Evolutionary riddles and phylogenetic twiddles: the ground plan and early diversification of the sternum V gland in Amphiesmenoptera (Trichoptera + Lepidoptera)

MARIE DJERNÆS[*] AND FELIX A. H. SPERLING

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9
E-mail: djernaes@ualberta.ca
([*]) *corresponding author*

Abstract

Despite the sternum V gland being a well known synapomorphy for Trichoptera and Lepidoptera, its ancestral configuration is uncertain. We investigated the sternum V gland in a wide variety of Trichoptera and Lepidoptera to resolve this question. We propose the ground plan to be constituted as follows: The gland is invaginated from sternum V with a slit-like opening and a U-shaped (in cross section) gland duct just inside the opening. Opening muscles originate anteromedially on sternum VI and insert on the walls of the gland duct just inside the opening. The gland reservoir is pressed against the cuticle of sternum IV and in females this area of cuticle is hyaline and perforated. A distinctive arrangement of muscle fibres originates around the perforated cuticle; they insert on the walls of the gland reservoir and facilitate secretion of gland products through the perforated cuticle. Other significant findings were the presence of 2 nonhomologous types of opening muscles in Trichoptera and the scattered distribution of retained ancestral gland features; the latter might imply that these features have been retained as genetic pathways without being physically present in all ancestors of the extant species exhibiting the features.

Key words: Amphiesmenoptera, Trichoptera, Lepidoptera, homology, re-evolution

Introduction

Trichoptera + Lepidoptera (Amphiesmenoptera) is one of the best supported superordinal clades in the insects, and 1 of the supporting synapomorphies is the sternum V gland. The sternum V gland is generally a pair of invaginations from sternum V in the imago, known to be present throughout the Trichoptera and in 5 families of basal Lepidoptera (Davis 1975; Kristensen & Nielsen 1979; Ivanov & Melnitsky 1999, 2002). However, some questions remain about the ancestral form of the gland present in those long extinct amphiesmenopterans that eventually evolved into the familiar Trichoptera and Lepidoptera of today.

Gland opening
Kristensen and Nielsen (1979), Kristensen (1984) and Nielsen and Kristensen (1996) investigated the sternum V gland in basal Lepidoptera and, based on SEM studies, found the gland opening to be a slit in the cuticle of sternum V. However, Ivanov and Melnitsky (2002) reported that in Trichoptera the gland opening was not a slit, but a perforated membrane. Ivanov and Melnitsky's (2002)

conclusion was also based on SEM studies, in this case of species of Beraeidae, Hydroptilidae, Limnephilidae, Polycentropodidae and Stenopsychidae. Recently Hashimoto and Kobayashi (2009) reported the gland opening in 3 other species of Trichoptera (in Limnephilidae, Phryganeidae and Stenopsychidae) to be slit-like, although sometimes with associated 'notches' in the cuticle.

Gland opening muscles

Gland opening muscles[1] were observed by Nielsen (1980) in representatives of 2 families of Trichoptera (Hydroptilidae and Limnephilidae). He described the limnephilid gland opening muscles as fan-like and noted that the opening muscles found in hydroptilids were completely different as they originated on the antecosta of sternum VI. While he examined females of a number of families and found the sternum V gland to be present in species from several families besides Hydroptilidae and Limnephilidae, he did not report gland opening muscles to be present in any of these.

In Lepidoptera, gland opening muscles are present in all families with sternum V glands (Kristensen 1984). They originate on the anteromedial margin of segment VI and Kristensen argued that they most likely represent a neoformation as they co-exist with a full complement of ventrolongitudinal abdominal muscles (Kristensen 1984, Nielsen & Kristensen 1996). Kristensen (1984) assumed that trichopterans possessed homologous opening muscles and therefore attributed Lepidoptera-type opening muscles to the amphiesmenopteran ground plan.

Hashimoto and Kobayashi (2009) reported a concentration of muscle fibres on the gland duct just inside the gland opening in a phryganeid and presumed this to be homologous to, and thus function like the gland opening muscle described by Kristensen (1984) in micropterigids.

Fenestrae

Fenestrae, defined as transparent patches of cuticle, occur on sternum IV in females of Philopotamidae (Trichoptera), Eriocraniidae, Neopseustidae and Nepticulidae (Lepidoptera) (Davis 1975; Ivanov & Melnitsky 1999, 2002). Transparent patches also occur on sternum V in some female philopotamids and on sternum II in *Catapterix crimaea* Zagul. & Sinev (Lepidoptera: Acanthopteroctetidae) (Ivanov & Melnitsky 1999, 2002).

In eriocraniids the fenestrae are associated with the reservoirs of normally developed sternum V glands, while in neopseustids and nepticulids the fenestrae apparently occur independently (Davis 1975, 1978). However, one nepticulid species [*Stigmella malella* (Stainton)] has been shown to produce pheromones of the same type as those produced by the sternum V gland in trichopterans and other lepidopterans (Tóth *et al*. 1995, Zhu *et al*. 1995, Kozlov *et al*. 1996, Löfstedt & Kozlov 1997). While pheromone production in *Stigmella* was not linked to any specific body part, it does raise the possibility that the fenestrae in nepticulid females are somehow associated with functional sternum V glands.

In some philopotamids, Ivanov and Melnitsky (1999, 2002) found secretory tissue to be directly connected to the fenestra without the presence of either a gland sac or gland duct. As this is structurally simpler than the more widespread configuration of a gland duct and gland sac surrounded by secretory tissue, Ivanov and Melnitsky (1999, 2002) proposed this type to be the ancestral type originally found in Amphiesmenoptera.

Objectives of the present study

The aim of this study is to elucidate the ground plan of the sternum V gland in Amphiesmenoptera. We investigate whether the gland opening in extant taxa is: 1) a slit (Kristensen 1984; Hashimoto &

---

1. Although Nielsen (1980) called them 'obturator,' closing, muscles, he clearly described them as opening the gland duct on p. 89.

Kobayashi 2009); 2) a porous membrane (Ivanov & Melnitsky 2002); or 3) a slit in Lepidoptera and a membrane in (some) Trichoptera. We examine the occurrence of gland opening muscles in Trichoptera to determine: 1) how widespread they are; 2) whether there are 2 completely different types present in Trichoptera as reported by Nielsen (1980); and 3) if any trichopteran opening muscles are homologous to those found in Lepidoptera. We examine the fenestrae to determine whether they are: 1) primitive glands, as suggested by Ivanov and Melnitsky (1999, 2002); 2) a very reduced form that is nonetheless still functional and secreting the pheromones found by Tóth *et al.* (1995); 3) simply a non-functional remnant; or 4) associated with normal sternum V glands which have been overlooked. Based on our observations in extant taxa, we then propose ground plan features for the sternum V gland in Amphiesmenoptera.

Materials and methods

Taxon sampling

Representatives of 38 families of Trichoptera were examined, including all annulipalpian families, all "spicipalpian" families and most integripalpian families. The families relevant and thus most closely studied for the present paper were Rhyacophilidae, Glossosomatidae and Hydroptilidae from "Spicipalpia" and Philopotamidae and Psychomyiidae from Annulipalpia. Members of seven families of basal Lepidoptera were examined: Micropterigidae, Agathiphagidae, Heterobathmiidae, Eriocraniidae, Lophocoronidae, Neopseustidae and Nepticulidae. The families relevant for the present paper were Eriocraniidae, Neopseustidae and Nepticulidae. A list of the species examined from Rhyacophilidae, Glossosomatidae, Hydroptilidae, Philopotamidae, Psychomyiidae, Eriocraniidae, Neopseustidae and Nepticulidae is shown in Table 1.

TABLE 1. Taxa studied in Trichoptera and Lepidoptera families of particular interest with respect to sternum V gland opening muscles and fenestra/perforated patches, including the treatments employed for each species. The table also shows the presence/absence of the sternum V gland, the type of opening muscles present and the presence or absence of perforated cuticle on sternum IV. '?' denotes that a structure either could not be detected with the treatment(s) used, and/or that the structure was absent, and the absence was presumed to be due to artefacts. Pore sizes are only given for species with perforated cuticle which were also subjected to SEM of external structures. F, female; M, male; Lep-type, Lepidoptera-type; Trich-type, Trichoptera-type.

