

# The previously unknown pupa and adult male of *Neobezzia fittkai* Wirth & Ratanaworabhan (Diptera, Ceratopogonidae)

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**ABSTRACT.** The previously unknown pupa and adult male of *Neobezzia fittkai* Wirth & Ratanaworabhan (Diptera, Ceratopogonidae). The pupa of *Neobezzia fittkai* Wirth & Ratanaworabhan, 1972, collected from a mat of floating fern (*Salvinia auriculata* Aubl., Salviniaceae) in Ilha da Marchantaria near Manaus, Brazil and the reared adult male are described, photographed and illustrated for the first time. This is the first detailed pupal description for the genus *Neobezzia* Wirth & Ratanaworabhan.

**KEYWORDS.** Amazon; immatures; male adult; *Neobezzia fittkai*.

**RESUMO.** Pupa e adulto macho previamente desconhecidos de *Neobezzia fittkai* Wirth & Ratanaworabhan (Diptera, Ceratopogonidae). A pupa de *Neobezzia fittkai* Wirth & Ratanaworabhan, 1972 foi coletada em macrófitas aquáticas flutuantes (*Salvinia auriculata* Aubl., Salviniaceae) na Ilha da Marchantaria próximo a Manaus, Brasil e o adulto macho criado foi descrito, fotografado e ilustrado pela primeira vez. Esta é a primeira descrição para a pupa do gênero *Neobezzia* Wirth & Ratanaworabhan.

**PALAVRAS-CHAVE.** Adulto macho; Amazonas; imaturos; *Neobezzia fittkai*.

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Ceratopogonidae are one of the most common and diverse fly families in the world, with 5989 extant and 265 fossil species presently known (Borkent 2009). However, knowledge of their immatures is limited, with fewer than 15% of the named species for the Neotropical Region described in even one stage (Borkent & Spinelli 2007).

The genus *Neobezzia* Wirth & Ratanaworabhan (1972), exclusively Neotropical, is distributed from Nicaragua to northeastern Argentina, with six of the eight known species inhabiting Amazonas (Borkent & Spinelli 2007). It is placed in the tribe Sphaeromiini, and the adult females are characterized by the unarmed femora, fore femur slender, equal claws with pointed external basal tooth, and wing with one radial cell, anal angle poorly developed, and long costa; adult males are characterized by the wing with one radial cell and gonocoxite and tergite 9 elongate. The pupa of only one species, *N. amnicola* (Macfie, 1940), has been previously but rather incompletely described by Mayer (1959), avoiding any reference to most of the cephalothoracic tubercles and setae and mentioning the tubercles of the fourth abdominal segment with scarce detail.

One of the known species, *Neobezzia fittkai* Wirth & Ratanaworabhan, 1972, was described based on two females from Tiririca, Rio Preto, and Amazonas, Brazil. The purpose of this paper is to provide the first description of male adult and describe the pupa of this species, representing as well the first detailed description of the pupa of the genus *Neobezzia*, based on material collected during a recent survey of immatures of Ceratopogonidae near Manaus, Brazil.

## MATERIAL AND METHODS

The pupae of *Neobezzia fittkai* were collected with a pipette from mats of the floating aquatic fern (*Salvinia auriculata* Aubl., Salviniaceae) (Fig. 2) in a lagoon on Ilha da Marchantaria, Município de Iranduba, Amazonas (Fig. 1). Mats were placed into white buckets. The pupae were transported to the laboratory individually in vials with a drop of water. Observations were made daily until adult emergence. Adults were allowed to harden for 24 hours before being preserved to ensure their pigmentation was complete.

Pupal exuviae and adults were examined using a binocular compound microscope (BCM) after being slide-mounted in Canada balsam following the technique described by Borkent & Spinelli (2007). Pupal exuviae were mounted dorsoventrally. Ink illustrations were made with a camera lucida. A pupa was examined with a Scanning Electron Microscope JSM6360LV using the technique of Ronderos *et al.* (2000), except that we used 30% glycolid acid and the exposure time of cleaning was increased to 24 hours, and posteriorly using the technique of Boltovskoy (1976) to mount the specimen. Photomicrographs were taken with a Pentax Optio Power Shot S60 digital camera through a Leitz SM-Lux (10 or 40×).

Terminology of the pupa follows Borkent & Craig (2001) and terms for adults are those in the Manual of Nearctic Diptera (McAlpine *et al.* 1981). Specimens are deposited in the following collections: Instituto Nacional de Pesquisas da Amazonia (INPA), Manaus, Brazil; Museo de La Plata, Argentina (MLP) and the Canadian National Collection of Insects (CNCI).

