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Morphology of the adult male and pupal exuviae of *Glyptotendipes* (*Glyptotendipes*) *glaucus* (Meigen 1818) (Diptera, Chironomidae) using scanning electron microscope (SEM)

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Abstract

In this paper, a study of the morphology of the pupa and male imago of *Glyptotendipes* (*G.*) *glaucus* (Meigen 1818) was carried out, with the aid of a scanning electron microscope (SEM). The SEM provided additional valuable information on the morphology of the species. Adult male head, antenna, wing, leg, abdomen, hypopygium, pupal cephalothorax and abdomen were examined. It is emphasized that SEM was not often used in Chironomidae studies. The present results confirm SEM as a suitable approach in carrying out morphological and taxonomical descriptions of Chironomidae species.

Key words: *Glyptotendipes* (*G.*) *glaucus*, pupal exuviae, male, morphology

Introduction

The imago of *Glyptotendipes* (*G.*) *glaucus* was first described by Meigen (1818) as *Chironomus glaucus*. Later morphological descriptions of the adult male of *G. (G) glaucus* were given by Edwards (1929), Michailova & Contreras-Lichtenberg (1995), and Langton & Pinder (2007). Pupal exuviae were described by Kalugina (1963), Pankratova (1983), and Michailova & Contreras-Lichtenberg (1995). All imago and pupal descriptions were based on observation with a light microscope. However, species of the genus *Glyptotendipes*, especially those that belong to group A (Pinder & Reiss 1986), are very similar and difficult to distinguish with the aid of light microscopy alone. Up to date, there are no descriptions of adults and pupal exuviae using SEM, its importance in morphological studies of Chironomidae has been discussed in detail by Kownacki *et al*. (2015, 2016).

In 2010, in the Goczałkowice Reservoir (southern Poland), an egg mass was found, from which all stages of *G. (G.) glaucus* were reared. A description of the cytogenetics, biology and morphology of the larvae of this species on the basis of SEM was presented by Kownacki *et al*. (2016). The aim of the study is to describe pupal exuviae and adult male of *Glyptotendipes* (*G.*) *glaucus* using the SEM. Our goal is to find new morphological details with taxonomical value.

Material and methods

An egg mass of *G.* (*G.*) *glaucus* was collected from the Goczałkowice Reservoir (southern Poland) on 11 August

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2010 and reared in laboratory using the method of Forbes & Cold (2005). Pupae and males were captured and preserved in 94 ml of 45% ethanol, 6 ml of glycerol, and thymol for further analysis with SEM and light microscope. For SEM investigation 5 pupal exuviae and 4 males were fixed in 2.5% glutaraldehyde GLU in 0.1 phosphate buffered saline PBS for 2 hours, rinsed with PBS 2x10 min and dehydrated in graded alcohols, then the specimens were placed in 100% acetone and transferred to Critical Point Drier, CPD E3000/E3100 Quorum Technologies. As a last step the specimens were coated with gold using JFC—1100E Ion sputter, Jeol. For coating, the materials were placed on the holder with conductive carbon adhesive tabs, Electron Microscopy Sciences. Morphological characters were analyzed by means of SEM, JSM—5410 operated at accelerating voltages of 15 kV in the Scanning Microscopy Laboratory of the Jagiellonian University. For light microscopy analysis some specimens of pupal exuviae and male were mounted with Faure liquid. Pictures were taken using a Nikon- Eclipse 50x light microscope fitted with a Digital sight DS-U1 camera. The morphological terminology according Sæther (1980) and Langton (1991) was used.

Results

Adult male. Medium-size species with a total length of about 10.0 mm. Wing length 5.0 mm. Body colour uniformly dark brown; legs brown; wing light with some brown veins.

Head (Figs. 1a–f). General view of head (Fig. 1a). Eye bare, with strong, parallel-sided dorsomedial extension (Figs. 1b,c). Frontal tubercles missing. Clypeus with about 50 long setae regularly distributed over entire surface (Figs. 1b,d). Maxillary palpus consisting of five palpomeres (Pm1–Pm5) (Fig. 1b). Palpomere lengths: 64, 96, 274, 269, 391 μm. First palpomere slightly chitinous (not visible in SEM, Fig. 1b), with one seta, which is difficult to see, palpomeres 2–5 chitinous, with long setae and densely covered with rows of microtrichia. Lacinia (La), slender, blade-like, carrying seta-like projection at tip and external surface (Fig. 1e). Labial lonchus (hypopharynx) (LL) has the shape of a triangular plate with numerous sharpened setae-like processes on the edge (Figs. 1e,f).