Taxa	Treatments	Sternum V gland	Opening muscles	Perforated cuticle (Pore size)
Trichoptera				
<u>Annulipalpia</u>				
Philopotamidae				
Chimarra aterrima Hagen F + M	external SEM	present	?	present in F only (100-400 nm)
C. obscura (Walker) F + M	wholemount, ext. SEM, histology, + int. SEM of F	present	Lep-type	present in F only (90-430 nm)
Dolophilodes sp. F	wholemount	present	?[a]	present
D. novusamericanus (Ling) F + M	wholemount	present	F: ?[a] M: Lep-type	present in F only
D. pallidipes Banks M	wholemount	present	Lep-type	absent

...... continued on the next page

TABLE 1 (continued)

Taxa	Treatments	Sternum V gland	Opening muscles	Perforated cuticle (Pore size)
Philopotamus montanus (Donovan) M	wholemount	absent	n/a	absent
Wormaldia arizonensis (Ling) F + M	wholemount, ext. SEM	present	Lep-type + Trich-type	present in F only (50-150 nm)
W. gabriella (Banks) F + M	wholemount	present	Lep-type	present in F only
W. planae (Ross & King) F + M	wholemount, ext. SEM	present	?[a]	absent
W. occidea (Ross) F + M	wholemount	present	Lep-type	present in F only
Psychomyiidae				
Lype diversa (Banks) F + M	wholemount	present	absent	present in F only
Psychomyia flavida Hagen F	wholemount, ext. & int. SEM, histology	present	absent	present (100-200 nm)
Tinodes sigodanus (Ross & Merkley) F + M	wholemount, ext. SEM, histology	present	absent	present in F only (40-260 nm)
"Spicipalpia"				
Glossosomatidae				
Agapetus walkeri (Betten & Mosely) F + M	wholemount	present	Lep-type	absent
Anagapetus debilis (Ross) F + M	wholemount, ext. SEM, + int. SEM of F	present	Lep-type	absent
Protoptila cana Flint F + M	wholemount, + ext. SEM of F	present	Lep-type	absent
Hydroptilidae				
Hydroptilinae sp. F + M	wholemount, ext. SEM	present	Lep-type	absent
Agraylea multipunctata Curtis F + M	wholemount, ext. SEM	present	Lep-type	absent
Palaeagapetus guppyi Schmid F + M	wholemount, ext. SEM	present	Lep-type	absent
Rhyacophilidae				
Himalopsyche phryganea (Ross) F + M	wholemount, + ext. SEM of M	present	Lep-type	absent
Rhyacophila arnaudi Denning F + M	wholemount, ext. SEM, histology	present	Lep-type	absent
Lepidoptera				
Eriocraniidae				
Dyseriocrania subpurpurella (Haworth) F + M	wholemount	present	Lep-type	present in F only
Eriocrania cicatricella (Zetterstedt) F + M	wholemount, ext. SEM, + int. SEM, histology of F	present in F absent in M	Lep-type in F, n/a in M	present in F only (20-220 nm)
E. semipurpurella (Stephens) F + M	wholemount, + histology of F	present	Lep-type	present in F only
Neopseustidae				
Synempora andesae Davis & Nielsen F	Wholemount, ext. & int. SEM, histology	present w/o duct	n/a	present (20-50 nm)
Nepticulidae				
Nepticulidae spp. F	wholemount, ext. SEM, histology	present w/o duct	n/a	present (30-200 nm)
Ectoedemia heringiella Doets F	internal SEM	?	?	present

[a] ventrolongitudinal musculature missing as well, so absence is likely due to decomposition.

Wholemounts, SEM, histology
For all species, both wholemount preparations and Scanning Electron Microscopy (SEM) of both sexes were used when possible (Table 1).

Wholemount preparations were of the ventral midabdomen, lightly stained with highly diluted chlorazol black and otherwise prepared using standard techniques and mounted in Canada balsam. Wholemounts were examined using brightfield and polarised light microscopy.

Specimens for SEM were prepared using critical point drying and gold coating. Standard SEMs were of the exterior (to show surface cuticular structures), but, for some species, SEMs were taken of the interior (partially cleared with 10% KOH) as well, showing cuticular structures and sometimes soft tissue (muscles, gland cells).

Histological sections were prepared from whole specimens or abdomens fixed in Bouin's or 70% ethanol. The abdomen (for larger specimens, just the ventral mid-abdomen) was embedded in paraffin wax, and sectioned at 8 μm. Sections were stained either in Masson's Trichrome Stain (Harris' hematoxylin, Ponceau-acid fuchsin and acetic aniline blue) or in Harris' Hematoxylin and acidified eosin. One *Synempora andesae* Davis & Nielsen (Neopseustidae) female was embedded in resin, sectioned at 1 μm and stained with Richardson's stain (methylene blue and azure II). Drawings based on wholemounts and histological sections were made using a camera lucida.

Observations

Gland opening
SEM studies showed the gland opening to be a single slit in the sclerotised part of sternum V (Fig. 1A–C). The only exception was in *Psychomyia flavida* Hagen females (Trichoptera: Psychomyiidae) where the slit-like gland opening was situated in the membranous cuticle between sternum V and IV (Fig. 1D). No trace of pores was found, even at magnifications where pores of the reported size (0.15 μm, Ivanov & Melnitsky 2002) should be impossible to miss. The gland duct just inside the gland opening is U-shaped in cross section (Fig. 2H) with thickened cuticle, especially the inner wall. When the gland duct is compressed, the gland opening is closed.

Gland opening muscles
Most trichopterans possessing the sternum V gland have gland opening muscles originating on the cuticle of sternum V mesad of the gland opening and inserting on the walls of the gland duct just inside the gland opening. The opening muscle consists of 1 to several bundles of muscle fibres which are typically fan-shaped with the tip of the fan inserting on the gland duct (Fig. 3A).

Some trichopterans (all species examined in Glossosomatidae, Hydroptilidae, Philopotamidae and Rhyacophilidae) have gland opening muscles that originate on the anteromedial margin of sternum VI (Fig. 3B). A single species, *Wormaldia arizonensis* (Ling) (Philopotamidae), possesses both types simultaneously (Fig. 3C). A single family, Psychomyiidae, had no gland opening muscles in any of the species examined, although the sternum V gland is well developed in this family (Fig. 4C). All species examined from the 5 lepidopteran families known to possess the sternum V gland had gland opening muscles originating on the anterior margin of sternum VI.

Fenestrae/perforated patches
SEM investigations showed the sternum IV fenestrae in females of Philopotamidae (Trichoptera), Eriocraniidae, Neopseustidae and Nepticulidae (Lepidoptera) to consist of perforated cuticle (Figs 2E, F; 5A, B, E-J) as well as being transparent, the latter not being visible with SEM. Furthermore,

our investigations of families without fenestrae showed perforated patches to be present on sternum IV in female psychomyiids (Figs 2A, B; 5C, D), but to be otherwise absent in investigated species.
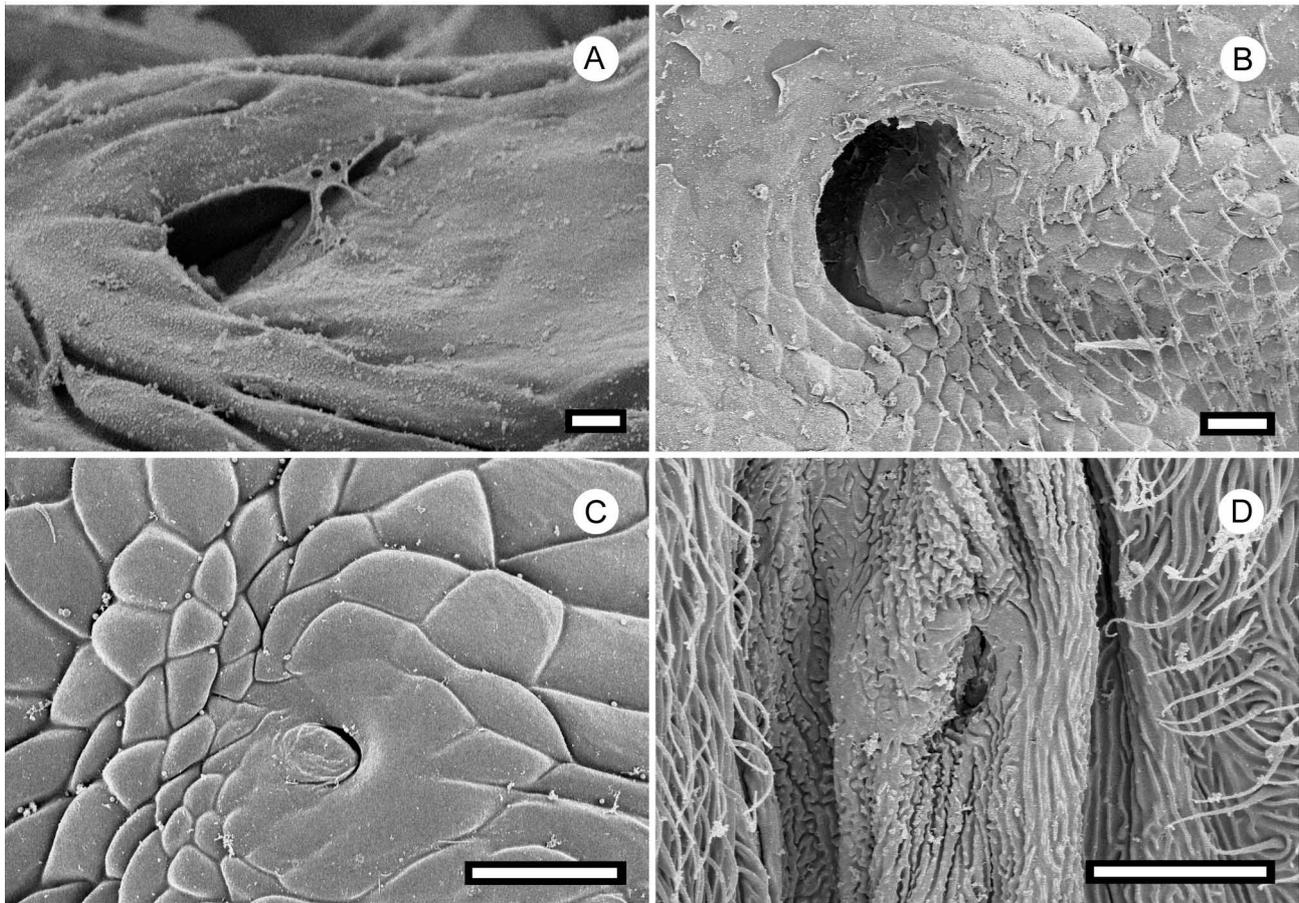

FIGURE 1. SEMs of Trichoptera gland openings. Openings are clearly slit-like. A: *Agraylea multipunctata* Curtis female (Hydroptilidae). B: *Limnephilus secludens* Banks female (Limnephilidae). C: *Wormaldia planae* Ross & King male (Philopotamidae). D: *Psychomyia flavida* Hagen female (Psychomyiidae). Scale bars: A = 1 μm; B-D = 10 μm.