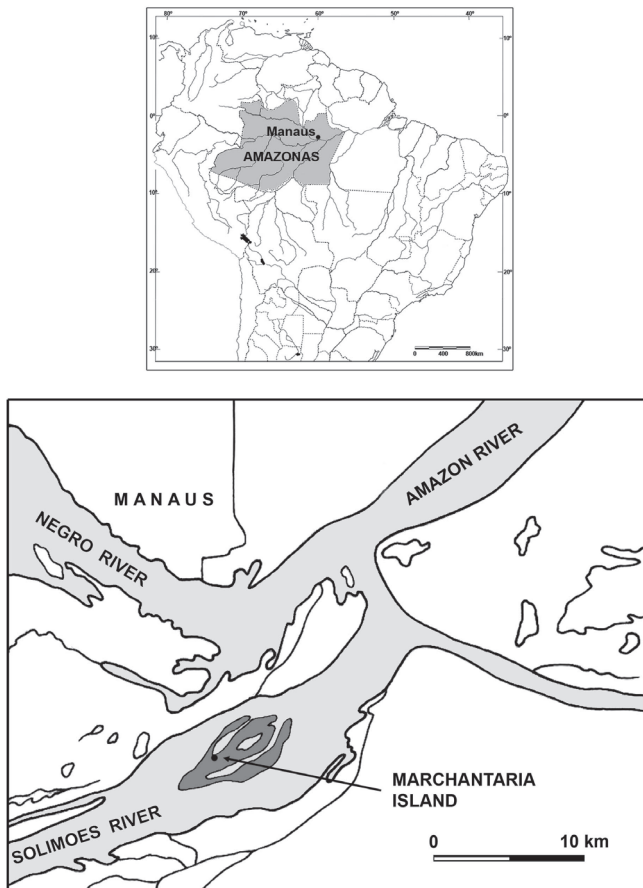


Fig. 1. Collecting sites at Marchantaria Island, in the Solimões River, Manaus, Amazon, Brazil.

## RESULTS

### *Neobezzia fittkau* Wirth & Ratanaworabhan

(Figs. 1–37)

*Neobezzia fittkau* Wirth & Ratanaworabhan, 1972: 489 (female; Brazil); Borkent & Wirth 1997: 120 (in world catalog); Borkent & Spinelli 2000: 59 (in catalog of species south of USA); Borkent & Spinelli 2007: 91 (in Neotropical catalog); Borkent 2009: 151 (online catalog).

**Diagnosis.** Male adult: the only species of *Neobezzia* with mid and hind tibiae entirely dark brown, forefemur yellowish on proximal half, stout aedeagus and with the tip of the fused parameres bulbous. Female adult: the only species of *Neobezzia* with wing length about 2.6 mm, costal ratio 0.93–0.95. Pupa: the only species of *Neobezzia* with two ventrolateral setae and lateral pore.

**Description of adult male.** Head. Dark brown (Fig. 29). Eyes bare, separated by distance equal to diameter of seven ommatidia. Flagellum brown, except flagellomeres 11–13 dark brown; four distal flagellomeres elongate (Figs. 30, 33); antennal ratio 0.67–0.80 (0.73,  $n = 6$ ). Palpus brown, short (Figs. 29, 34), third segment with 3–4 subapical sensilla on inner margin; palpal ratio 1.17–1.50 (1.32,  $n = 8$ ).

Thorax. Dark brown, scutum with few strong hairs, scutellum with 4–5 strong setae, seven smaller ones. Legs (Fig. 31) dark brown; coxae, trochanters, basal ½ of forefemora paler; tarsi pale except tarsomeres 5 infuscated, hind tibial comb with 3–4 bristles; prothoracic TR 1.68–1.94 (1.84,  $n = 6$ ), mesothoracic TR 2.00–2.35 (2.11,  $n = 6$ ), metathoracic TR 2.08–2.40 (2.29,  $n = 6$ ); ventral palisade in one row on tarsomeres 1 of midleg and tarsomere 2 of hind leg, in two rows on tarsomere 1 of hind leg; tarsomeres 4 subcylindrical; tarsomeres 5 unarmed, claws small, equal-sized, with bifid tips. Wing (Fig. 32) length 1.36–1.50 mm (1.44,  $n = 6$ ), width 0.40–0.43 mm (0.42,  $n = 5$ ); membrane slightly infuscated; anterior veins brown, apical portion of  $M_1$ ,  $M_2$ ,  $CuA_1$ ,  $CuA_2$  pale brown; anal angle poorly developed; costal ratio 0.82–0.86 (0.84,  $n = 6$ ). Halter pale brown.

Abdomen. Dark brown. Genitalia (Figs. 35): tergite 9 progressively narrowing distally, posterior margin notched, not reaching level of apex of gonocoxite; sternite 9 0.25 times longer than broad, distal margin deeply excavated; sternite 10 broad, spiculate, rounded distally. Gonocoxite slender, 3.7 times longer than greatest breadth, inner margin with anterior pointed protuberance; gonostylus 0.5 as long as gonocoxite, slender, slightly curved with pointed tip. Parameres (Fig. 36) fused on distal 3/4; basal arch high; basal arms slender, subparallel, well sclerotized, apex bulbous. Aedeagus (Fig. 37) triangular, 1.3 times longer than greatest (basal) breadth, tapering to rounded caplike tip; basal arch extending to 0.2 of total length; basal arms slender, well sclerotized; cercus elongate.