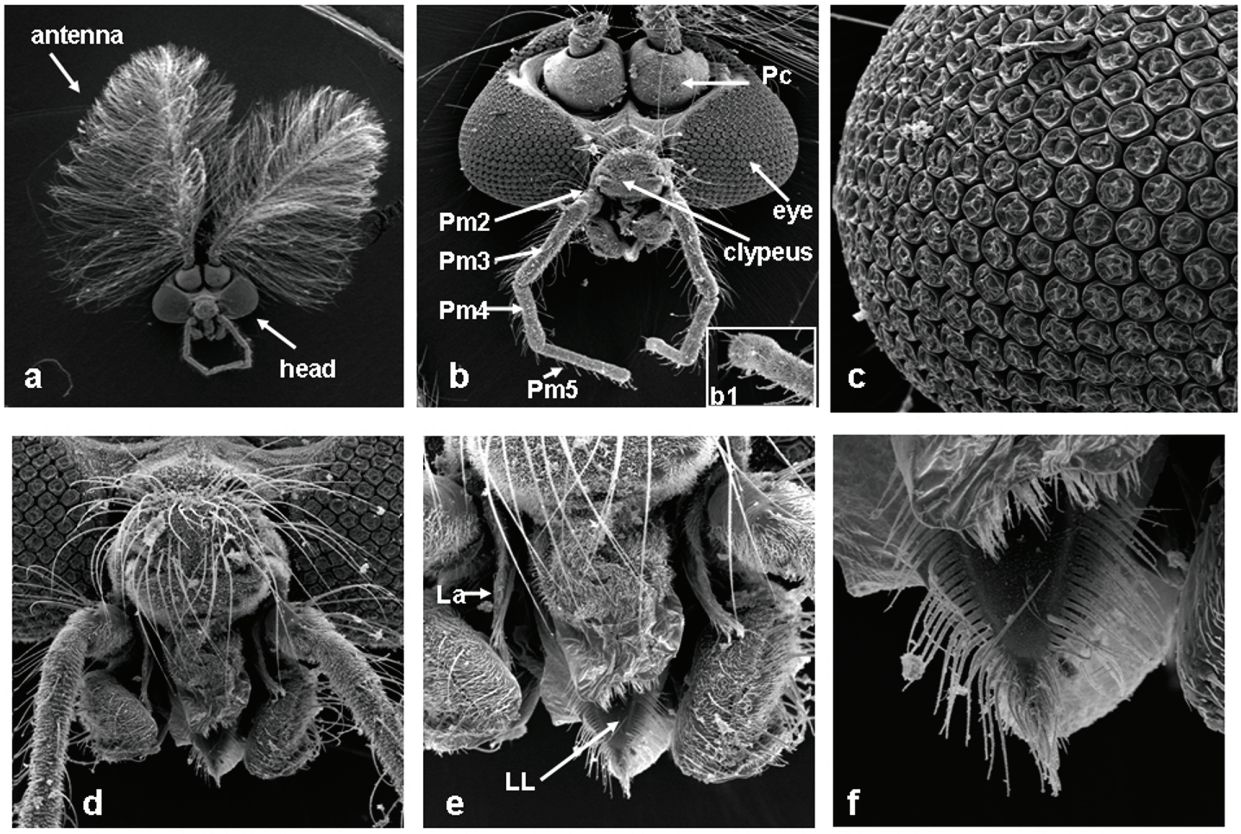

FIGURE 1. *Glyptotendipes glaucus*—male (SEM); a—Head and antenna, dorsal view (15x); b—Head (75x); b1—End of Pm5; c—Ommatidium of eye (350x); d—Clypeus (200x); e—General view of mouthparts (350x); f—Labial lonchus (hypopharynx) (1000x). Abbreviations: LL—labial lonchus; La—lacinia; Pc—pedicel; Pm2-Pm5—palpal segments.