FIGURE 2. SEMs of Trichoptera and Lepidoptera internal structures. A-B: *Psychomyia flavida* Hagen female (Trichoptera: Psychomyiidae). A: Gland reservoir with excurrent duct, the reservoir has been separated from the body wall during preparation; on the body wall the internal surface of the perforated patch can be seen. B: Close-up of internal surface of perforated patch. C-D: *Eriocrania cicatricella* (Zetterstedt) female (Lepidoptera: Eriocraniidae). C: Gland reservoir with external duct *in situ* showing close association with body wall. D: Here the gland reservoir has been removed, showing the internal surface of the perforated patch with 'sunburst' musculature. E-F: *Ectoedemia heringiella* Doets female (Lepidoptera: Nepticulidae). E: Internal surface of fenestra. F: Close-up of same. G: *Synempora andesae* Davis & Nielsen female (Lepidoptera: Neopseustidae), gland reservoir with secretory tissue and muscle fibres, note lack of excurrent duct. H: *Gumaga griseola* (McLachlan) female (Trichoptera: Sericostomatidae), U-shaped (in cross section) gland duct just inside gland opening. S IV, sternum IV; S V, sternum V; Ppi, perforated patch, internal; Rs, gland reservoir; Dt, excurrent duct; Mf, muscle fibres; Sc, secretory cells. Scale bars: A, D, E, H = 10 μm; B, F = 1 μm; C = 100 μm; G = 20 μm.
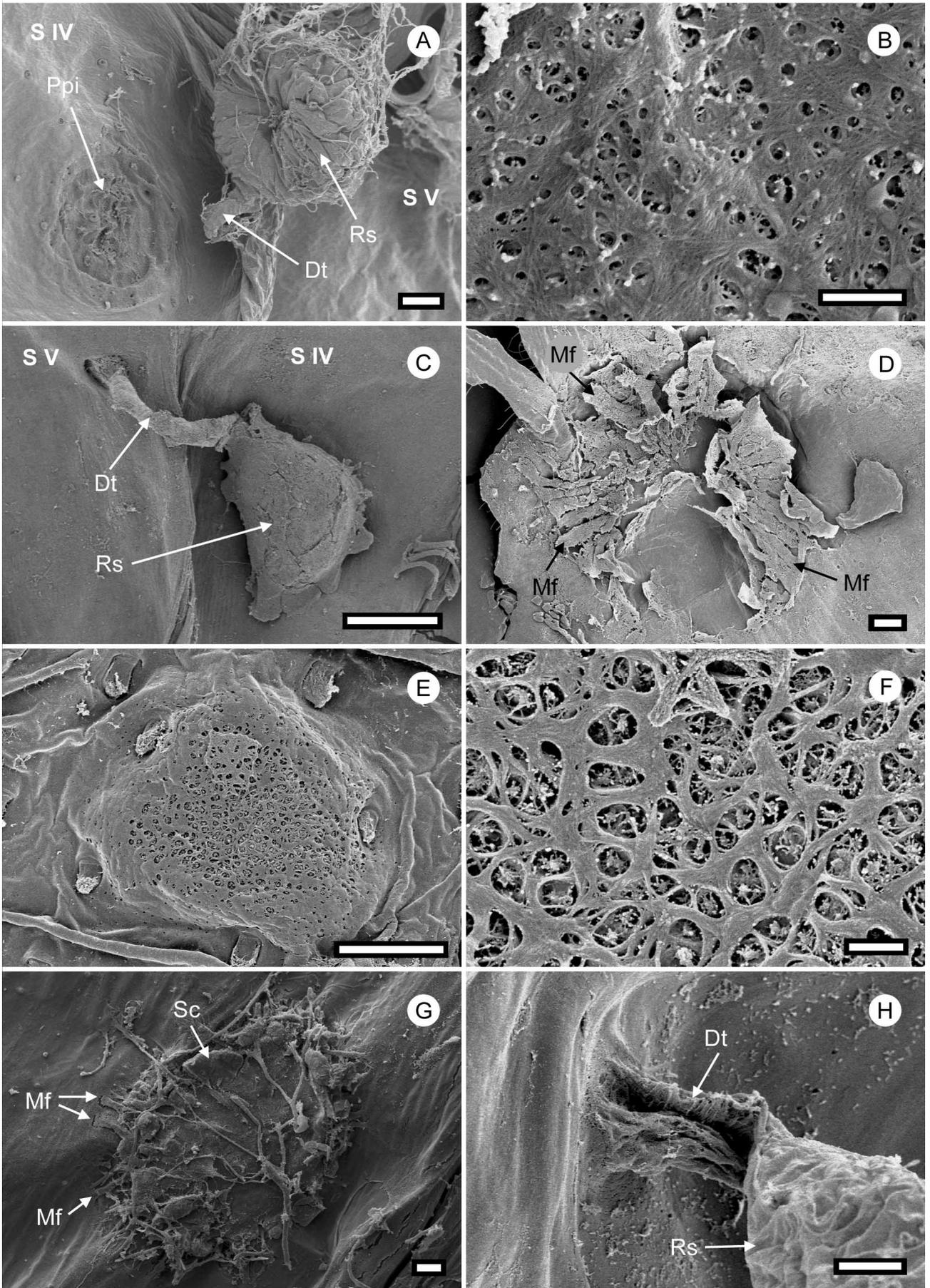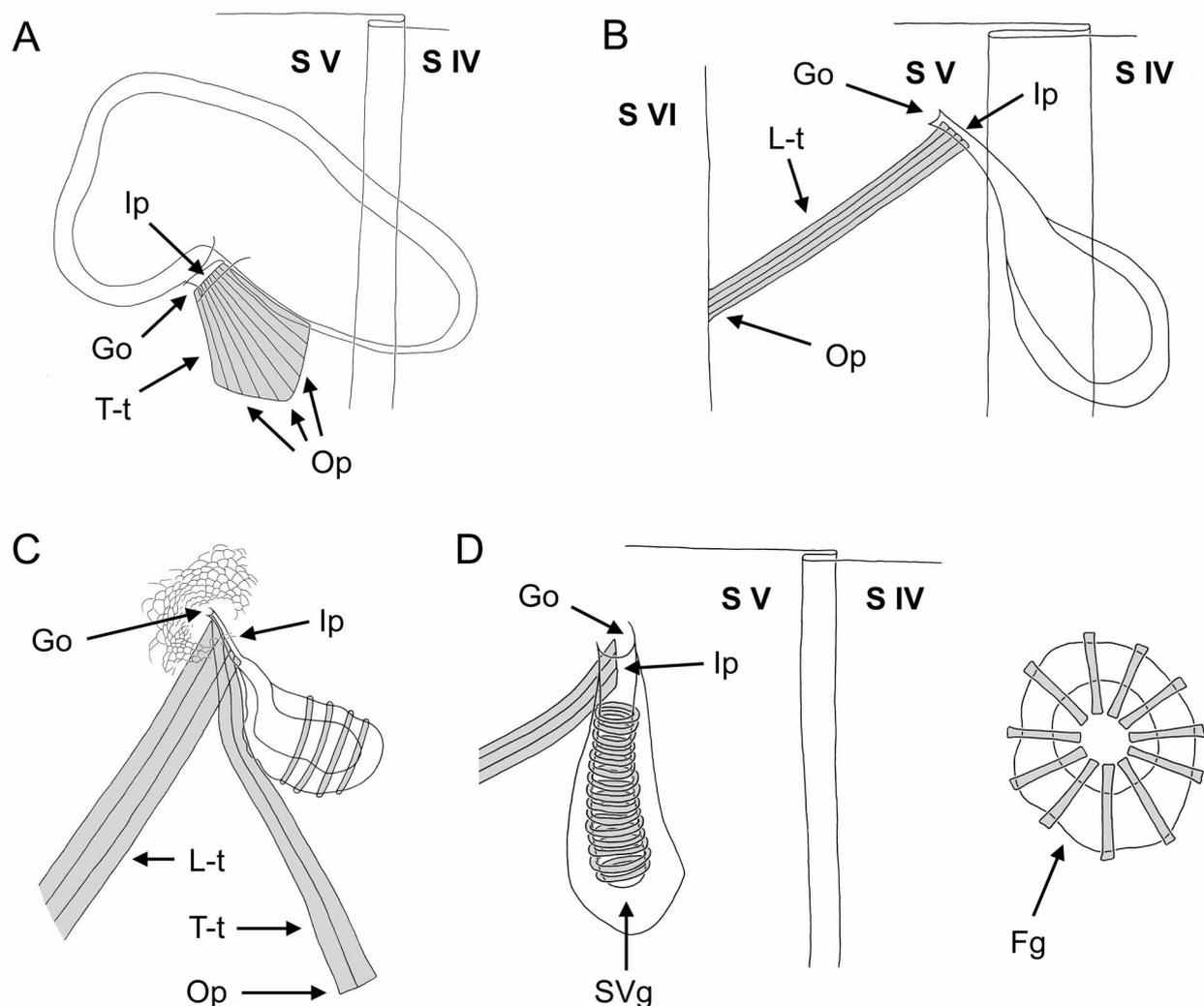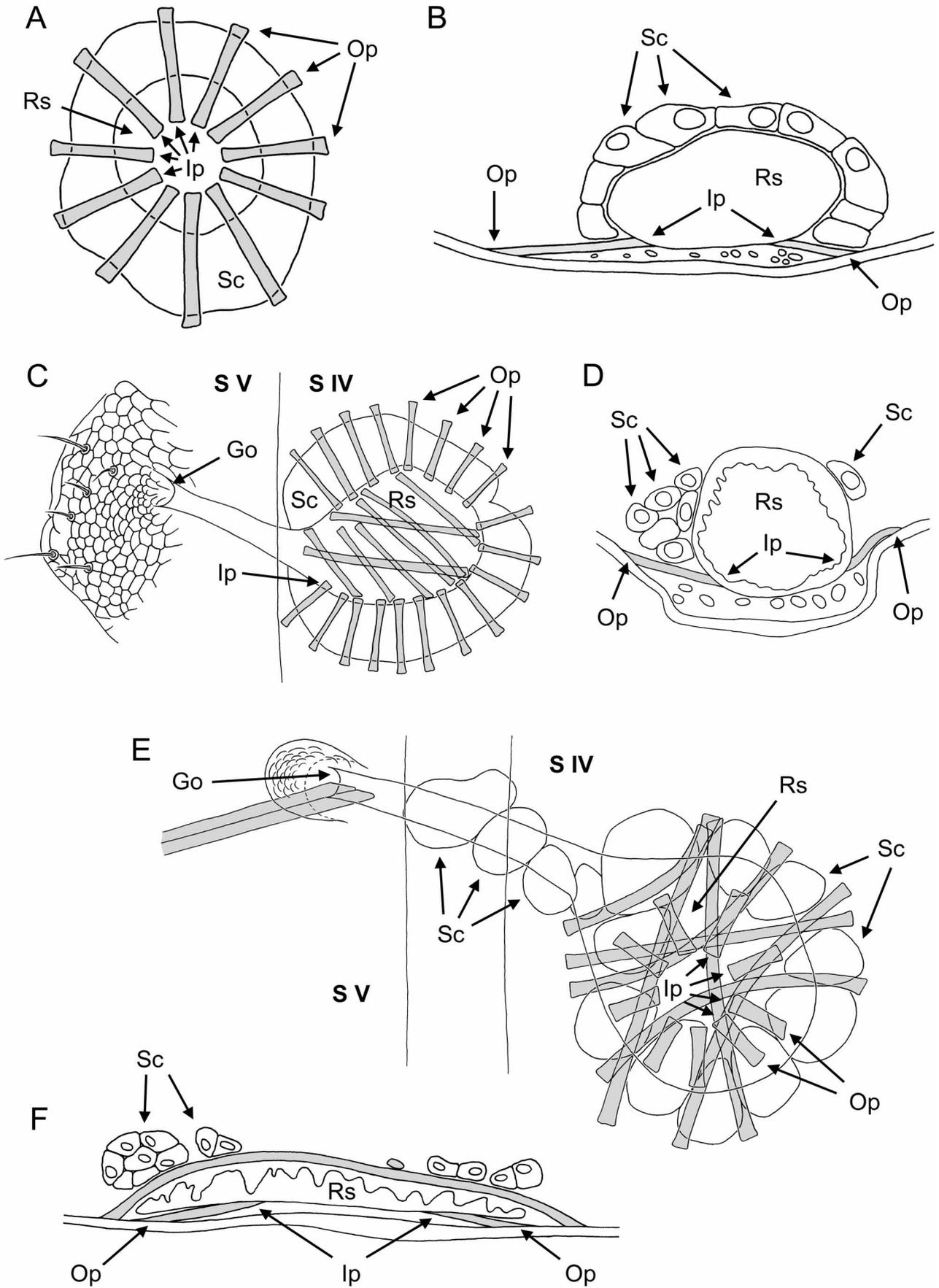
FIGURE 3. Drawings of Trichoptera sternum V gland and associated structures based on wholemounts. The structures are viewed through the cuticle and the animals are facing right with down being mesad. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A: *Limnephilus secludens* Banks male (Trichoptera: Limnephilidae), Trichoptera-type opening muscle originating mesally on cuticle of sternum V and inserting on the gland duct wall just inside gland opening. B: *Rhyacophila arnaudi* Denning female (Trichoptera: Rhyacophilidae), Lepidoptera-type opening muscle originating medioanterially on sternum VI and inserting on the gland duct wall just inside gland opening. C: *Wormaldia arizonensis* (Ling) female (Trichoptera: Philopotamidae), co-occuring Lepidoptera-type and Trichoptera-type opening muscles. D: *Chimarra obscura* (Walker) female (Trichoptera: Philopotamidae), co-occuring normal sternum V gland and fenestral gland. S IV, sternum IV; S V, sternum V; S VI, sternum VI; Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; T-t, Trichoptera-type opening muscle; L-t, Lepidoptera-type opening muscle; SVg, sternum V gland; Fg, fenestral gland.

