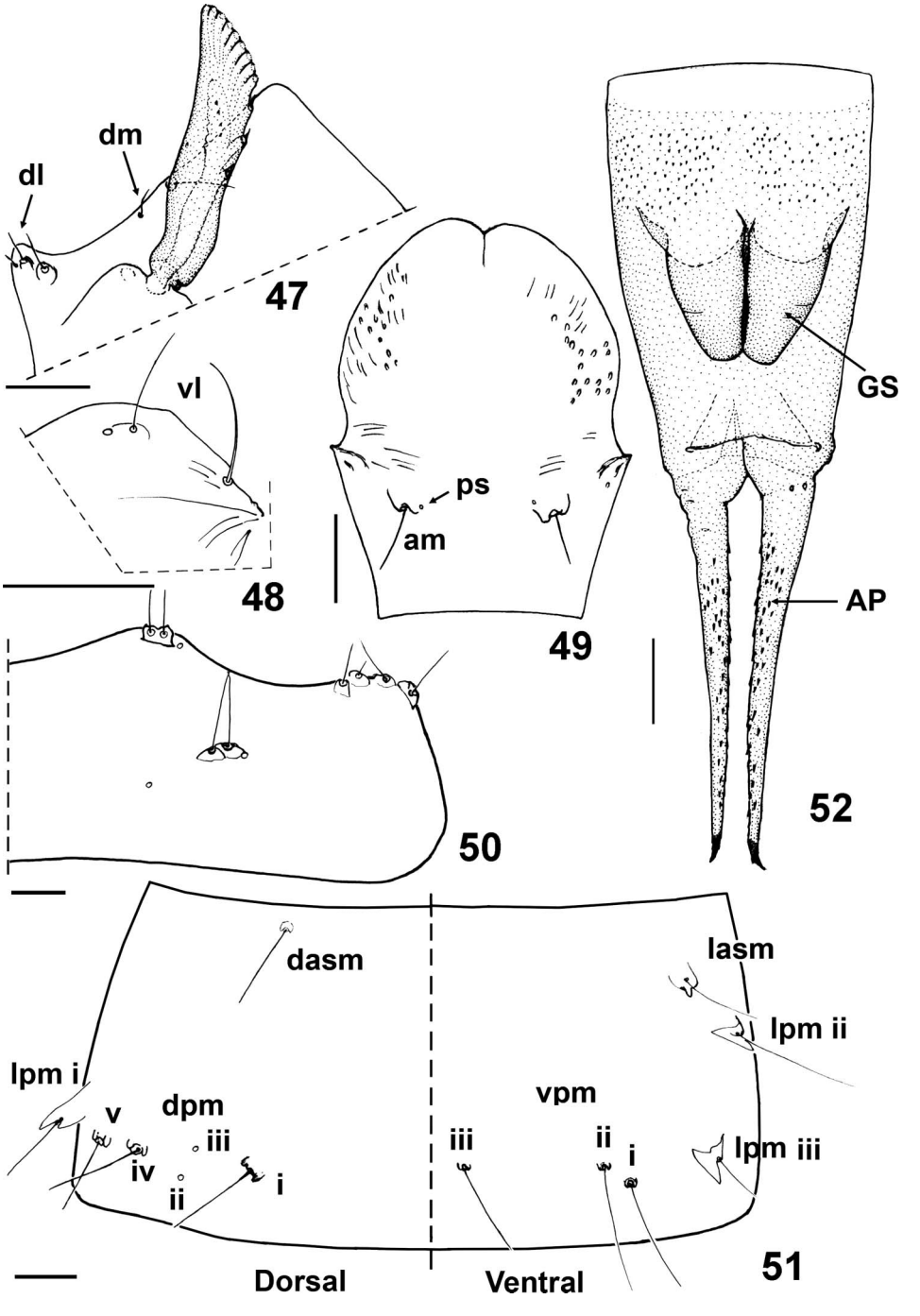
Figures 31–36. *Stilobezzia enigma* n. sp., fourth instar larva; (31) detail left side epipharynx; (32) epipharynx, ventral view; (33) mandible, lateral view; (34) hypostoma, ventral view; (35) detail epipharynx, ventral view; (36) caudal segment, ventral view.

than first; r-m cross-vein as long as petiole of M; stout macrotrichia on costa, radial veins, weaker macrotrichia on distal half of M_1, apex of M_2, scarce on apex of cells r_3, few in m_1, m_2. Halter brown.