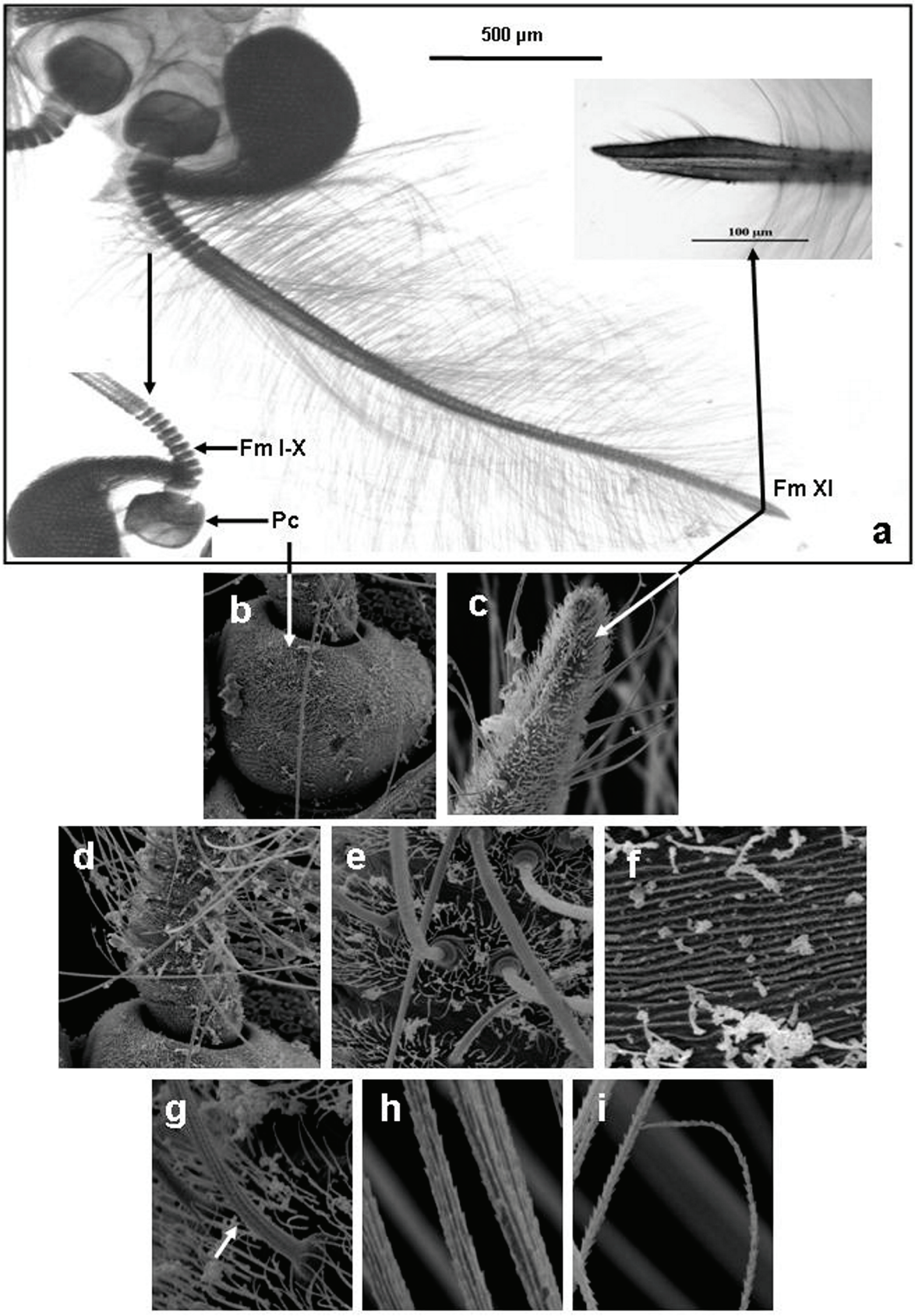

FIGURE 2. *Glyptotendipes glaucus* male – antenna; a—Antenna with flagellomeres I-X and apical flagellomere Fm XI (light microscope); b—Pedicel (SEM, 350x); c—Apex of antenna (SEM, 1500x); d—Flagellomere I-VI (SEM, 350x); e—Setae of antenna plume and small bristle which covered the segment (SEM, 1500x); f—Intersegment (SEM, 5000x); g—Lower part of setae (SEM, 3500x); h—Middle part of setae (SEM, 5000x); i—Apical part of setae (SEM, 3500x). Abbreviations: Fm I-X—flagellomeres I-X; Fm XI—flagellomere XI; Pc—pedicel.

Antenna (Figs. 1a, 2 a–i). Antenna plumose (Fig. 1a), flagellum with 11 flagellomeres (Fig. 2a). Flagellomere lengths (in μm): 133, 40, 40, 40, 40, 40, 40, 40, 40, 40, 2787. Antennal ratio AR 5.6. Large globular pedicel without setae, covered with microtrichia (Figs. 2a,b). The first flagellomere with whorls of short setae not longer than a segment (Fig. 2d). Remaining flagellomeres with whorls of long setae and thickly covered with short microtrichia (Figs. 2d,e). Apical flagellomere (Fm XI) with tip slightly extended, covered with short and slightly curved sensilla chaetica, apex sharp, on whole length visible groove (Fig. 2c). Intersegment without setae and microtrichia, with characteristic horizontal structure (Fig. 2f). Flagellomere setae in basal part smooth, in middle and apical part serrated (Figs. 2g–i).

Wing (Figs. 3a–f). Wing membrane light, without macrotrichia, unmarked, densely covered with very small microtrichia (Fig. 3b). Margin of the wing fringed with setae. Anal lobe (AnL) rounded (Fig. 3c) with long dagger setae, longer than remaining setae on the edge of wing (Figs. 3c1). Squama (Sq) rounded with many long setae on margin (Fig. 3a). Alula (Al) without setae on margin (Figs. 3a). Veins: C, R, M, R_{2+3}, R_{4+5} light brown, remaining veins light. Costa (C) on whole length thickly covered with setae, veins R and R_{2+3} with single row of setae, R_{4+5} in the end part with 15 setae. Costa not extended R_{4+5}. Scopula alaris densely covered with hooked microtrichia of different length (Fig. 3d). Fork of radius (FR) (Fig. 3g). Brachiolum (B) with 5 long setae and row of 10 sensilla campaniformia (SCf) (Fig. 3f) in basal part and ±1/4 in distal part of vein. Arculus (Ar) L-shaped found at tip of brachiolum (Fig. 3e).

Legs (Figs. 4a–i). Legs uniformly brown densely covered with long setae and microtrichia. Fore tibia without spurs and comb. Mid and hind tibiae equipped with 2 spurs (TS) and combs (TC) consisting of a group of strong, dagger setae, fused basally (Figs. 4b–e). The 5th tarsomere (Figs. 4f) apically with 2 strong black claws (Fig. 4i). Claws in basal part (1/3 length) covered by setae, middle and end part bare (Fig. 4g). Paired large, pad-like pulvilli are present between claws, about ½ as long as claw, their external surface densely covered by long setae (Fig. 4h). Empodium (visible in light microscope) in basal part with incision (± 10) (Fig. 4i). Leg measurements (in μm):

	Coxa	femur	Tibia	tarsus 1	tarsus 2	tarsus 3	tarsus 4	tarsus 5	LR (leg ratio)
P 1	382	1645	1793	-	-	-	-	-	-
P 2	292	1843	1938	989	627	464	318	243	0.51
P 3	250	1944	2390	1450	879	657	383	212	0.60

Abdomen. All tergites are uniformly dark brown, without markings, and covered with very long setae, as long as tergite and densely covered with microtrichia. Setae rising on pale areas without microtrichia.

Hypopygium (Figs. 5a–f). Tergite IX with anal tergite bands brown, V-type, separated medially, median area oval with about 30 long setae (visible only in light microscope). Anal point (AnP) in dorsal view slender, narrow in middle part, apex rounded (Fig. 5a), and in lateral view strong, curved on ventral side, sharpened at end, naked, only in basal part with setae (Figs. 5c,d). Gonocoxite (Gc) shorter than gonostylus (Gs), both with very long setae and covered with microtrichia (Fig. 5c). Gonostylus rounded apically, with very small spurs at the tip, in ventral part with row of short setae (5–6) (Figs. 5a,b). Inferior volsella (Ivo) club-shaped, with curved long setae in distal part, covered with microtrichia (Figs. 5b,e). Superior volsella (Svo) shorter than inferior volsella, slightly curved, hooked apically (visible in light microscope), in basal part largely with group of setae, central and ending part without setae (Figs. 5e,f).