The fenestrae in female eriocraniids are known to be associated with the gland reservoir (Davis, 1978) (Figs 2C, 4F). Our investigations showed the same to be true for the perforated patches in female psychomyiids. The fenestrae in female philopotamids were associated with glandular structures consisting of a cuticular gland sac surrounded by secretory cells (Figs 4A, B). Although the sac is lined with cuticle, it does not have any excurrent duct. Furthermore, the fenestral gland structure in female philopotamids co-exists with normally developed sternum V glands (Fig. 3D). Male philopotamids possess only normal sternum V glands. The fenestrae in female neopseustids and nepticulids are also associated with glandular structures consisting of a cuticular gland sac surrounded by secretory cells (Figs 2G; 4G, I). Like the fenestral glands in female philopotamids, these lack excurrent ducts.
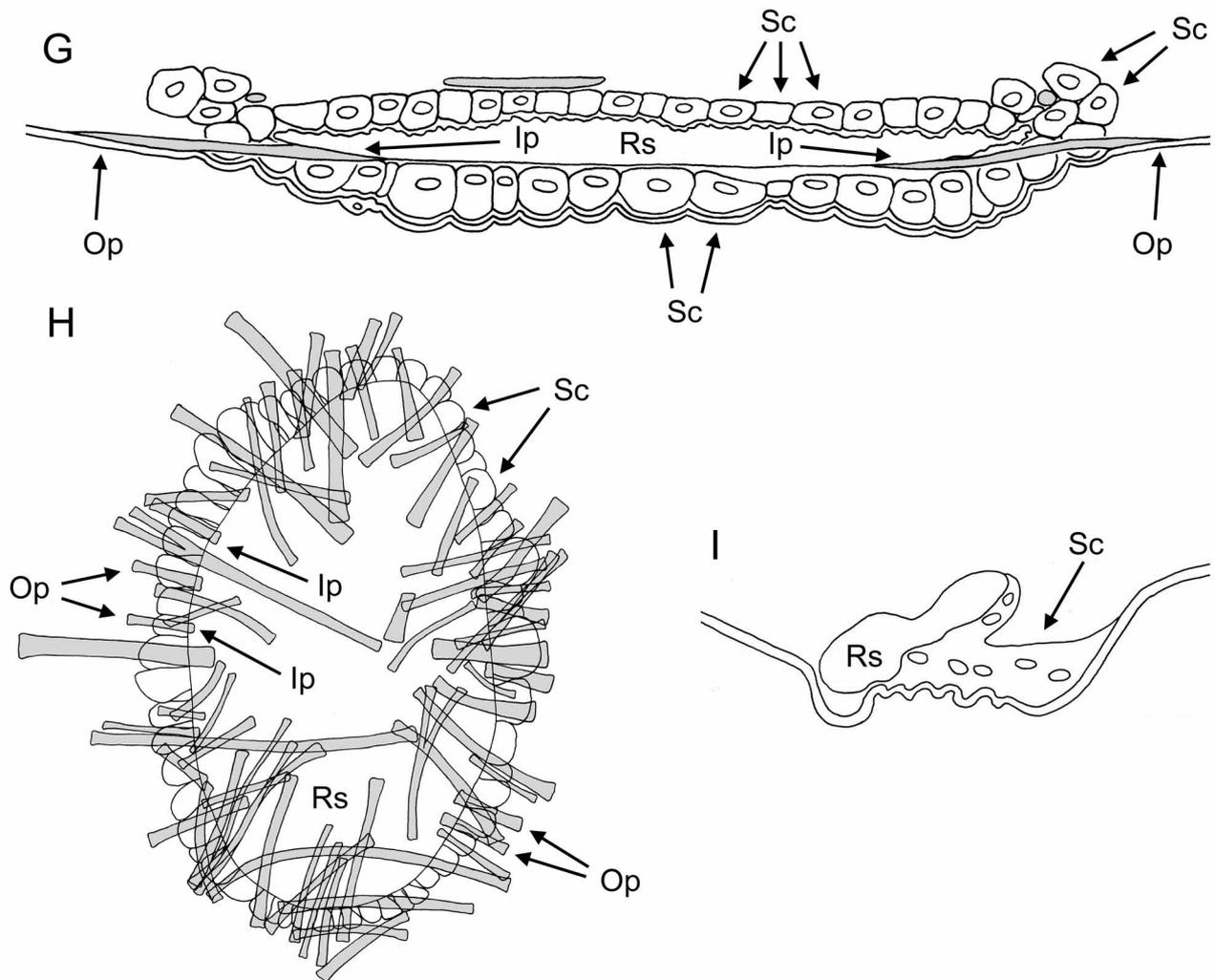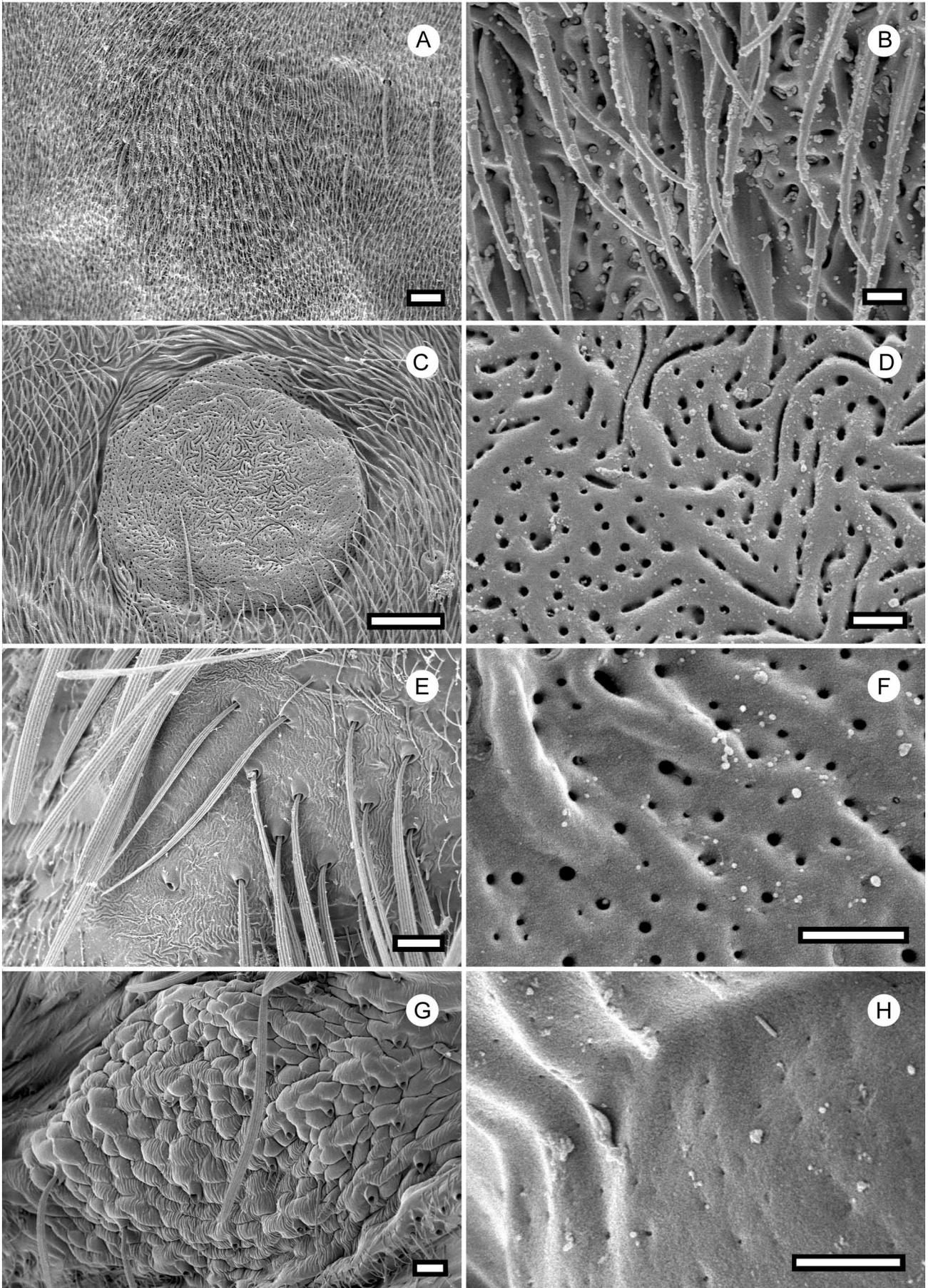
FIGURE 4. Drawings of Trichoptera and Lepidoptera gland structures associated with fenestrae and perforated patches. Drawings are based on wholemounts (A, C, E and H) or histological sections (B, D, F, G and I). The drawings based on wholemounts are viewed through the cuticle and the animals are facing right with down being mesad. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is not indicated. All drawings show the gland/fenestral reservoir with secretory tissue. A-H also show musculature associated with the reservoir, including 'sunburst' musculature. In B, D, F and G note how the 'sunburst' muscle fibres insert on the reservoir. A-B: *Chimarra obscura* (Walker) female (Trichoptera: Philopotamidae). C-D: *Tinodes sigodanus* Ross & Merkley female (Trichoptera: Psychomyiidae), note lack of gland opening muscle. E-F: *Eriocrania cicatricella* (Zetterstedt) female (Lepidoptera: Eriocraniidae). G-H: *Synempora andesae* Davis & Nielsen female (Lepidoptera: Neopseustidae). I: Nepticulidae sp. female (Lepidoptera). S IV, sternum IV; S V, sternum V; Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells.

