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Morphology, function and evolution of the sternum V glands in Amphiesmenoptera

by

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Abstract

I investigated the paired sternum V glands in thirty-eight trichopteran families and all lepidopteran families possessing the gland or associated structures. Using my morphological data and literature data on sternum V gland secretions, I examined phylogenetic trends in morphology and gland products and reconstructed ancestral states. I investigated correlations between gland products, between morphological traits and between chemistry and morphology. The gland is present in twenty-five trichopteran families. It is generally present in Annulipalpia, except Dipseudopsidae, and in Spicipalpia. It is widespread in Plenitentoria, but is often absent in Brevitentoria, especially in males. In Lepidoptera, I present the first report on the reduced, but functional glands in Neopseustidae and Nepticulidae. The gland is typically an invagination from sternum V with a duct leading to a reservoir surrounded by secretory tissue. An opening muscle inserts just inside the opening. I found two non-homologous opening-muscle types, one in Lepidoptera and some Trichoptera, another in the remaining Trichoptera. Muscle fibres often surround the reservoir, sometimes also the secretory tissue. Exceptions are found in Psychomyiidae (no opening muscle), female Philopotamidae (fenestra with separate glandular complex), Agathiphagidae (several unique features), Neopseustidae and Nepticulidae (gland present without gland opening). Using variations in gland structure, I identified phylogenetically useful characters from the superorder to the species level. The fenestrae in female Philopotamidae, Eriocraniidae, Neopseustidae and Nepticulidae are perforated, and perforated

patches are present in female Psychomyiidae. The perforated patches are associated with a reservoir, secretory tissue and a distinctive ‘sunburst’ musculature in both Trichoptera and Lepidoptera. The probable ancestral gland compounds are heptan-2-ol, 4-hepten-2-one, 4-hepten-2-ol, nonan-2-one, 6-nonen-2-one and 6-nonen-2-ol, making pheromone production a plausible ancestral function. The most widespread gland compounds are heptan-2-one, heptan-2-ol, nonan-2-one and nonan-2-ol, but these are absent from Apataniidae + Limnephilidae, which instead produce methylated 3-ketones and -ols, unique within Trichoptera. These compounds all probably function as pheromones. Both large and small glands in females can function in sex pheromone production, while large glands in male *Hydropsyche* (Hydropsychidae) are likely linked to male aggregation pheromone production. Relative sizes of regular gland reservoirs and fenestral gland reservoirs in female philopotamids suggest a complementary function.

Acknowledgements

I am grateful to N. P. Kristensen for suggesting this interesting project, and to my supervisor F. A. H. Sperling for encouraging me to expand the project and study not only Lepidoptera, but also Trichoptera. I appreciate having had numerous discussions on the intricacies of the sternum V gland with both. I am also enormously grateful to my husband T. J. Simonsen who has provided companionship, practical assistance above and beyond and been a willing discussion partner.

This entire project would not have been possible without the loan of specimens from several collections and the collection efforts of a number of individuals. Thus I am grateful to Clemson University (C. J. Geraci and I. Stocks), Los Angeles County Museum (J. P. Donahue and W. Xie), Natural History Museum of Denmark (O. Karsholt and N. P. Kristensen), Smithsonian Institution (C. J. Geraci and O. Flint) and University of Minnesota Insect Collection (R. Holzenthal and D. Robertson) for the loan of specimens. Futhermore, I thank R. Brown, J. Dombroskie, G. Gibbs, O. Karsholt, J.-F. Landry, E. van Nieukerken, G. Pohl, G. S. Robinson and F. A. H. Sperling for collecting or providing specimens. I thank Gatineau Park, Québec, for a permit to collect and conduct experiments on *Epimartyria auricrinella*. I am grateful to N. P. Kristensen for supplying prepared slides of various Lepidoptera as well as TEM micrographs of *Eriocrania semipurpurella*.

In addition to specimens, a PhD project cannot be completed without the help of a large number of people. I thank C. Löfstedt for supplying unpublished data on gland compounds and T. Garland for advice on phylogenetically independent contrasts. I am grateful to I. Lusebrink who went above and beyond to get me a copy of Ansteeg's thesis. I gratefully acknowledge B. S. Heming and D. A. Craig for help with microscopy. I thank A. Nimmo for help in identifying specimens, G. Braybrook for assistance with SEM and M. Eberhard for preparing the 1µm sections of *Synempora andesae* and *Agathiphaga vitiensis*.

I thank the members of the extended Sperling lab for being good company, and making me feel at home in Edmonton. I thank the members of my PhD committee, B. Heming, M. Evenden and J. Spence, for being willing to serve, and for many useful discussions and pieces of advice over the years.

Finally, a project like this requires funding. This study was funded by an NSERC grant to Felix A. H. Sperling, an Alberta Ingenuity Scholarship to M. Djernæs, and operation money from the Danish Council for Independent Research/Natural Sciences (grant no 272-08-0480). Further support for travel to conferences came from FGSR (Marie Louise Imrie award), from the Department of Biological Sciences and from the Graduate Students' Association.