Pupa. Large pupa, 12–13 mm long, exuviae pale brown, cephalothorax partly brown, on lateral margin of abdominal tergites II–VI brown line near muscle marks.

Cephalothorax (Figs. 6a–g). Frontal apotome (FA) rounded with conical apices. Cephalic tubercles (CT) short and broad with long frontal setae (FS) (Fig. 6a). Dorsal part of thorax strongly granulose anteriorly and weak to evanescent medially (Figs. 6e,f). Thoracic horn (TH) richly branched, plumose (Fig. 6b,c). Basal ring of thoracic horn kidney-shaped with 2 fused tracheal marks (visible only in light microscope not in SEM) (Fig. 6g). Wing sheaths without pearl row. Ventral thorax smooth with 3 pairs of leg sheaths (ls) (Fig. 6b), between first pair of leg sheaths two processes (Figs. 6d).

Abdomen (Figs. 7a–i, 8a–f). Tergite I bare without shagreen. Tergite II (Fig. 7a) with various shagreens (Fig. 7b), anteriorly small and short (Fig. 7b1), posteriorly bigger and longer covering whole surface (Fig. 7b2). Hook row on tergite II continuous, with 55–60 intersegmental hooklets (HL) (Fig. 7c), occupying segment between muscle marks.

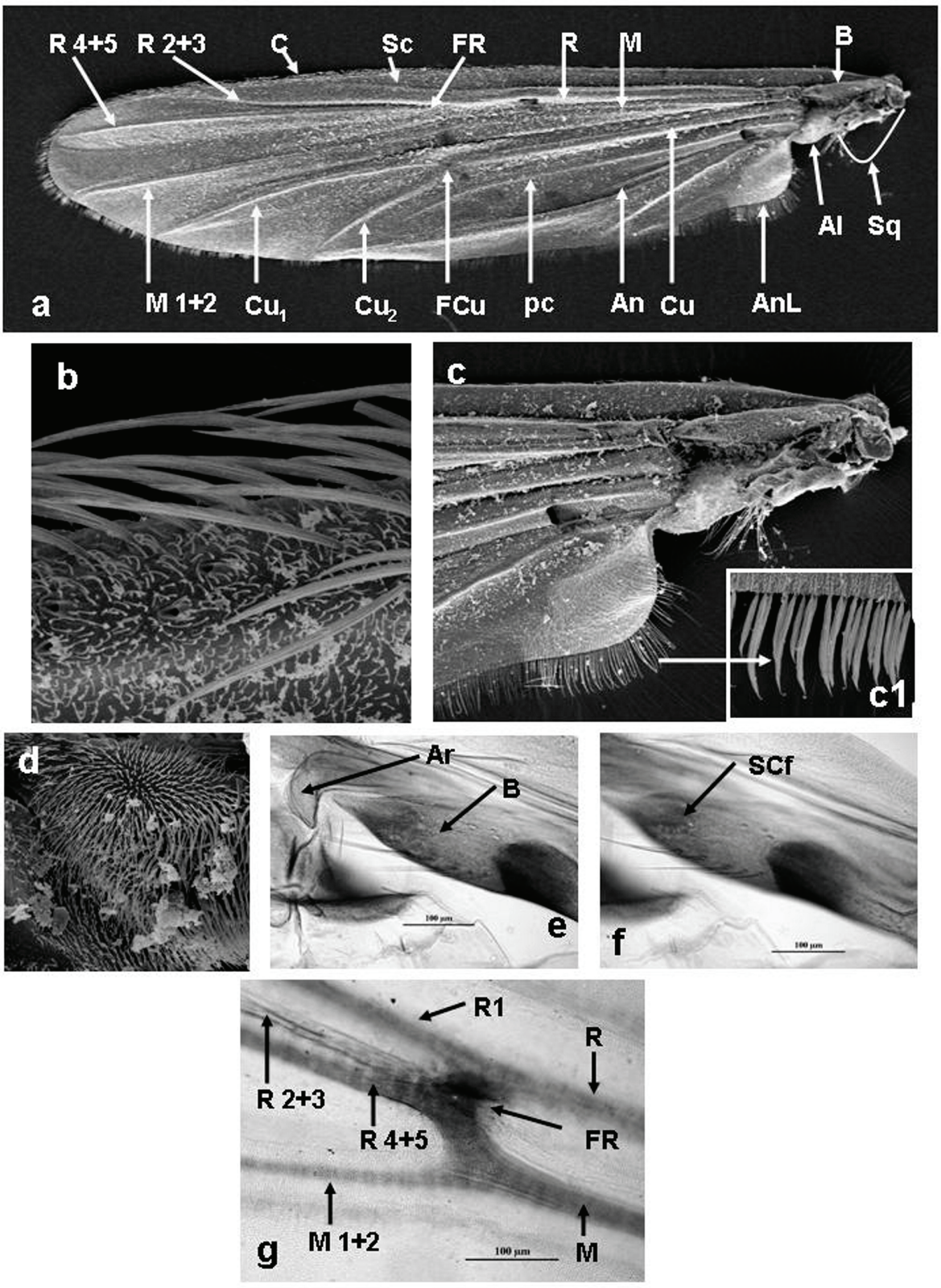

FIGURE 3. *Glyptotendipes glaucus*—wing; a—General view (SEM 5x); b—Wing membrane covered by density microtrichia (SEM 1500x); c—Anterior part of wing (SEM 75x); c1—Hair on the edge of the wing (SEM 1000x); d—Scopula alaris (SEM 1000x); e—Arculus and end of basal vein (brachiolum) (light microscope); f—Sensilla campaniformis (annular organs) (light microscope); g—Fork of radius (light microscope). Abbreviations: An—anal veins; Ar—arculus; B—basal vein (brachiolum); C—costa; Cu—cubitus and its branch Cu_1 and Cu_2 (Cu_1 also is named M_{3+4}); FCu—fork of cubitus; FR—fork of radius; M—media and its branched M_{1+2}; pc—post cubitus; R—radius; Sc—subcosta; SCf—sensilla campaniformis; Sq—squama; Al—alula.