In philopotamids, psychomyiids and eriocraniids a distinctive 'sunburst' musculature is associated with the fenestrae/perforated patches. This musculature consists of muscle fibres originating on the cuticle around the fenestrae/perforated patch and inserting on the wall of the gland reservoir (Figs 2D, 4A–F). A more irregular version of this arrangement is associated with the fenestrae in *Synempora andesae* (Neopseustidae) (Figs 4G, H) while no muscle fibres were found to be associated with the fenestrae in nepticulids.
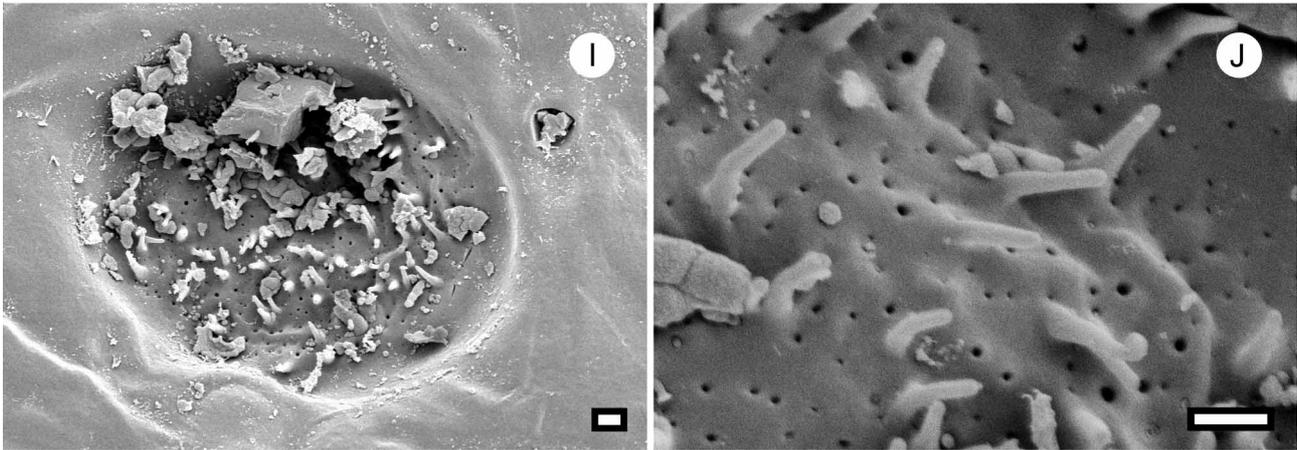
FIGURE 5. SEMs of Trichoptera and Lepidoptera fenestrae/perforated patches. A, C, E, G and I are overviews showing the whole fenestra/perforated patch, B, D, F, H and J are close-ups showing the perforations. A-B: *Chimarra aterrima* Hagen female (Trichoptera: Philopotamidae). C-D: *Psychomyia flavida* Hagen female (Trichoptera: Psychomyiidae). E-F: *Eriocrania cicatricella* (Zetterstedt) female (Lepidoptera: Eriocraniidae). G-H: *Synempora andesae* Davis & Nielsen female (Lepidoptera: Neopseustidae). I-J: Nepticulidae sp. female (Lepidoptera). Scale bars: A, C, E, G = 10 μm; B, D, F-J = 1 μm.

Discussion

Gland opening

The gland opening on sternum V was a slit in all investigated species without any differences between Trichoptera and Lepidoptera. This supports Kristensen's (1984) and Hashimoto and Kobayashi's (2009) observations as well as Kristensen's (1984) conclusion that the ancestral form of the gland in Amphiesmenoptera is an invagination from sternum V. No support was found for Ivanov and Melnitsky's (2002) assertions that the gland opening in Trichoptera is a porous membrane.