Abdomen. Dark brown. Sternite 8 (Figure 6) stout with posteromedian V-shaped excavation. Two ovoid, unequal spermathecae with short necks (Figures 5, 6), measuring 45–52 (48.5, $n = 2$), by 40–42 (41, $n = 2$) μm, 38



Figures 37–46. *Stilobezzia enigma* n. sp., pupa; (38–44) female; (37, 45, 46) male. (37) Entire pupa, ventrolateral view; (38) cephalothorax, anterodorsal view; (39) dorsal setae, right side cephalothorax; (40) dorsomedial seta, anterolateral view; (41) detail of dorsolateral setae, anterolateral view; (42) detail of anterodorsal seta, anterodorsal view; (43) ventromedian setae, anteroventral view; (44) posterior portion of thorax and anterior portion of first abdominal segment, dorsolateral view; (45) anterior portion of anal segment, ventrolateral view; (46) anal segment with apicolateral processes, ventrolateral view.



Figures 47–52. *Stilobezzia enigma* n. sp., pupa; (47, 48, 50, 51) female; (49, 52) male. (47) Right respiratory organ, lateral view, dorsomedial and dorsolateral setae; (48) ventrolateral setae; (49) operculum; (50) first abdominal segment, dorsal view; (51) fourth abdominal segment setae, views as shown; (52) anal segment, ventral view.