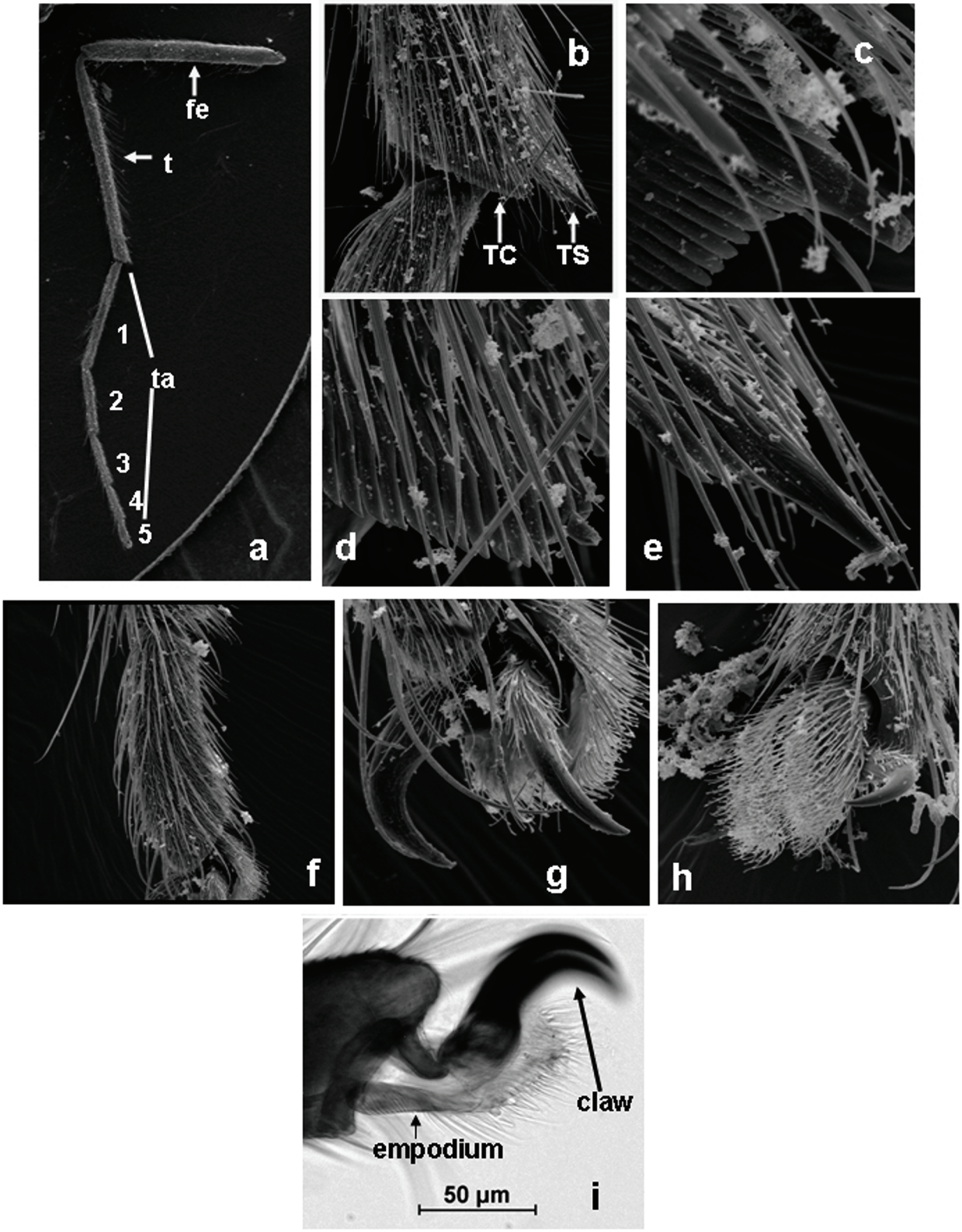

FIGURE 4. *Glyptotendipes glaucus*—leg; a—Leg (SEM, 15x); b—Tibial comb (SEM, 350x); c—Tibial comb and spur (SEM, 1000x); d—Comb (SEM, 2000x); e—Tibial spur (SEM, 2000x); f—Tarsomere 5 (SEM, 350x); g—Claws and pulvillus (SEM, 1000x); h—Pulvillus (SEM, 750x); i—Empodium (light microscope). Abbreviations: fe—femur; t—tibia; ta—tarsomeres 1–5; TC—tibial comb; TS—tibial spur.