The gland duct just inside the opening is U-shaped in cross section and consists of unsclerotised and likely elastic cuticle. This combination constitutes an effective closing mechanism for the gland duct, similar to that described for silk gland ducts by Snodgrass (1935, figure 165F). The opening muscles insert on the wall of the gland duct in the bottom of the U, thus contraction of these muscles will cause the gland duct to open, allowing gland products to be released.

Gland opening muscles

Our study found gland opening muscles to be widespread in Trichoptera. All species examined with gland openings on sternum V possessed gland opening muscles, except species in Psychomyiidae. Previously gland opening muscles in Trichoptera had been reported only by Eltringham (1934, figure 3), Nielsen (1980) and Hashimoto and Kobayashi (2009) in representatives of 4 families, although Eltringham did not recognise them as such. With the present study, gland opening muscles are now known in representatives of 24 of 38 investigated families of Trichoptera, as well as in representatives of 5 families of Lepidoptera (Djernæs, in press, in prep.).

We found 2 completely different types of gland opening muscles to be present in Trichoptera, confirming Nielsen's (1980) observations. One type originates anteromedially on sternum VI (Fig. 3 B) and is found in representatives of 3 "spicipalpian" families (Glossosomatidae, Hydroptilidae and Rhyacophilidae) and of 1 annulipalpian family (Philopotamidae). The other type originates mesally on sternum V (Fig. 3A) and was found in all other trichopterans with a gland opening muscle. The former type is indistinguishable from the opening muscles found in Lepidoptera, and is likely

homologous. The latter type is most likely what was observed by Hashimoto and Kobayashi (2009) as they were looking at a phryganeid (they did not investigate where the muscle originated), and is thus not homologous to the gland opening muscles found in Lepidoptera, although its function is equivalent.

If one of the 2 types of opening muscles in Trichoptera is homologous to that found in Lepidoptera, the distribution of this type within Trichoptera raises some interesting questions. It is possible that Rhyacophilidae, Glossosomatidae and Hydroptilidae (all "spicipalpians") belong at the very base of Trichoptera with the trichopteran gland opening muscle as a later development. "Spicipalpia" are probably not monophyletic (their paraphyly indicated here by quotation marks), but are consistently placed at the base of the trichopteran tree even if their placements differ in other respects (e.g., Frania & Wiggins 1997, Morse 1997, Holzenthal *et al.* 2007). However, this scenario does not explain the presence of the lepidopteran type in philopotamids, which are annulipalpians. Furthermore, one philopotamid species, *Wormaldia arizonensis*, possesses both the lepidopteran type and the trichopteran type, showing that the two types can co-exist. It is possible that there have been multiple origins of morphologically indistinguishable muscles (Fig. 6A), but we regard this as highly unlikely. Presuming only 1 origin of each type of opening muscle, the type found in Lepidoptera and some Trichoptera would have been present in ancestral Amphiesmenoptera while both types would have been present in stem-group Trichoptera with a later loss of one or the other in different lineages (Fig. 6 B). While the multiple origins scenario is most parsimonious if gains and losses are regarded as equally likely, if losses are regarded as more than $1\frac{1}{3}$ as likely as gains[1], then the single origin scenario becomes most parsimonious.

The presence of a typical trichopteran opening muscle in *W. arizonensis* as well as the lepidopteran type otherwise typical of philopotamids is a puzzle in its own right. Although *Wormaldia* is likely basal within Philopotamidae (Kjer *et al.* 2002, Holzenthal *et al.* 2007), no other species in the genus shows this muscle configuration (several species were investigated upon the discovery of both muscle types in *W. arizonensis*). This might be an instance where a structure has been lost, but its genetic pathway has been retained and, if expressed, is still capable of producing the structure. Although this is not viewed as a common occurrence, several examples have been reported (e.g., Whiting *et al.* 2003, Kohlsdorf & Wagner 2006), and it is increasingly viewed as an important mechanism (Collin & Miglietta 2008).

Fenestrae/perforated patches
We found the fenestrae on sternum IV to be perforated in all investigated species (females of Philopotamidae, Eriocraniidae, Neopseustidae and Nepticulidae) and furthermore showed that female psychomyiids have perforated patches on sternum IV despite the cuticle being as sclerotised as that surrounding the patch. In addition we found a specialised 'sunburst' arrangement of muscle fibres associated with the perforated cuticle in philopotamids, psychomyiids, eriocraniids and, to some degree, in neopseustids. As these muscle fibres originate on the body wall and insert on the wall of the gland reservoir, their contraction stretches the cuticle side of the reservoir, presumably rendering it (more) permeable to the gland products (in a manner similar to that of a rubber sheet with tiny holes in it being stretched). This would bring the gland products into contact with the perforated cuticle, thereby releasing them through the cuticle. While this scenario is speculative, it is the most parsimonious way for gland products to be released from the fenestral glands in philopotamids and neopseustids as these lack an excurrent duct.

1.   Fig. 6A: 7 losses and 5 gains, Fig. 6B: 11 losses and 2 gains:
      7 losses + 5 gains = 11 losses + 2 gains   <=>   1 gain = $1\frac{1}{3}$ loss
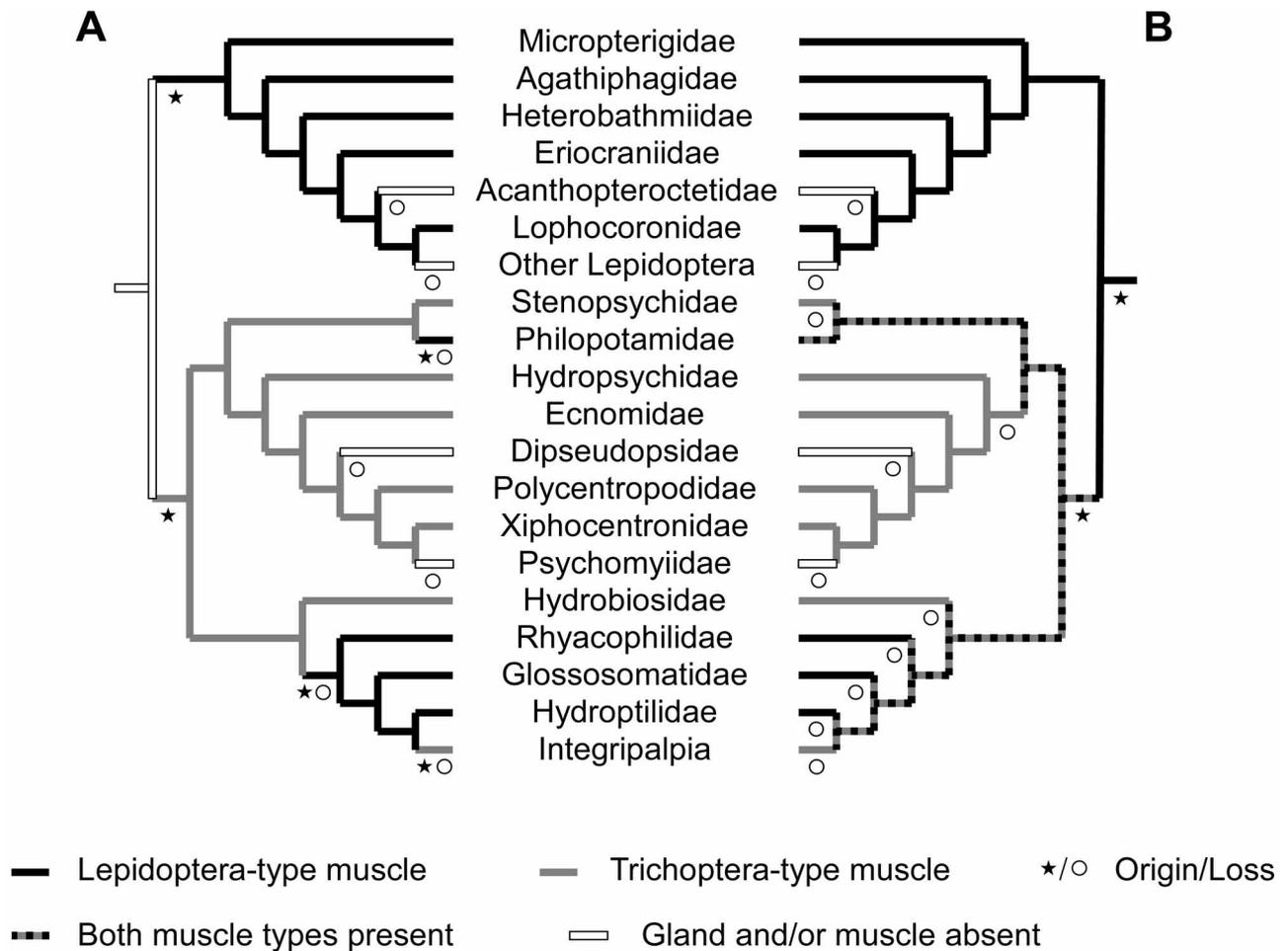
FIGURE 6. Mapping of gland opening muscles on a phylogeny of Amphiesmenoptera from Kristensen and Skalski (1998) (Lepidoptera) and Holzenthal *et al.* (2007) (Trichoptera) A: Multiple origins of both Lepidoptera-type and Trichoptera-type. B: Single origin of each type with both types or genetic pathway for both types present in ancestral Annulipalpia and "Spicipalpia" + Integripalpia. The codings for Philopotamidae reflect the fact that most philopotamids have Lepidoptera-type opening muscles, while a single species, *Wormaldia arizonensis* (Ling), has both types of opening muscles. See text for probability of losses vs. gains under the single origin scenario.