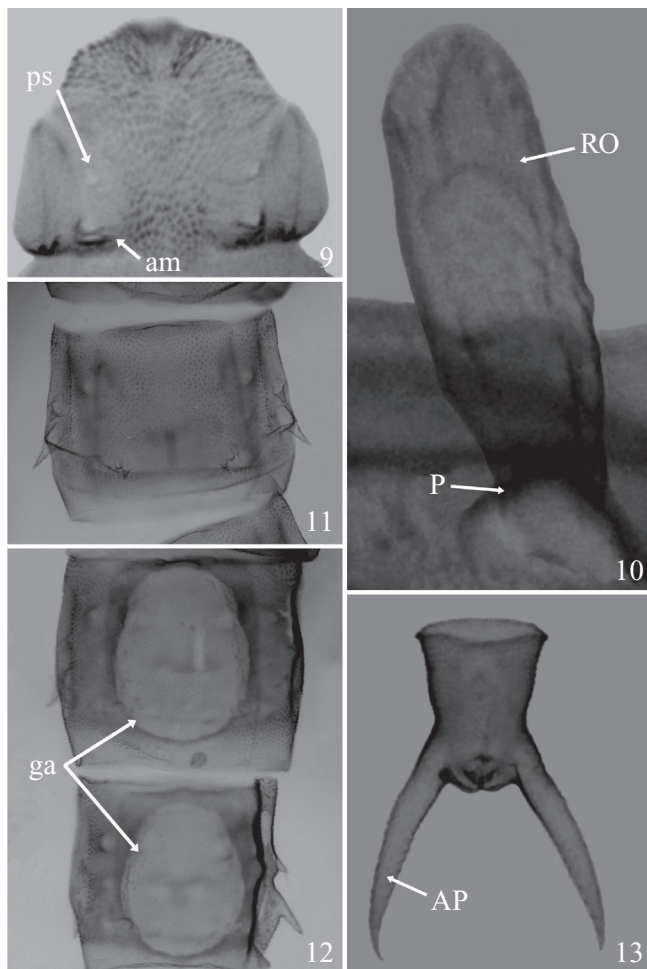
Male pupa (Figs. 3–8, 14–28). Total length 4.06–4.64 mm (4.2,  $n = 5$ ). Exuviae brown (Fig. 4). Operculum triangular (Figs. 5, 23) with disc broader than long, OL 0.10–0.11 mm (0.11,  $n = 7$ ), OW 0.18–0.20 mm (0.19,  $n = 7$ ), OW/OL 2.00–2.66 (2.16,  $n = 7$ ); anterior margin pointed, surface covered with small, rounded tubercles; each side of mesal portion with pair of raised areas, ventral one blunt, with anteromarginal seta, dorsal one with large pore. Two stout ventromedian setae (Figs. 4, 6, 14–15, 25), one longer than other; two elongate ventrolateral setae (Figs. 14–15, 25) thinner than vm, lateral pore present. Respiratory organ (Figs. 3, 14, 16, 20) relatively short, broad, 2.75 times longer than broad, RO length 0.13–0.16 mm (0.14,  $n = 7$ ), RO width 0.032–0.044 mm (0.038,  $n = 7$ ), surface smooth, apex with 13 spiracles (sp) in two rows; pedicel (P) short, stout, pedicel length 0.014–0.030 mm (0.018,  $n = 7$ ), P/RO 0.11–0.21 (0.15,  $n = 7$ ). Cephalothorax (Figs. 6, 14) length 1.28–1.68 mm (1.39,  $n = 8$ ), width 0.74–0.90 mm (0.80,  $n = 8$ ); medial crest medium-sized, crenulate, extending between bases of respiratory organs; cephalothoracic setae (Figs. 6, 21–22) as follows: two anterodorsal setae (Figs. 6, 22), one peg, one pore; one stout, short dorsolateral setae (Figs. 6, 21); three dorsomedian setae (Figs. 6, 21); one long, thin, other peg, other pore; six dorsal setae (Figs. 3, 17, 24) on rounded tubercles: i–iii peg, iv medium-sized, thin, v–vi pore. Abdominal segments covered with small pointed spicules, each segment with small pigmented anterolateral spiracular scar. Abdominal tergites



Figs. 2–8. 2, *Salvinia auriculata* Aubl, collecting site; 3–8, *Neobezzia fittkaui* Wirth & Ratanaworabhan, male pupa: 3, entire pupa (specimen in alcohol), lateral view; 4, entire pupa (slide mounted specimen), ventral view; 5, operculum; 6, cephalothorax, ventral view; 7, 4<sup>th</sup> abdominal segment, dorsal view; 8, anal segment. [Legends: anterodorsal setae (ad); anteromarginal setae (am); antenna (AN); anal segment (AS); apicolateral processes (AP); dorsal anterosubmarginal setae (d.a.s.m.); dorsal posteromarginal setae (d.p.m.); dorsal sensillae (d); dorsolateral seta (dl); dorsomedian setae (dm); foreleg (fl); glandular areas (ga); genital sac (GS); midleg (ml); operculum (o); pedicel (P); pore at tubercle base (ps); respiratory organ (RO); ventromedian setae (vm)].