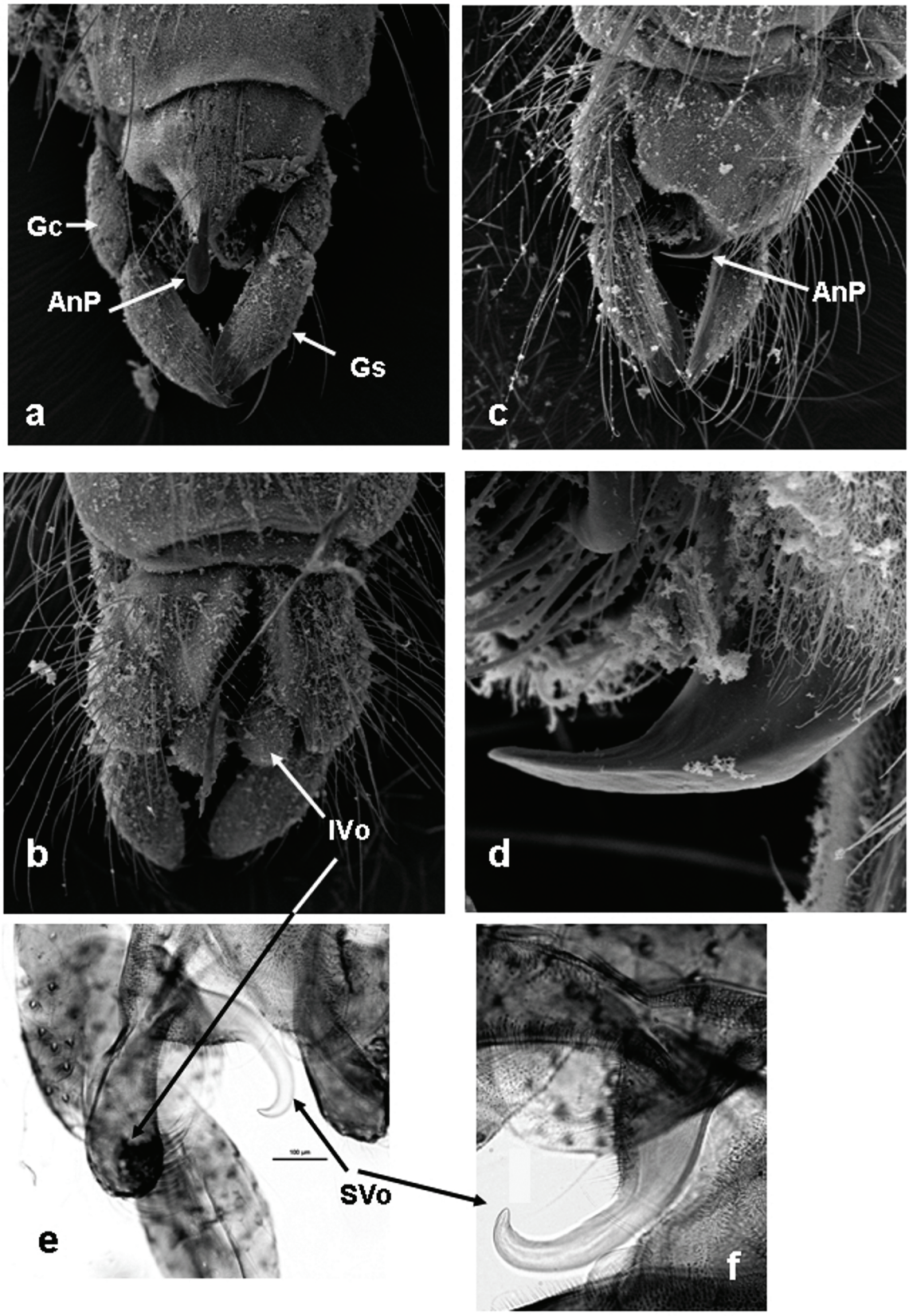

FIGURE 5. *Glyptotendipes glaucus* male—hypopygium; a—Hypopygium, dorsal view (SEM, 150x); b—Hypopygium, ventral view (SEM, 150x); c—Hypopygium, lateral view (SEM, 150x); d—Anal point, lateral view (SEM, 1000x); e—Hypopygium (light microscope); f—superior volsella (light microscope). Abbreviations: AnP—anal point; Gc—gonocoxite; Gs—gonostylus; IVo—interior volsella; SVo—superior volsella.