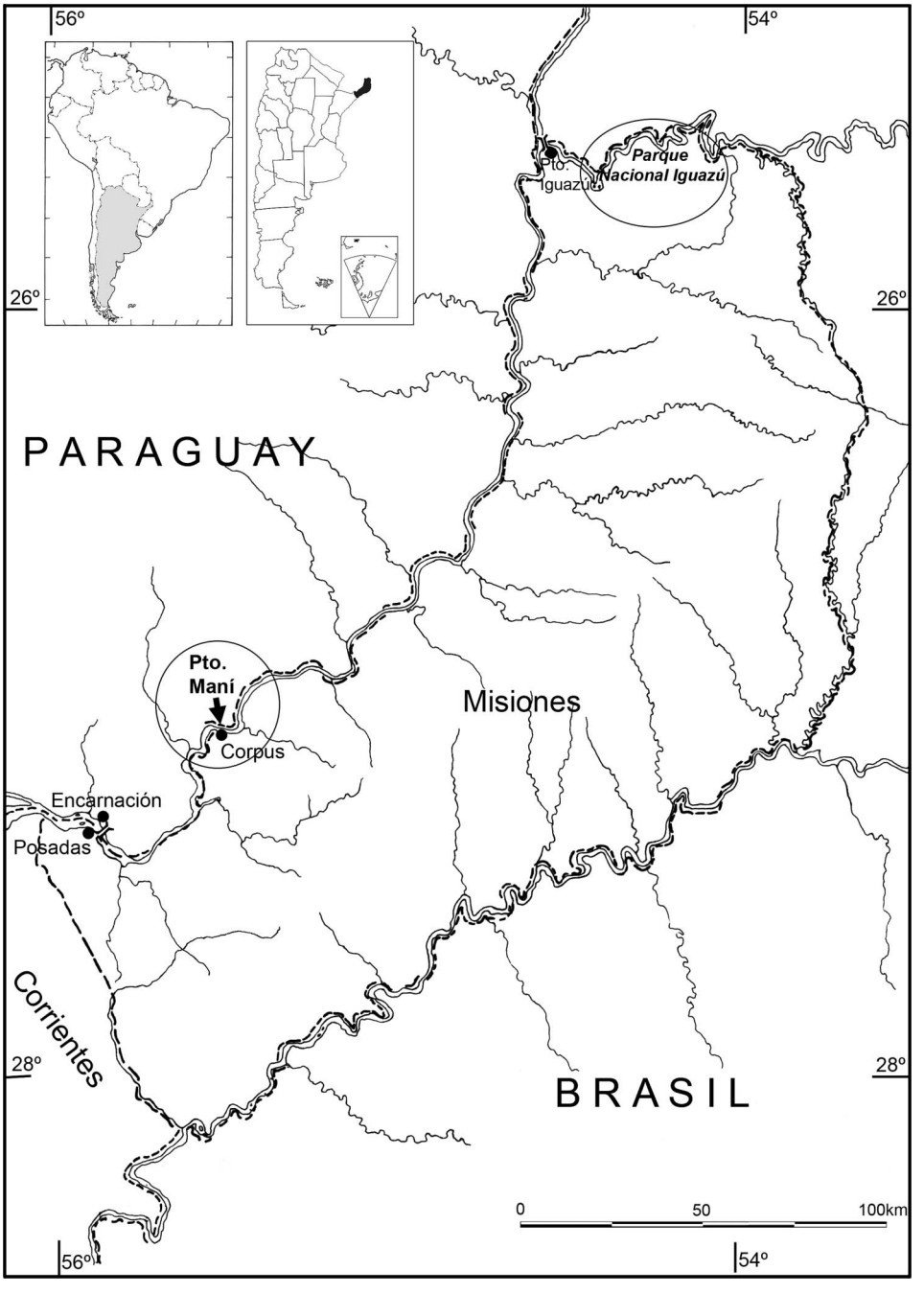


Figure 53. Distribution map.

($n = 2$) by 30 ($n = 2$) μm; a third rudimentary, moderately well developed, rounded spermatheca.

Fourth instar larva (Figures 14–29, 31–36).

Length 5.43–6.33 (5.81, $n = 3$) mm. Antennae cylindrical, medium-sized (length

0.02 mm, $n = 1$) (Figures 18, 26). Head capsule pale brown, elongate, tapering to rounded apex (Figures 14–16, 26–27); chaetotaxy as in Figures14–16; HL 0.28–0.32 (0.29, $n = 13$) mm; HW 0.15–0.19 (0.17, $n = 7$) mm; HR 1.56–1.84 (1.76, $n = 7$); SGW 0.11–0.13 (0.12, $n = 7$) mm; SGR 3.07–3.19 (3.17, $n = 2$). Labrum (Figures 14, 15, 17, 19, 26, 27) 0.6 times longer than width; palatum (Figures 17–19, 26) with three pairs of sensilla styloconica on anterior edge, pair of small parallel furrow immediately underneath, one sensilla trichoidea lateral to each furrow, one sensilla campaniformia behind later one, one pair of medium-sized, stout, sensilla trichoidea anterior to scopae (Figures 16–22); well developed, sclerotised messors underneath, lateral to scopae; well developed scopae with six strong, lanceolate, teeth (Figures 16, 17, 19, 21); palatal bar present under scopae (Figures 17, 21). Maxilla (Figures 15, 17, 18, 20, 22) sclerotised, with short, slender papilla; galeolacinia with bundle of three setae; maxillary palpus cylindrical, complex in structure, flap-like lobe lying alongside, lacineal sclerites 1, 2 with elongate, conspicuous setae. Mandible (Figures 15–19, 22, 26, 27, 29, 33) bidentate, thin, curved, strongly sclerotised; well-defined, elongate, apical tooth, proximal tooth short; evident fossa mandibularis on ectal surface, with two medium-sized setae on basal portion near hypocondyle, MDL 0.06–0.07 (0.065, $n = 12$) mm. Hypostoma (Figures 15–17, 19–22, 34) with straight smooth mesal elevation with mesal groove, flanked by serrate margins, with several uncountable teeth. Epipharynx (Figures 26–28, 31, 32, 35) less massive, with three combs: comb 4 (= dorsal comb) massive, its posterior margin irregular, bearing six to eight lanceolate teeth; other two combs (= ventral comb) overimposed, reduced; each lateral arms without curtain or fringe; LAW 0.072–0.084 (0.075, $n = 4$), DCW 0.048 ($n = 4$) mm, median sclerite with auxiliary sclerite bearing comb of eight or nine small, lanceolate teeth. Hypopharynx (Figures 26–28) slender, sclerotised, posterior end of each arm irregular, without hypopharyngeal fringe, labium small, broad. Thoracic pigmentation diffuse yellowish. Abdominal segment whitish, without defined pigmentation, with slender setae (Figures 23–25, 36). Caudal segment: chaetotaxy as in Figures 24, 25; one pair of subequal, medium-sized, thin setae 'o' 0.13 mm; CSL 0.56–0.71 (0.62, $n = 7$) mm, CSW 0.13–0.24 (0.17, $n = 7$) mm; CSR 3.86 (3.10–4.30, $n = 5$), OD 0.50 ($n = 1$) mm.