1–7 with d.a.s.m. i, d.p.m. i on short, sclerotized tubercles, each of these tubercles arising directly from surrounding cuticle. First abdominal segment (Figs. 3–4, 26) with setae as follows: two anterior setae: i medium-sized, ii long, thin, iii pore on mesal portion; four posterior setae: i minute, ii pore, iii peg, iv, long, thin setae; three lateral sensilla: one medium-size, one long thin seta and one pore. Second abdominal segment similar to first, except all setae longer, and

one small lateral seta. Fourth abdominal segment (Figs. 3–4, 7, 27) with two dorsal anterosubmarginal setae and one pore: i, medium-sized, thin seta, ii long, thin seta, iii pore; four dorsal posteromarginal setae and one pore: i, iv peg, ii absent, iii pore, v long, thin seta; three lateral posteromarginal setae (Fig. 18): i, short, thin on stout triangular tubercle with minute spines, ii long, thin seta on rounded small base, iii minute on small base; one lateral anterosubmarginal seta (Fig.



Figs. 9–13. *Neobezzia fittkai*, female pupa: 9, operculum; 10, respiratory organ; 11, 4<sup>th</sup> abdominal segment (ventral view); 12, six and seven abdominal segment; 13, anal segment. [Legends: anteromarginal setae (am); apicolateral processes (AP); glandular areas (ga); pedicel (P); pore at tubercle base (ps); respiratory organ (RO); spiracular scar (sp.s); lateral antersubmarginal seta (l.a.s.m.); lateral posteromarginal setae (l.p.m.); ventral posteromarginal setae (v.p.m.).]

18), i, long, thin on strong triangular tubercle; three ventral posteromarginal setae (Fig. 18), i medium-sized, thin, ii long, thin, iii thin, shorter than i, ii; ii-iii on bifid tubercles. Abdominal tergites six and seven with large disc-like glandular areas (Fig. 4). Anal segment (Figs. 3–4, 8, 19, 28) 2.6 times longer than greatest width, ASL 0.49–0.58 mm (0.53,  $n = 7$ ), ASW 0.18–0.21 mm (0.19,  $n = 7$ ); lateral margins concave, ventral surface with abundant rounded small, posteriorly directed tubercles not extending to bases of apicolateral processes. Apicolateral processes (Figs. 3–4, 8, 19, 28) stout, curved, divergent, with pointed spinules, base broad, one ventral pore on each base, tip sharply pointed, length 0.29–0.36 mm (0.32,  $n = 7$ ).

Female pupa (Figs. 9–13) – Similar to male with sexual differences: Total length 4.86–4.98 mm (4.92,  $n = 2$ ). Exuviae brown. Operculum (Fig. 9) as in male except sexual differences: operculum anterior end rounded with small notch;

OL 0.06–0.07 mm (0.07,  $n = 2$ ); OW 0.17–0.18 mm (0.17,  $n = 2$ ); OW/OL 2.5–2.8 (2.65,  $n = 2$ ). Respiratory organ (Fig. 10) length 0.13–0.14 mm (0.14,  $n = 2$ ); width 0.04–0.05 mm (0.05,  $n = 2$ ); pedicel length 0.015 mm ( $n = 2$ ); P/RO 0.115–0.12 (0.12,  $n = 2$ ). Cephalothorax length 1.66–1.70 mm (1.68,  $n = 2$ ), width 0.86–0.92 mm (0.89,  $n = 2$ ). Fourth abdominal segment as in Fig. 11. Abdominal segments 6–7 as in Fig. 12. Anal segment (Fig. 13) length 0.54–0.62 mm (0.58,  $n = 2$ ); width 0.21–0.22 mm (0.22,  $n = 2$ ), apicolateral processes length 0.34 mm ( $n = 2$ ) (Fig. 13).

Distribution. BRAZIL (Amazonas).

Specimens examined. BRAZIL, Amazonas, Município de Iranduba, Ilha da Marchantaria, 03°15' S 59°58' W, 28-V-2009, Ronderos – Ferreira-Keppler, 1 male (with pupal exuvia), collected as pupa, reared in laboratory (INPA). Same data except 17-VI-2009, Ferreira-Keppler, 2 males (with pupal exuviae) (INPA). Same data except Ilha da Marchantaria, Lago Camaleão, 27-VII-2010, Marino, Díaz & Torreias, 4 males, 2 females (with pupal exuviae), on *Salvinia auriculata* (2 males, 1 female in INPA; 1 male, 1 female in MLP; 1 male in CNCI). Same data except Díaz, Marino & Torreias, 1 male (with pupal exuvia) (INPA), on *Pistia stratiotes* and *Salvinia auriculata*.

Specimen examined with SEM. BRAZIL, Amazonas, Município de Iranduba, Ilha da Marchantaria, Lago Camaleão, 27-VII-2010, Díaz, Marino & Torreias, 1 male pupa (MLP).