Nepticulids have glandular tissue and a reservoir associated with each fenestra, but no indications of a 'sunburst' musculature. This might be due either to reduction or that the animals are so small that while the muscles are present, they do not show up in histological sections. Despite the apparent lack of gland musculature, the glands are likely functional as the fenestrae are distinctive depressions with numerous perforations. Thus the glandular complexes associated with the fenestrae are good candidates for the production site for the pheromones found by Tóth *et al.* (1995) as these were structurally similar to substances known to be produced by the sternum V gland in trichopterans and other lepidopterans, but different from those found in lepidopterans without the sternum V gland (Zhu *et al.* 1995; Kozlov *et al.* 1996; Löfstedt & Kozlov 1997).

As noted in Observations, the fenestral glands in female philopotamids co-occur with normally developed sternum V glands. However, the histological structure shows a cuticular reservoir which must have originated (both evolutionarily and presumably developmentally) as an epidermal invagination. Furthermore, their histological structure shows extensive similarities with that of psychomyiids. This argues for the fenestral glands being derived from the sternum V gland, probably

through increasing compartmentalisation, eventually leading to complete physical separation of the part of the gland secreting through the gland duct and the part secreting through the fenestra.

Ansteeg (1989) observed the concurrence of both separate glandular structures on sternum IV in female *Wormaldia copiosa* (McLachlan) and normally developed sternum V glands. He suggested that the sternum IV structures were homologous to the structures found on sternum IV in female *Eriocrania semipurpurella* (Stephens) by Philpott (1925). Philpott's figure 5 shows *E. semipurpurella* with the gland reservoirs detached from the fenestrae, hence the sternum V gland and the fenestra appear to be separate structures. Ansteeg's homologization is likely based on this preparation artefact, thus he viewed the fenestrae as structures not connected with the sternum V gland.

Ivanov and Melnitsky (1999, 2002) noticed the presence of glandular tissue associated with the fenestrae in philopotamids. However, they overlooked the reservoir associated with the fenestra, and the co-occurrence of normally developed sternum V glands. Instead they reported fenestral glands without reservoirs or excurrent ducts on sternum V in some philopotamids while we found only normally developed glands on this segment. However, in philopotamids the gland opening is often situated in a transparent patch of cuticle, and in some instances the cuticle surrounding the gland opening has a scaled appearance (denoted as 'callus' in the literature) (Fig. 1C). Based on their observations, Ivanov and Melnitsky (1999, 2002) proposed that the fenestral gland without reservoir or excurrent duct was the ancestral type of gland, retained only in female philopotamids. Contrary to this, our observations indicate that the fenestral glands are derived from normal sternum V glands with associated perforated patches, which support Kristensen's (1984) conclusion that a saccular invagination from sternum V is the ancestral form of the gland.

Nevertheless, the occurrence of fenestrae/perforated patches, and in most cases also a distinctive and specialised muscular structure, in both Trichoptera and Lepidoptera, suggests that this arrangement originated in the common ancestor, with losses occurring in several members of both lineages (Fig. 7 B). An alternative is to hypothesize 5 independent origins of perforated cuticle and 4 independent origins of the associated 'sunburst' musculature (Fig. 7A)[1]. A single origin does not necessarily imply that the fenestral complex has been physically present in all the ancestors of the extant taxa having the trait. It does require that it was developed (and physically present) in the common ancestor of Trichoptera and Lepidoptera, and that stem group Trichoptera and Lepidoptera both inherited at least the genetic pathway for the fenestral complex.

Finding fossil Trichoptera/Lepidoptera with fenestrae from the lineages predicted to have fenestral complexes based on the present distribution would support the hypothesis of a single origin. While the fenestrae/perforated cuticle and associated structures might be a very elusive character in fossils, it should be possible to detect unsclerotised cuticle as well as a raised patch of cuticle in amber fossils; the latter is associated with the perforated cuticle in *Psychomyia flavida* and *Synempora andesae*. As all investigated extant species with paired fenestrae on sternum IV were found to have associated reservoirs and secretory tissue, similar fenestrae/raised patches in fossils should indicate the presence of the associated structures.

---

1. Again, if gains and losses are regarded as equally likely, the multiple origins scenario is most parsimonious. In this case losses must be more than 4.14 times as likely as gains to make the single origin scenario most parsimonious:
   Fig. 7 A: 9 gains, Fig. 7 B: 2 gains and 29 losses
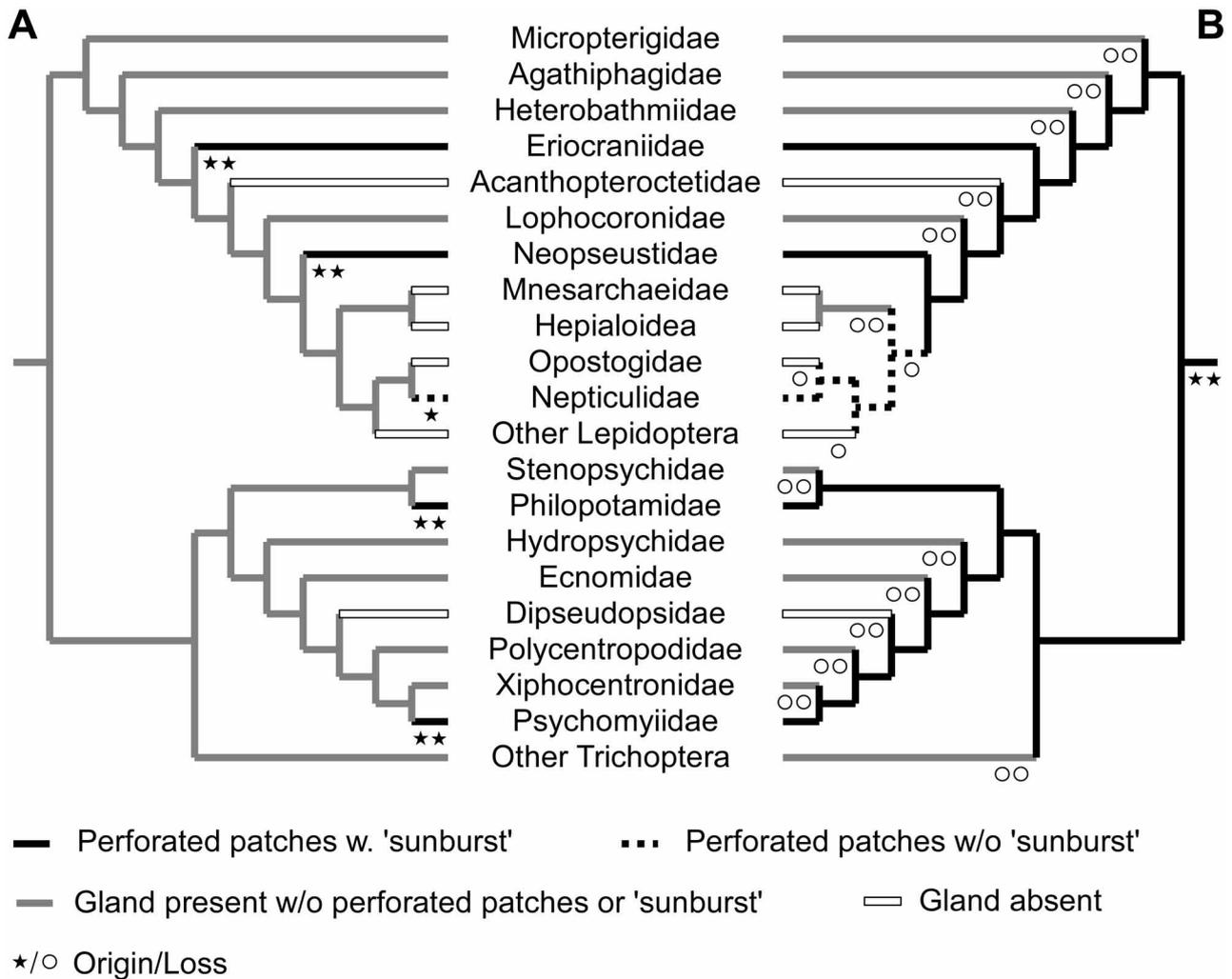   9 gains = 2 gains + 29 losses   <=>   1 gain ≈ 4.14 loss

FIGURE 7. Mapping of fenestrae/perforated patches and associated 'sunburst' musculature on a phylogeny of Amphiesmenoptera from Kristensen and Skalski (1998) (Lepidoptera) and Holzenthal *et al.* (2007) (Trichoptera). A: Multiple origins of both fenestrae/perforated patches and 'sunburst' musculature. B: Single origin of fenestrae/ perforated patches and 'sunburst' musculature with the structures or genetic pathway for the structures present in the ancestral Trichoptera and Lepidoptera and present in the trunk of the basal lepidopteran phylogeny and the trunk of the annulipalpian phylogeny. Due to the presence of fenestrae, but no 'sunburst' musculature in Nepticulidae, origin/loss of fenestrae and/or 'sunburst' musculature are treated as separate events. See text for probability of losses vs. gains under the single origin scenario.