Second and third instar larvae.

Similar to fourth instar larva, with differences in size and some proportions. For measurements and ratios of head capsule and caudal segment see Table 1.

Female pupa (Figures 30, 38–44, 47, 48, 50, 51).

Length 3.77 (3.61–4.11, $n = 3$) mm. General coloration uniformly pale brown. Exuviae pale brown, body surface smooth. Operculum (Figures 30, 38) rounded dorsally, with dorsomedial notch; disc covered by stout pointed spicules, except central bare area; well-developed anteromarginal seta (am) (Figures 30, 38) located on triangular tubercle, with pore at tubercle base; posterior margin slightly convex, smooth, OL 0.132 mm; OW 0.168 mm; OW/OL 1.27. Thorax surface smooth, with medial crest extending between bases of respiratory organs. One long, thin anterodorsal seta with associated pore, on short tubercle (ad) (Figure 42); two dorsomedial medium-sized seta (dm) (Figures 38, 40, 47); three medium-sized, stout dorsolateral setae (dl), two thick, one thin (Figures 38, 41, 47); five dorsal sensilla (d) (Figures 38, 39): i, ii, iv elongate, thin setae, v, vi each a pore. Respiratory organ (Figures 38, 47) pale brown, short, with 12–16 pores opening at tubercles on curved lateral margin, other lateral margin nearly straight; length 0.18–0.192 (0.184, $n = 4$) mm; pedicel slightly darker,

Table 1. *Stilobezzia enigma* n.sp., larva. Measurements (in mm with SEM) and ratios of head capsule and caudal segment.

	Larval instars		
	II	III	IV
Head capsule			
HL	0.153	0.172	0.240
HW	0.094	0.126	0.135
HR	1.66	1.62	1.27
SGW	0.072	0.079	0.106
SGR	1.65	2.11	3.13
Caudal segment			
CSL	0.54	0.55	0.77
CSW	0.098	0.11	0.15
CSR	5.00	5.00	5.17
OL	X	X	0.35
OD	0.34	0.521	0.538

HL, head length; HW, head width; HR, head ratio = HL/HW; SGW, subgenal width; SGR, subgenal ratio; CSL, caudal segment length; CSW, caudal segment width; CSR, caudal-segment ratio = CSL/CSW; OL, length of caudal setae 'o'; OD, distance between bases of caudal setae 'o'.

smooth, short, length 0.076 (0.069–0.082, $n = 2$) mm; P/RO 0.045–0.062 (0.052, $n = 3$). Two medium-sized, thin ventromedian setae (vm), two elongate thin vl setae and one associated pore (Figure 43). Abdominal segments integument smooth with scarse spicules on anterior margin, tubercles short, with single, pointed apex or, if very small, some bifid. First abdominal segment (Figure 50) with setae as follows: two anterior setae, mesal one slightly stouter, with associated pore; four lateral setae; two posterior setae, mesal one slightly stouter, one posterior pore medial to posterior setae. Fourth abdominal sensillar pattern (Figure 51), as follows: one dorsal anterosubmarginal (d.a.s.m.) long, thin seta; five dorsal posteromarginal (d.p.m.) sensillae, i, iv, v long, thin setae, ii, iii pores; one lateral anterosubmarginal (l.a.s.m.) medium-sized, thin seta; three lateral posteromarginal (l.p.m.), elongate setae on strong, raised bases; three ventral posteromarginal (v.p.m.) medium-sized setae each on very small tubercle, ii longer, thinner than others. Anal segment dorsal surface smooth, only a few spicules on anterior edge, apicolateral processes triangular, very long, base wide, parallel, darkish extreme tips, each with one dorsal, one lateral pore; length 0.515 mm, 0.50 mm, width 0.020 mm, apicolateral process length 0.24 mm, 0.25 mm.

Male pupa (Figures 37, 45, 46, 49, 52).