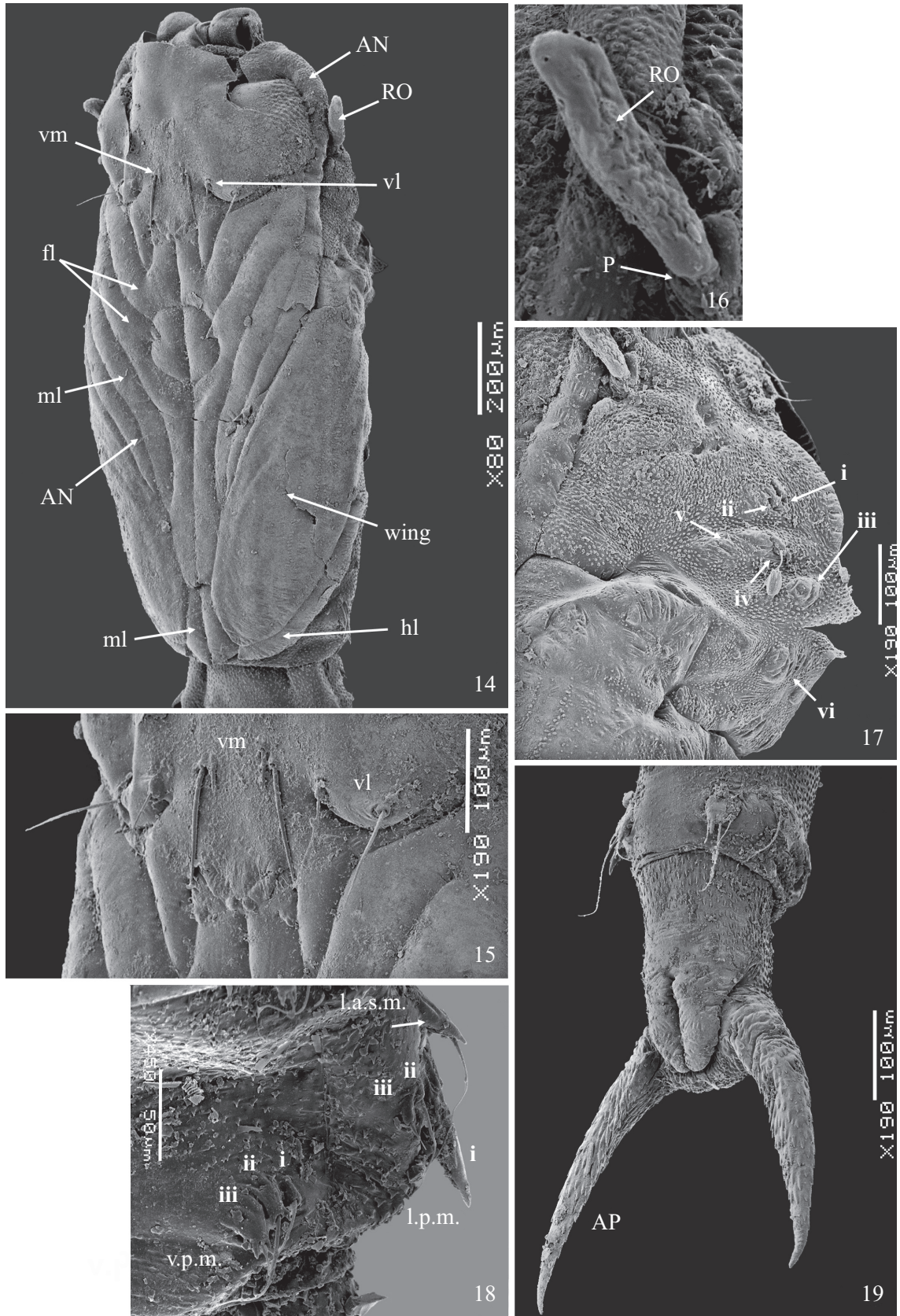
Remarks. The pupae of *N. fittkai* were collected with pupae of *Dasyhelea* sp., *Alluaudomyia punctivenosa* Wirth & Grogan, 1988, *Monohelea* sp., *Stilobezzia* sp., *Palpomyia* sp., *Paryphoconus aemulus* Macfie, 1940, and *Paryphoconus* sp.

The freshly collected pupae were very dark. When kept in the laboratory, they lost some of this pigmentation, probably as a result of changes in the laboratory environment. The presence of the adhesive sternites in the Sphaeromiini pupae indicates that they climb out of the water to stick to emergent vegetation (Borkent, pers. obs.). The pupae wiggle and twist until they enter the meniscus of emergent vegetation and then they push their way upward and out of the water. They then become completely quiescent and do not move at all. We observed the same behavior in *Neobezzia fittkai*. However, when the pupae were removed from the aquatic mats, and placed them in water in white buckets without any vegetation, they moved actively. The developmental times of the pupae to adult hood was 3 days in the laboratory at 17°–20° C and 90% humidity.