Biography

I was born on December 12, 1978, and grew up on a dairy farm in the western part of Denmark. I was an extremely curious child. At around age two I started asking 'why' about the world around me: "Why are ducks brown?", "why are only female ducks brown?" I appreciate the patience of my parents in answering my questions, but also think they were relieved when I learned to read well enough that I could look up the answers myself.

But my curiosity often outstripped my reading ability or the literature in the school library. Growing up on a dairy farm, I knew cows, and I had a passion for horses. I could see that cows and horses were in many respects similar, but did wonder how cows got cloven hooves and horses did not. Looking at cow and horse feet, I concluded that if the two halves of a cow foot were to fuse, the result would look very like a horse's hoof. Soon after that I found a book explaining the evolution of feet in hoofed mammals, and I had to reject my theory.

Eventually looking up the answers wasn't really enough anymore, and I started looking at a career in research. I began studying biology at the University of Copenhagen in 1999, knowing that I wanted to study evolution, but thinking that I would be working with vertebrates.

My first introduction to the wonderful world of entomology came in the third year at university when we had to do undergraduate research projects. We were offered several standard and mainly literature based projects, but Klaus-Dieter Klass and Rudolf Meier from the Natural History Museum of Denmark had two small research projects suitable for students, one on flies and one on cockroaches. With a fellow student I went for the cockroach project, not attracted by the cockroaches, but by the promise of being allowed to do real research. This was also the time when I met my future husband Thomas J. Simonsen, truly a time for starting long term relationships. The cockroach project resulted in a talk that I gave at the 22nd International Congress on Entomology in Brisbane, Australia, my first conference talk ever!

I spent a year (2002/2003) as an exchange student at University of California, Riverside where I took my first entomology courses and continued my studies on cockroaches in Daphne Fairbairn's lab. Back at the University of Copenhagen I took a course in molecular evolution. After the course we students were offered small relevant research projects. This was when I was confirmed as an entomologist: Among the different projects offered were tracing paternity in elephants or trying to place the newly described insect order Mantophasmatodea. I went for Mantophasmatodea, supervised by Jakob Damgaard, and never really considered working on vertebrates after that. The Mantophasmatodea project ended up being about intron positions in EF-1α in the entire Hexapoda, but I still learned valuable new skills about sequencing, data mining, and aligning and analysing gene sequences.

In early 2004, I started my Master's thesis, a molecular phylogeny of cockroaches, at the Natural History Museum of Denmark. Originally I was supervised by Nils Møller Andersen and Jakob Damgaard. Upon the untimely death of Nils Andersen, Niels Peder Kristensen and Jakob Damgaard supervised my thesis work. In the fall of 2004, I married Thomas Simonsen. I defended my MSc in May 2005 before moving to Alberta two weeks later.

In September 2005 I started my PhD in Felix A. H. Sperling's lab, going back to morphology after my molecular stints. In addition to learning new skills and concepts, my time in Alberta provided me with many new friends, the experience of doing long collecting trips, and a generally good time. Let's hope the future brings as much!

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List of Abbreviations

EAD Electroantennographic detection: electroantennography coupled with gas chromatography, also known as GC-EAD

EAG Electroantennography: do antenna react to a given substance or mixture of substances

SEM Scanning electron microscopy

SEMs Scanning electron micrographs

TEM Transmission electron microscopy

TEMs Transmission electron micrographs

Chapter 1

General introduction

Thorough comparative morphological studies are the foundation for reconstructing evolutionary history and correlating form and function. This type of study is also essential for identifying new morphological characters to help reconstruct the 'Tree of Life'. The sternum V glands are one of the supporting synapomorphies of Trichoptera + Lepidoptera (Amphiesmenoptera), one of the best supported superordinal clades in the insects (Kristensen, 1981). The glands are present throughout Trichoptera and in representatives of five basal lepidopteran families (Davis 1975; Kristensen & Nielsen 1979; Kristensen, 1984; Nielsen & Kristensen, 1996; Ivanov & Melnitsky 1999, 2002). The general structure of the sternum V glands are a pair of invaginations from the anterolateral part of sternum V (Figure 1-1), each with a duct leading to a reservoir with associated secretory cells (Kristensen, 1984; Ivanov & Melnitsky 1999, 2002). The morphology of the sternum V glands has been the subject of several studies (Philpott, 1925; Le Cerf, 1926; Eltringham, 1931, 1934; Razowski, 1975; Ansteeg, 1989; Ivanov and Melnitsky, 1999, 2002; Hashimoto & Kobayashi, 2009) and has been included in several morphological studies of basal lepidopterans (mainly Davis, 1975, 1978; Kristensen & Nielsen, 1979; Kristensen, 1984; Nielsen & Kristensen, 1996) and in a study of the female abdomen in Trichoptera (Nielsen, 1980). However, studies employing large taxon sampling (Davis, 1975; Nielsen, 1980; Ansteeg, 1989; Ivanov and Melnitsky, 1999, 2002) have generally been focused on gross morphology of cuticular structures. Thus, despite a large number of studies, the detailed investigations of a large number of taxa necessary for meaningful comparisons of structures and functions, as well as identification of phylogenetically informative characters, are still missing.