Similar to female with sexual differences; Operculum (Figure 49) as in female, with dorsomedial notch more evident; OL 0.24–0.27 (0.25, $n = 5$) mm; OW 0.18–0.216 (0.206, $n = 5$) mm; 1.12–1.128 (1.123, $n = 5$). Respiratory organ (Figure 26) length 0.156–0.19 (0.17, $n = 10$) mm; pedicel length 0.006–0.012 (0.0096, $n = 5$) mm; P/RH 0.045–0.062 (0.052, $n = 2$). Anal segment (Figures 45, 46, 52) length 0.576–0.648 (0.602, $n = 5$) mm, width 0.192–0.216 (0.206, $n = 5$) mm, apicolateral processes length 0.252–0.312 (0.280, $n = 5$) mm; 0.18–0.216 (0.199, $n = 5$) mm.

Type material examined

Holotype male with larval and pupal exuviae, labelled "Holotype Stilobezzia enigma Ronderos, Spinelli and Borkent", "Argentina, Misiones, Corpus, 24-XI-2005, E.

Lestani'' (MLPA); allotype female; Corpus, Puerto Maní, VI-2000, F. Krsticevic - S. Araki (MLPA). Paratypes, 13 males, 2 females, 16 larvae, 3 pupae, 3 pupal exuviae, 1 fourth larval exuviae, as follows: same data as holotype except 22-X-1999, Pascual-Araki, 1 male, 1 female, 3 pupal exuviae, 1 fourth larval exuviae (CNCI); same data as allotype, 1 male (with pupal exuviae) (MLPA); same data except in tacuara, 3 males (each with pupal exuviae), 6 fourth instar larvae (CNCI); Parque Nacional Iguazú, in *Guadua chacoensis* (Rojas) (Bambuseae), 29-X-2005, M. Mogi - R. Campos, 6 males (3 with pupal exuviae), 1 female (with pupal exuviae), 6 fourth instar larvae (on 2 slides, 3 larvae on each slide) (MLPA); Puerto Iguazú, V-2006, R. Campos, 2 males (with pupal exuviae) (USNM and FSCA); same data except V-2007, 2 male pupae, 1 female pupa, 4 larvae (on SEM stubs, MLPA).

Distribution and bionomics

This new species is known only from Misiones province, Argentina (Figure 53). Larvae and pupae were collected from water accumulated in the internodes of two species of bamboo, *Guadua chacoensis* and *Guadua trinii*. Other taxa collected in the bamboo internodes were *Forcipomyia* sp., *Dasyhelea* sp., *Culicoides bambusicola* Lutz and *Palpomyia guarani* Lane. Most studies on phytotelmata bamboo habitats concern broken or cut internodes that are filled with rainwater instead of plant secretions. The majority of the investigations carried out on these microhabitats have been undertaken in Asia and have been particularly focused on the study of different aspects of mosquito biology (Mogi and Suzuki 1983). Within the Neotropical Region Louton et al. (1996) (Peruvian Amazon) and Lozovei (2001) (Brazilian Atlantic) studied the aquatic fauna of bamboo internodes. Louton et al. (1996) examined the entire macrofauna encountered in living culms of *Guadua* bamboo with naturally occurring lateral apertures, pointing out that '*Guadua* is a rapid coloniser by means of underground lateral shoots in areas disturbed by erosion or tree fall, and individual stems typically live about three years. Recurved spines protruding from the foliage internode joints allow the tall culms to remain suspended in the canopy, and made it difficult to pull down the cut stems'.

Live larvae exhibited the typical rapid, undulating swimming motion of other members of the subfamily Ceratopogoninae. When live pupae were placed in a Petri dish with some loose substrate, they exhibited the typical, semi-circular, slow abdominal movement of most other ceratopogonid pupae. They used their elongate apicolateral processes to thrust themselves forward against the substrate. The respiratory organ of pupae lacks scale-like spines, which were considered a common feature of tree-hole breeding species by Ronderos and Spinelli (2000) and Huerta et al. (2001). Pupae use their respiratory organs to obtain oxygen at the water surface.