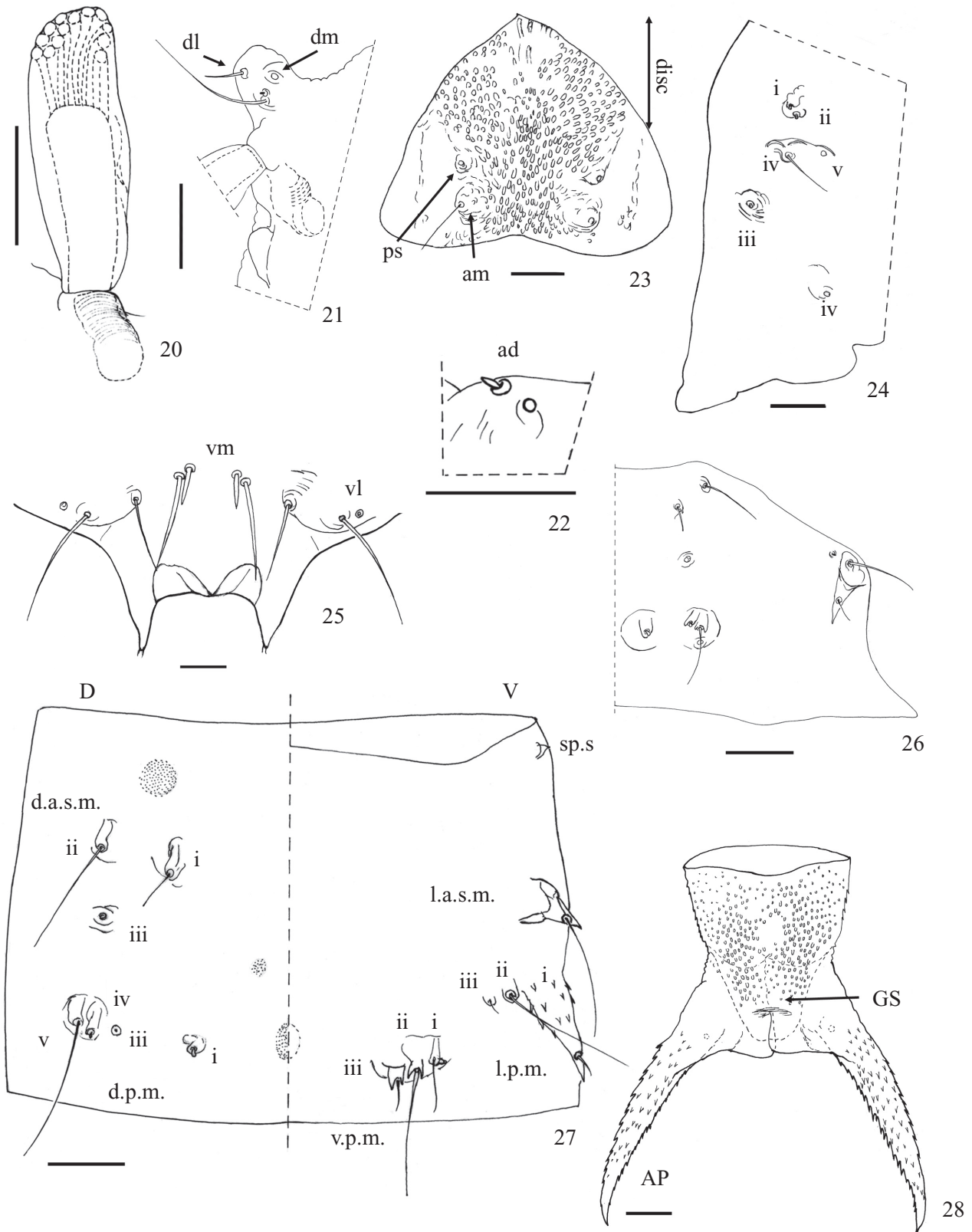
## DISCUSSION

The adult female runs to *N. fittkai* in the key to females provided by Wirth and Ratanaworabhan (1972). Of the eight recognized species of *Neobezzia*, only the males of *N. amnicola* and *N. wirthi* Spinelli & Felipe-Bauer, 1990 are known. The male of *N. fittkai* is easily recognized from those species by its stouter aedeagus and the shape of the tip of the fused parameres (a spatulate lobe in *N. amnicola*, blunt and not bulbous in *N. wirthi*).