We have shown that the functional components (perforated cuticle and 'sunburst' musculature) of the fenestral complex can be present without the presence of traditional fenestra (transparent cuticle). Thus it is possible that fenestral complexes could be present, but overlooked in representatives of additional groups. We specifically looked for perforated cuticle in all the species investigated with SEM, but there were species for which females were not available. Extant species of particular interest in this regard would be additional species of *Heterobathmia* as *H. pseuderiocrania* Kristensen & Nielsen showed a gland structure very similar (gland reservoir round and pressed against cuticle) to that of female eriocraniids and psychomyiids, except for the lack of fenestra/perforations and sunburst musculature (Djernæs, in prep.). Also of interest would be any species of Acanthopteroctetidae (Lepidoptera), including *Catapterix crimaea* Zagulaev & Sinev where patches of transparent cuticle are present on sternum II (Zagulaev & Sinev 1988, in Ivanov &

Melnitsky 1999, 2002). This family was not included in the present study as no gland associated structures have been observed on sternum V or IV (Davis 1975, 1978). Any females of Agathiphagidae and Lophocoronidae should be examined as only males were included in the present study. Additional species of Micropterigidae should be included, along with any species of Mnesarchaeidae, although females in the latter family have pheromone producing structures on both the hindwing and thorax (Löfstedt & Kozlov 1997, Kristensen 1998 and references therein). Species of Hepialoidea and Opostegidae should be examined, although females of Hepialidae also have pheromone producing structures on the hindwing, and hepialoid males often have scent organs on wings or hindlegs (Kristensen 1998 and references therein).

Ground plan features of Amphiesmenoptera
In conclusion, we propose the following ground plan for the sternum V gland in Amphiesmenoptera: The gland forms a pair of invaginations from sternum V. The gland openings are slit-like, with U-shaped (in cross section) gland ducts just inside the openings. A pair of gland opening muscles originates on the anteromedial margin of sternum VI and insert in the bottom of the U on the gland duct wall just inside the gland opening. The gland ducts expand into saccular reservoirs, which subtend the cuticle of sternum IV. Opening into the reservoir is a layer of type 3 secretory cells (as defined by Noirot & Quennedy 1974). In females, the cuticle against which the reservoir is pressed is perforated and likely transparent. Muscle fibres originate around the perforated cuticle and insert on the walls of the gland reservoir; contraction of these fibres facilitate secretion of gland products through the cuticle.

References

Ansteeg, O. (1989) *Abdominale exokrine Drüsen der Trichoptera: Ihre Morphologie und Chemie*. Thesis, Universität Bayreuth, Germany, 108 pp.
Collin, R & Miglietta, M.P. (2008) Reversing opinions on Dollo's Law. *Trends in Ecology and Evolution*, 23, 602–609.
Davis, D.R. (1975) Systematics and zoogeography of the family Neopseustidae with the proposal of a new superfamily (Lepidoptera: Neopseustoidea). *Smithsonian Contributions to Zoology*, 210, 1–45.

Davis, D.R. (1978) A revision of the North American moths of the superfamily Eriocranioidea with the proposal of a new family, Acanthopteroctetidae (Lepidoptera). *Smithsonian Contributions to Zoology*, 251, 1–131.

Djernæs, M. (in press) Structure and phylogenetic significance of the sternum V glands in Trichoptera. *Zootaxa*.

Djernæs, M. (in prep.) The sternum V gland in Lepidoptera.

Eltringham, H. (1934) On some further remarkable structures in Trichoptera. *Transactions of the Royal Entomological Society of London*, 82, 409–413 and plate XV.

Frania, H.E. & Wiggins, G.B. (1997) Analysis of morphological and behavioural evidence for the phylogeny and higher classification of Trichoptera (Insecta). *Life Sciences Contributions, Royal Ontario Museum*, 160, 1–67.

Hashimoto, Y. & Kobayashi, Y. (2009) Morphology of sternum V gland in three caddisfly species, *Stenopsyche marmorata*, *Eubasilissa regina* and *Nemotaulius admorsus* (Insecta: Trichoptera). *Entomological Science*, 12, 298–307.

Holzenthal, R.W., Blahnik, R.J., Kjer, K.M. & Prather, A.L. (2007) An update on the phylogeny of caddisflies (Trichoptera). *In*: Bueno-Soria, J., Barba-Álvarez, R. & Armitage, B. (Eds.), *Proceedings of the XIIth International Symposium on Trichoptera*. The Caddis Press, Columbus, Ohio, pp. 143–153.

Ivanov, V.D. & Melnitsky, S.I. (1999) Structure of sternal pheromone glands in caddisflies (Trichoptera). *Entomological Review,* 79, 926–942. Translated from *Entomolgicheskoe Obozrenie*, 78, 505–526.

Ivanov, V.D. & Melnitsky, S.I. (2002) Structure of pheromone glands in Trichoptera. *Nova Supplementa Entomologica,* 15, 17–28.

Kjer, K.M., Blahnik, R.J. & Holzenthal, R.W. (2002) Phylogeny of caddisflies (Insecta, Trichoptera). *Zoologica Scripta*, 31, 83–91.

Kohlsdorf, T. & Wagner, G.P. (2006) Evidence for the reversability of digit loss: a phylogenetic study of limb evolution in *Bachia* (Gymnophthalmidae: Squamata). *Evolution*, 60, 1896–1912.

Kozlov, M.V., Zhu, J., Philipp, P., Francke, W., Zvereva, E.L., Hansson, B.S. & Löfstedt, C. (1996) Pheromone specificity in *Eriocrania semipurpurrella* (Stephens) and *E. sangii* (Wood) (Lepidoptera: Eriocraniidae) based on chirality of semiochemicals. *Journal of Chemical Ecology,* 22, 431–454.

Kristensen, N.P. (1984) The pregenital abdomen of the Zeugloptera (Lepidoptera). *Steenstrupia*, 10 (4), 113–136.

Kristensen, N.P. (1998) The homoneurous Glossata. *In*: Kristensen, N.P. (Ed.), *Lepidoptera, Moths and Butterflies. Volume 1: Evolution, Systematics, and Biogeography. In*: Fischer, M. (Ed.), *Handbuch der Zoologie, Band IV, Arthropoda: Insecta, Teilband 35*. de Gruyter, Berlin, pp. 51–63.

Kristensen, N.P. & Nielsen, E.S. (1979) A new subfamily of micropterigid moths from South America. A contribution to the morphology and phylogeny of the Micropterigidae, with a generic catalogue of the family (Lepidoptera: Zeugloptera). *Steenstrupia* 5(7), 69–147.

Kristensen, N.P. & Skalski, A.W. (1998) Palaeontology and phylogeny. *In*: Kristensen, N.P. (Ed.), *Lepidoptera, Moths and Butterflies. Volume 1: Evolution, Systematics, and Biogeography. In*: Fischer, M. (Ed.), *Handbuch der Zoologie, Band IV, Arthropoda: Insecta, Teilband 35*. de Gruyter, Berlin, pp. 7–25.

Löfstedt, C. & Kozlov, M. (1997) A phylogenetic analysis of pheromone production in primitive moths. *In*: Card, R. T. & Minks A. K. (Eds.), *Insect Pheromone Research: New Directions*. Chapman & Hall, New York, pp. 473–489.

Morse, J.C. (1997) Phylogeny of Trichoptera. *Annual Review of Entomology*, 42, 427–450.

Nielsen, A. (1980) A comparative study of the genital segments and genital chamber in female Trichoptera. *Biologiske Skrifter, Det Kongelige danske Videnskabernes Selskab*, 23(1), 1–200.

Nielsen, E.S. & Kristensen, N.P. (1996) The Australian moth family Lophocoronidae and the basal phylogeny of the Lepidoptera-Glossata. *Invertebrate Taxonomy,* 10, 1199–1302.

Noirot, C. & Quennedy, A. (1974) Fine structure of insect epidermal glands. *Annual Review of Entomology*, 19, 61–80.

Philpott, A. (1925) On an abdominal scent-organ (?) in *Sabatinca* and other primitive genera of Lepidoptera. *Transactions of the Royal Entomological Society of London*, 72, 457–461.

Snodgrass, R.E. (1935) *Principles of Insect Morphology*. McGraw-Hill, New York, pp. 768.

Tóth, M., Szöcs, G., van Nieukerken, E.J., Philipp, P., Schmidt, F. & Francke, W. (1995) Novel type of sex pheromone structure identified from *Stigmella malella* (Stainton) (Lepidoptera: Nepticulidae). *Journal of Chemical Ecology,* 21, 13–27.

Whiting, M.F., Bradler, S. & Maxwell, T. (2003) Loss and recovery of wings in stick insects. *Nature*, 421, 264–267.

Zhu, J., Kozlov, M.V., Philipp, P., Francke, W. & Löfstedt, C. (1995) Identification of a novel moth sex pheromone in *Eriocrania cicatricella* (Zett.) (Lepidoptera: Eriocraniidae) and its phylogenetic implications. *Journal of Chemical Ecology,* 21, 29–43.