Discussion

The generic placement of *S. enigma* is somewhat controversial. Even though we place it in *Stilobezzia*, it lacks the only synapomorphy reported for the genus, namely the presence of a medially split aedeagus. In most species of *Stilobezzia*, the aedeagus appears as two separate lateral rods that do not meet posteromedially (as they do in all other Ceratopogonidae with a complete aedeagus). In some other species of *Stilobezzia*, the aedeagus is very reduced, with the state not scorable; instead there is

a transverse bar between the gonocoxites near midlength and ventral to parameres. This latter group will be interpreted elsewhere (Borkent, in preparation). Even though technically the apex of the aedeagus of *S. enigma* is fused, the area between the two halves of the apex is more lightly sclerotised, so that, initially, under the dissecting microscope, it appears that the aedeagus is split. It is conceivable that this is an atavistic expression, or perhaps a superficial regression to the plesiomorphic state.

On the other hand, a pupal feature indicates that *S. enigma* is related to other species of the subgenus *Stilobezzia* Kieffer. *Baeodasymyia* Clastrier and Raccurt, 1979; *Monohelea* Kieffer, 1917; *Austrohelea* Wirth and Grogan, 1988; *Stilobezzia* and *Schizonyxhelea* Clastrier, 1984; have only three setae on the dorsum of the scutum (plus the pore) and generally these are on small to medium-sized tubercles. Within *Stilobezzia*, all species other than those with males with a transverse bar between their gonocoxites (e.g. *Stilobezzia bulla* Thomsen) have closely clumped dorsal setae. *Stilobezzia lutea* (Malloch) and a few others in the subgenus *Acanthohelea* Kieffer have the setae on low tubercles. All species of the subgenus *Stilobezzia* (12 species examined) have setae arising directly from the cuticle, without any tubercles; this is unique within the family and therefore a synapomorphy (also present in the Palaearctic species *Stilobezzia papillata* Remm and *Stilobezzia ochracea* (Winnertz) presently in *Acanthohelea* but probably belonging to the subgenus *Stilobezzia*). *Stilobezzia enigma* has three closely clumped dorsal setae arising directly from the cuticle and this indicates that this new species is related to other members of the subgenus *Stilobezzia*.

Other species of the subgenus *Stilobezzia* lack macrotrichiae on the wings (as is present in female *S. enigma*). However, as noted by Clastrier (1976), it seems unlikely to us that the presence or absence of macrotrichiae is an important feature for the recognition of subgenera.

Stilobezzia enigma has a number of distinctive features that in a phenetic framework would indicate separate generic status. These are all likely autapomorphies that are susceptible to homoplasy, and these are listed as follows in a morphological sequence:

- elongate spicules on eyes. Some *Stilobezzia* have spicules but not as long as in *S. enigma*. A scattered array of other Ceratopogonidae do have long spicules (e.g. *Baeodasymyia*, *Baeohelea* Wirth and Blanton, 1970; *Borkenthelea* Spinelli and Grogan, 1993; *Brachypogon* Kieffer, 1899; *Macrurohelea* Ingram and Macfie, 1931; *Notiohelea* Grogan and Wirth, 1979; *Parastilobezzia* Wirth and Blanton, 1970.
- large, broad palpal segment 3, with large, deep sensory pit. Some *Stilobezzia* have a palpal pit but when present it is relatively shallow and small. A large third palpal segment is present in a few other Ceratopogonidae such as *Notiohelea, Nannohelea* Grogan and Wirth, 1980; and *Rhynchohelea* Wirth and Blanton, 1970.
- loss of mandibular teeth in female. Some other *Stilobezzia* lack teeth but these are all very small species. Otherwise loss of mandibular teeth has occurred in numerous other genera and this feature is clearly susceptible to homoplasy.
- straight female claws. Only a few other species of *Stilobezzia*, in the subgenus *Acanthohelea*, have females with similar straight claws on each leg, as do some other Ceratopogonidae (even if in some others they are paired and not single as

in *S. enigma*), (e.g. *Baeodasymyia*, *Baeohelea*, *Leptohelea* Wirth and Blanton, 1970; *Macrurohelea*, *Notiohelea*, *Parastilobezzia*).

- sternite 10 and cerci pale, contrasting with an otherwise dark brown abdomen. This feature appears in a few other lineages of Ceratopogonidae [e.g. *Allohelea* Kieffer (1917), *Downeshelea* Wirth and Grogan (1988)].
- pupa with four setae on the lateral margin of T1. Other *Stilobezzia* have only three setae, but, these are smaller and at most, on very small tubercles (shorter than in this new species). Related genera also have only three setae but *Monohelea* has four setae, although these are arranged on a single rounded tubercle. This indicates that the presence of four setae in *S. enigma* is derived but as an autapomorphy it has no significance phylogenetically.
- pupa with elongate apicolateral processes. This feature occurs in some other *Stilobezzia*, some *Dasyhelea* Kieffer, 1911, some Sphaeromiini and some Palpomyiini. Clearly it is susceptible to homoplasy.