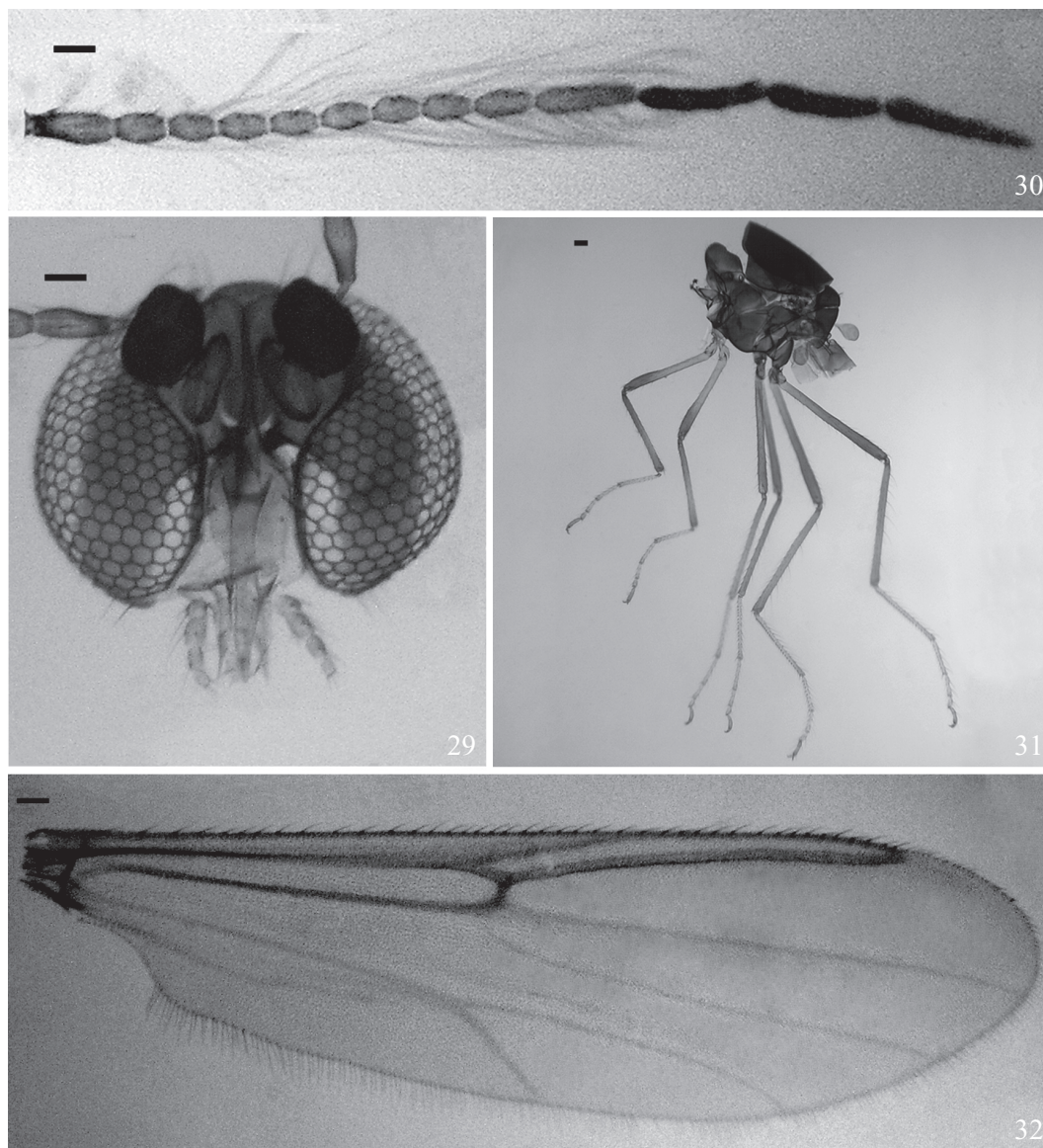
The pupa of *Neobezzia* is very similar to some species of the Holarctic genus *Probezia* Kieffer, 1906. Both groups have



Figs. 14–19. *Neobezzia fittkaui*, male pupa: 14, cephalothorax (ventral view); 15, ventromedian and ventrolateral setae; 16, respiratory organ; 17, dorsal setae (lateral view); 18, 4<sup>th</sup> abdominal segment (ventral view); 19, anal segment (ventral view). [Legends: antenna (AN); anal segment (AS); apicolateral processes (AP); foreleg (fl); genital sac (GS); hind leg (hl); midleg (ml); pedicel (P); respiratory organ (RO); spiracles (sp); ventrolateral setae (vl); ventromedian setae (vm); lateral anterosubmarginal seta (l.a.s.m.); lateral posteromarginal setae (l.p.m.); ventral posteromarginal setae (v.p.m.)].



Figs. 20–28. *Neobezzia fittkaui*, male pupa: 20, left respiratory organ; 21, dorsolateral and dorsomedian setae; 22, anterodorsal setae (ad); 23, operculum; 24, dorsal setae; 25, ventromedian and ventrolateral setae; 26, 1<sup>st</sup> abdominal segment; 27, 4<sup>th</sup> abdominal segment; 28, anal segment, dorsal view. Scale bars 0.05 mm. [Legends: anterodorsal setae (ad); anteromarginal setae (am); apicolateral processes (AP); dorsolateral seta (dl); dorsomedian setae (dm); genital sac (GS); pore at tubercle base (ps); spiracular scar (sp.s); 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 seta (l.a.s.m.); lateral posteromarginal setae (l.p.m.); ventral posteromarginal setae (v.p.m.).]



Figs. 29–32. *Neobezzia fittkau*, adult male: 29, head, frontal view; 30, flagellum; 31, thorax and legs; 32, wing. Scale bars 0.05 mm.