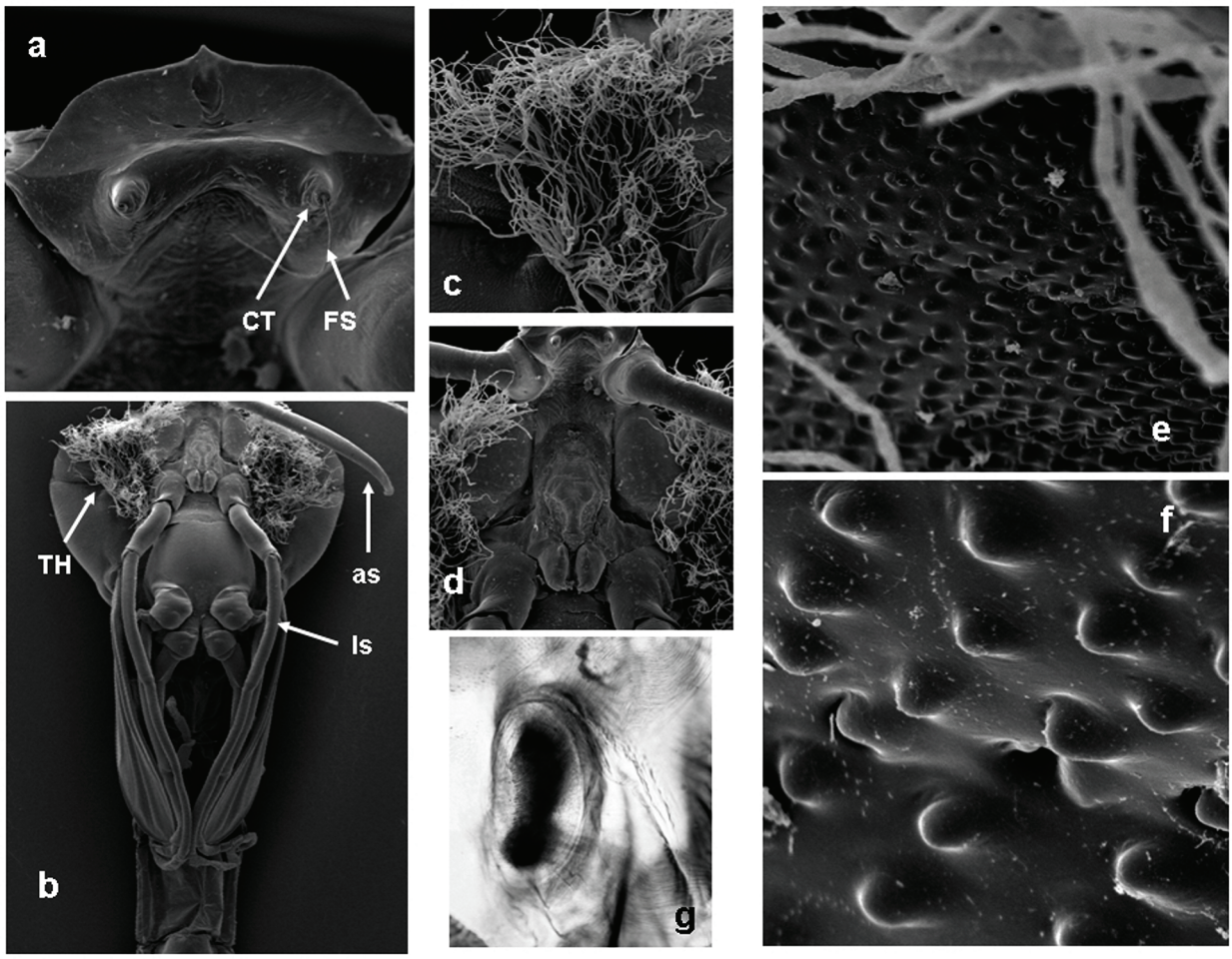

FIGURE 6. *Glyptotendipes glaucus*—pupal exuviae (SEM); a—Frontal apotome (200x); b—Thorax of ventral view (15x); c—Thoracic horn (100x); d—Thorax of ventral view (75x); e—Thorax granulate (500x); f—Thorax granulate (2000x); g—Thoracic basal ring. Abbreviations: as—antenna sheath; CT—cephalic tubercle; FS—frontal setae; ls—leg sheaths; TH—thoracic horn.

Epaulette (Ep) on tergite II very small and short (6x smaller than the length of the segment) (Fig. 7e). Shagreen on tergites III–V similar to tergite II, on tergite VI only two anterior patches of small shagreen, VII–VIII without shagreen. Epaulettes rocket-shaped, present on tergites II–VI with teeth apically and also on the surface (visible in light microscope). Epaulette on tergite VI long, about 4x longer than epaulette on tergite II (Figs. 7e–i). Epaulette on tergites V and VI are poorly visible in the SEM due to impurities accumulated along edging (Figs. 7j and 7j1). Anal lobe with fringe setae (Fig. 8a). Dorsally on segment VIII a small anal comb is visible (AC) (Figs. 8b–d), but ventrally this structure is very strong and massive (Figs. 8e,f). Segment I without lateral setae, II–IV with 3 slender setae (LS), V–VI with 4 lateral filament setae (LS) and VII–VIII with 5 LS setae.

Discussion

The use of SEM enabled to emphasize a number of morphological details, which were previously unknown or poorly known. The present results indicate that the morphology of male antennae is more complex than appears under the light microscope. An important detail are the intersegmental parts with a characteristic horizontal structure devoid of microtrichia and setae. The labial lonchus and lacinia were omitted in previous descriptions. Their structure is here emphasized: the labial lonchus has the shape of a triangular plate with numerous sharpened setae-like processes on the edge. Our observations using SEM indicate that these are very complex structures and can be helpful in a taxonomic study. The high magnification of SEM allowed to better analyze the structures of the legs and hypopygium, especially the anal point.