We are reluctant to propose new genera, especially when the phylogenetic relationships are unclear or conflicting, as in this instance. Therefore, considering all the above, we interpret *S. enigma* as a modified member of *Stilobezzia*.

Even though the only synapomorphy we identify here places *S. enigma* in the subgenus *Stilobezzia*, some other of its features are common in other species of *Stilobezzia* in the subgenus *Acanthohelea*, such as sexually dimorphic macrotrichiae on the wings, well-developed setae on R and R_3 on that of the female (hardly any or none on male wing), a small round third spermatheca and small straight claws, the latter two features of which are present in some species of *S.* (*Acanthohelea*).

Finally, both male and female *S. enigma* can be identified to the genus *Stilobezzia* in the key to Neotropical genera by Borkent and Spinelli (2007) if one does not apply the conditions regarding the aedeagus in couplet 38.

References

Borkent, A., and Craig, D.A. (2001), 'Submerged *Stilobezzia rabelloi* Lane (Diptera: Ceratopogonidae) pupae obtain oxygen from the aquatic fern *Salvinia minima* Baker', *Proceeding of the Entomological Society of Washington*, 103, 655–665.

Borkent, A., and Spinelli, G.R. (2007), 'Neotropical Ceratopogonidae (Diptera: Insecta)', in *Aquatic Biodiversity in Latin America (ABLA)* (Vol. 4), eds. J. Adis, J.R. Arias, G. Ru-Delgado and K.M. Wantzen, Sofia-Moscow: Pensoft, p. 198.

Clastrier, J. (1976), Étude systématique du genre *Stilobezzia* (Dipt. Ceratopogonidae). *Annales de la Société de France (N. S.)*, 12, 567–578.

Huerta, H., Ronderos, M.M., and Spinelli, G.R. (2001), 'Description of larva and pupa and redescription of the adult of *Culicoides albomaculus* Root & Hoffman (Diptera: Ceratopogonidae)', *Transactions of the American Entomological Society*, 127, 545–561.

Louton, J., Gelhaus, J., and Bouchard, R. (1996). 'The aquatic macrofauna of water-filled bamboo (Poaceae: Bambusoideae: Guadua) internodes in a Peruvian lowland tropical forest', *Biotrópica*, 28, 228–242.

Lozovei, A.L. (2001), 'Microhabitats de mosquitos (Diptera, Culicidae) em internódios de taquara na mata atlântica, Paraná, Brasil', *Iheringia, serie Zoologia*, 90, 3–13.

McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R., and Wood, D.M., (coords.). (1981), *Manual of Nearctic Diptera, Volume 1,* Agriculture Canada Monograph 27, Ottawa, ON: Agriculture Canada, p. 674.

Mogi, M., and Suzuki, H. (1983), 'The biotic community in the water-filled internode of bamboos in Nagasaki, Japan, with special reference to mosquito ecology', *Japanese Journal of Ecology*, 33, 271–1983.

Ronderos, M.M., Díaz, F., and Sarmiento, P. (2008), 'A new method using acid to clean and a technique for preparation of eggs of biting midges (Diptera: Ceratopogonidae) for Scanning Electron Microscope', *Transactions of the American Entomological Society*, 134, 471–476.

Ronderos, M.M., and Spinelli, G.R. (2000), 'The larva and pupa of *Culicoides bambusicola* Lutz observed with SEM, and additional notes on the adult (Diptera: Ceratopogonidae)', *Transactions of the American Entomological Society*, 126, 133–144.

Ronderos, M.M., Spinelli, G.R., and Sarmiento, P. (2000), 'Preparation and mounting of biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) to be observed with scanning electron microscope', *Transactions of the American Entomological Society*, 126, 125–132.

Szadziewski, R. (1996), 'Biting midges from Lower Cretaceous amber of Lebanon and Upper Cretaceous Siberian amber of Taimyr (Diptera: Ceratopogonidae)', *Studia Dipterologica*, 3, 23–86.