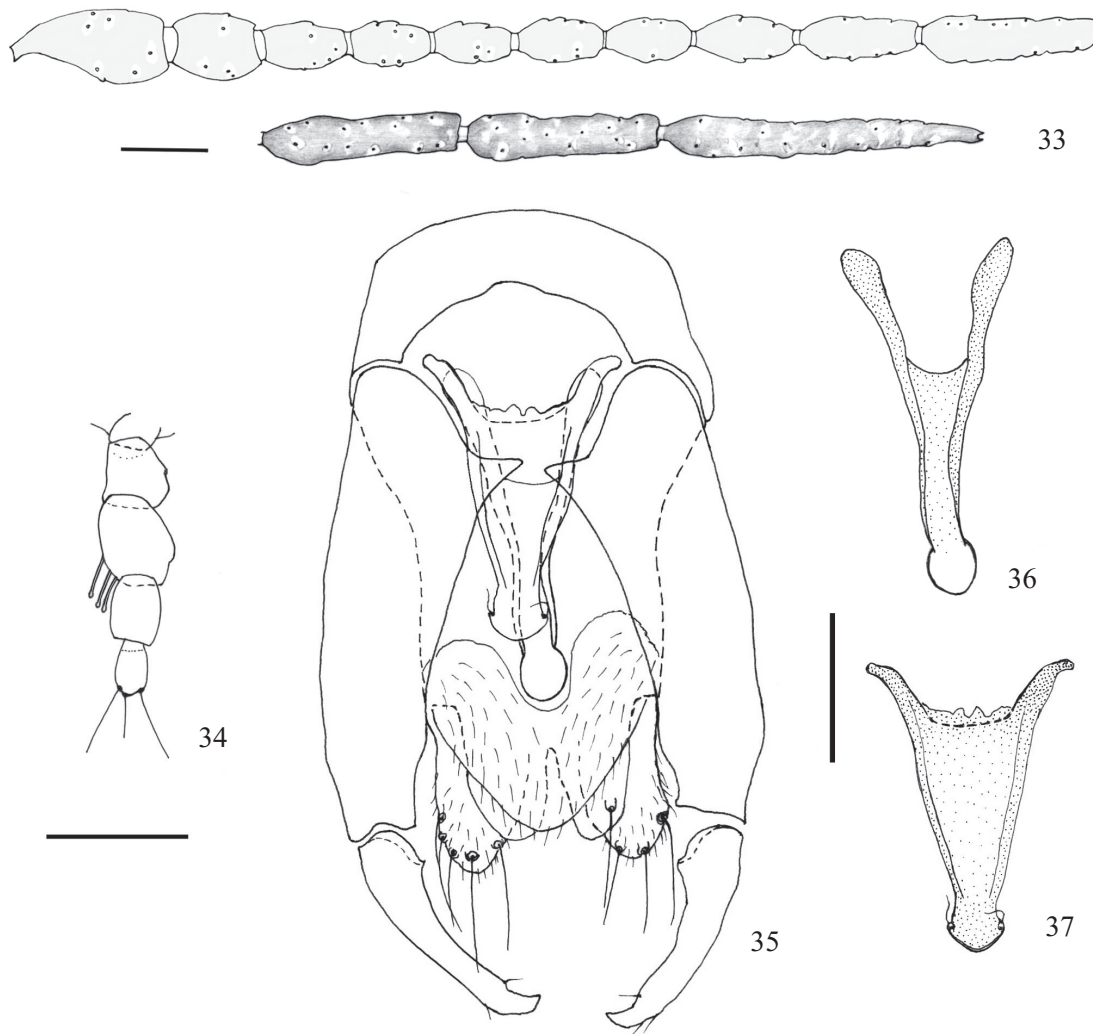
the abdominal tergites 1–7 with dorsal anterosubmarginal sensillae i and dorsal posteromarginal seta i on short sclerotized tubercles, and the fourth abdominal sternite with v.p.m. iii on a bifid tubercle. However, *Neobezzia* can be distinguished from these *Probezzia* by the lateral anterosubmarginal seta i on the fourth abdominal segment being a medium-sized, thin seta on a strong triangular tubercle (this seta short, thin, on small, apically rounded tubercle in *Probezzia fuscipennis* Wirth, 1971). A complete generic key for the pupae of Ceratopogonidae will be published elsewhere (Borkent, in prep.).

The description of the pupa of *N. amnicola* by Mayer (1959) is incomplete. The spiracles of the respiratory organ and the number of sensillae on the abdominal segments are apparently the same as those of *N. fittkau*; Mayer (1959) did not note the size or shape of the associated tubercles. *Neobezzia amnicola* apparently lacks the pore situated lat-

eral to the two ventrolateral setae. These are the only cephalothoracic setae mentioned by Mayer (1959). The second author (AB) has attempted to locate the original material described by Mayer but without success.

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Figs. 33–37. *Neobezzia fittkaui*, adult male: 33, flagellum (top, flagellomeres 1–10; bottom, flagellomeres 11–13); 34, palpus; 35, male genitalia (ventral view); 36, parameres; 37, aedeagus. Scale bars 0.05 mm.

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