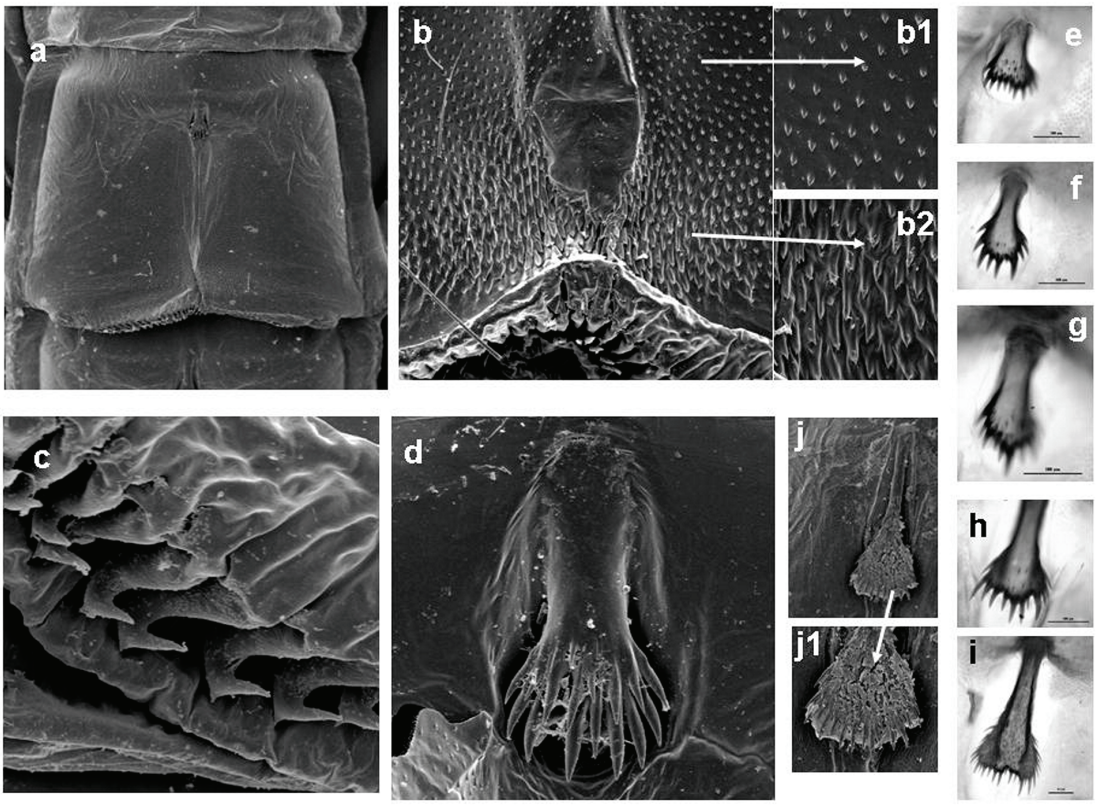

FIGURE 7. *Glyptotendipes glaucus*—pupal exuviae; a—Tergit II (SEM 75x); b—Epaulette of tergite II (SEM 500x); b1—Anterior granulate (SEM 1500x); b2—Posterior granulate (SEM 1500x); c—Hooklets (SEM 1000x); d—Epaulette on tergite II (SEM 350x); e–i—Epaulette on tergite II–VI (light microscope); j—Epaulette on tergite VI (SEM 150x); j1—Epaulette on tergite VI (SEM 350x).

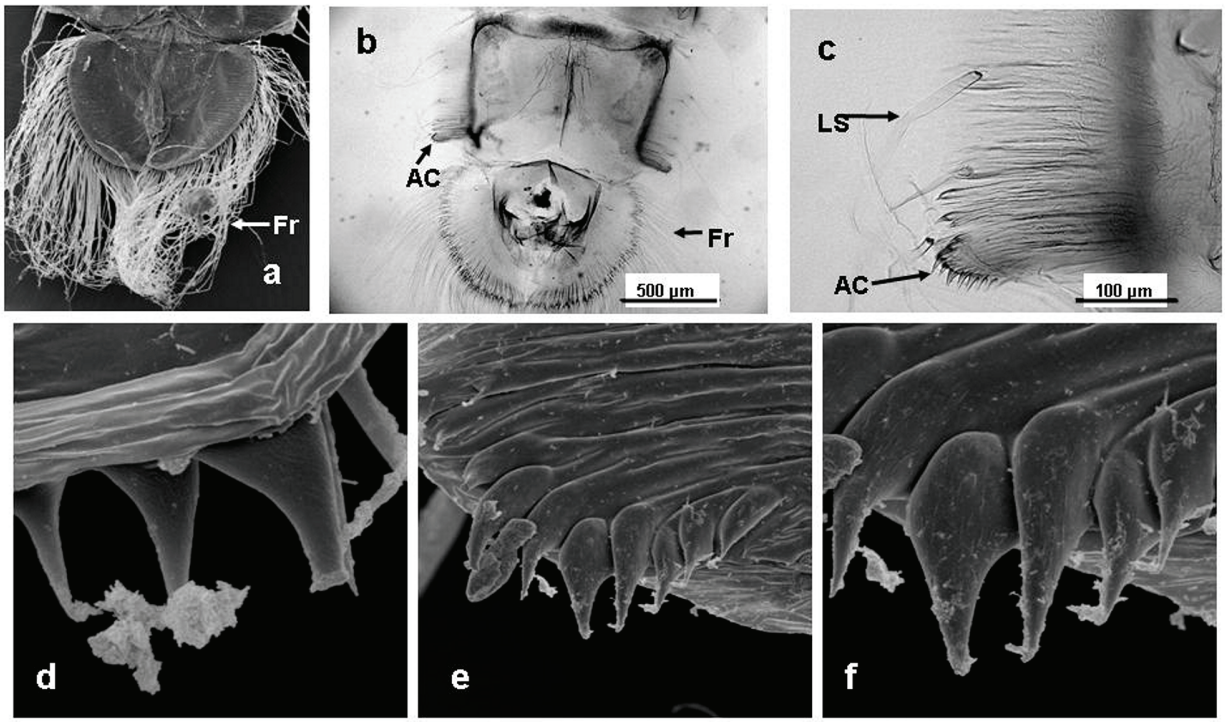

FIGURE 8. *Glyptotendipes glaucus*—pupal exuviae; a—Anal lobe (75x); b—Segment VIII and anal segment (light microscope); c—comb of segment VIII (light microscope); d—Comb of dorsal view (SEM, 3500x); e—Comb of ventral view (SEM, 1000x); f—Comb of ventral view (SEM, 2000x). Abbreviations: AC—anal comb; Fr—anal fringe of filament; LS—lateral seta.

The morphological structure of tergite granulation and epaulettes on the tergites of pupae were also well analyzed. The pupa of the genus *Glyptotendipes* was described as characterized by an anal comb small and composed of delicate teeth (Pinder & Reiss, 1986). Our observations show that under the light microscope only the ends of the anal comb are visible, the SEM study emphasize that the anal comb seen from the ventral side is a massive structure.

On the other hand the present research emphasizes that the SEM method has some limitations. The main problem is that dirt (e.g. algae, mud, debris, etc.) often occurs on the surface of external structures, especially those covered with hair or growths, worsening the picture. Another problem is the layered arrangement of structures. For example in the hypopygium gonocoxite and gonostylus covers the position of superior and inferior volsella.

To sum up, we believe that SEM is an important technique allowing a better understanding of the morphology of Chironomidae and can be useful in the taxonomy and phylogeny of particular species. A comparison of the described characters in other species of the genus and in other related genera is recommended.

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