GLAND MORPHOLOGY

The cuticle surrounding the gland opening is modified in many taxa; some of the most distinctive modifications are the long processes associated with the gland opening in some representatives of Hydropsychidae and Polycentropodidae (Trichoptera) (e.g. Eltringham, 1934; Nielsen, 1980; Ivanov & Melnitsky, 2002). Other modifications include a short protuberance with long setae found in representatives of Hydroptilidae (Trichoptera) and Micropterigidae (Lepidoptera) (Marshall, 1979; Kristensen, 1984), and thumb-shaped protuberances covered with domes in representatives of Heterobathmiidae and Eriocraniidae (Lepidoptera) (Davis, 1978; Kristensen & Nielsen, 1979).

The internal cuticular structures of the gland generally consist of a duct leading to a reservoir; in trichopterans this reservoir may be situated in segment IV or V while in lepidopterans it is always situated in segment IV (Kristensen 1984; Ivanov & Melnitsky 1999). Ivanov and Melnitsky (1999, 2002) found several different reservoir shapes in representatives of Trichoptera, and divided the sternum V gland into four different types based on reservoir shape: 1) ampulliform glands; 2) sacculous glands; 3) kidney-shaped glands; and 4) window or fenestral glands (no reservoir). Muscle fibres associated with the gland reservoir have been described in representatives of Stenopsychidae, Phryganeidae (Trichoptera), Micropterigidae and Lophocoronidae (Lepidoptera) (Kristensen, 1984; Nielsen & Kristensen, 1996; Ivanov & Melnitsky, 2002; Hashimoto & Kobayashi, 2009).

Ivanov and Melnitsky (2002) described the secretory cells as a layer surrounding the gland reservoir in their gland types 1-3. However, Eltringham (1931, 1934) described the secretory cells as being some distance from the reservoir in representatives of Glossosomatidae, Polycentropodidae and Hydropsychidae (Trichoptera), and only as closely associated with the reservoir in a representative of Limnephilidae (Trichoptera).

Ancestral gland structure

Despite the large number of studies of the morphology of the sternum V gland, there are still disagreements about essential points of gland structure. This in turns leads to inconsistent reconstructions of the theoretical ground plan of the gland in the ancestral amphiesmenopterans, the lineage which eventually evolved into the extant Trichoptera and Lepidoptera. The disagreements are mainly about the nature of the gland opening, the types of gland-opening muscles, and the distribution of these traits across taxa. Considerable disagreement also exists over the nature of the fenestrae, areas of transparent cuticle present on sternum IV in some taxa. The gland opening has been proposed to be either a slit (Kristensen & Nielsen 1979; Kristensen, 1984; Kristensen & Nielsen, 1996; Hashimoto & Kobayashi, 2009) or a perforated membrane (Ivanov & Melnitsky, 2002).

Nielsen (1980) observed two completely different kinds of gland-opening muscles[1] in representatives of Trichoptera; he observed one type in hydroptilids, the other type in Limnephilids. Kristensen (1984) investigated gland-opening muscles in representatives of Lepidoptera in which the opening muscles originate on the anterior edge of sternum VI. Kristensen (1984) assumed that the opening muscles found by Nielsen (1980) in representatives of Trichoptera were homologous to those he had observed in representatives of Lepidoptera. As a consequence, Kristensen (1984) attributed Lepidoptera-type opening muscles to the amphiesmenopteran ground plan. Hashimoto and Kobayashi (2009) found muscle fibres inserting on the gland duct in a phryganeid (Trichoptera) and assumed that these were homologous to the lepidopteran gland-opening muscles described by Kristensen (1984).

Fenestrae (a pair of patches of transparent cuticle) are present on sternum IV in females of Philopotamidae (Trichoptera), Eriocraniidae, Neopseustidae and Nepticulidae (Lepidoptera) (Davis 1975; Ivanov & Melnitsky 1999, 2002). Ivanov and Melnitsky (1999, 2002) reported secretory tissue directly connected to the fenestra in philopotamids without the presence of either a gland sac or gland duct.

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

This is structurally simpler than the typical gland configuration; hence Ivanov and Melnitsky (1999, 2002) suggested this as the gland type originally found in ancestral Amphiesmenoptera. The fenestrae in eriocraniids are associated with the reservoirs of normal sternum V glands (the reservoirs are pressed against the fenestrae), while fenestrae in neopseustids and nepticulids seemingly occur independently (Davis 1975, 1978). However, Tóth et al. (1995) found that a nepticulid female produced sex pheromones similar to those produced by the sternum V gland in other amphiesmenopterans (e.g. Löfstedt et al., 1994; Zhu et al., 1995; Kozlov et al., 1996; Bergman et al., 2001, 2002) which suggests that the fenestrae in female nepticulids are associated with functional sternum V glands.

GLAND FUNCTION

The function of the sternum V gland is reasonably well known in females in which it generally produces sex pheromones (Wood & Resh, 1984; Resh & Wood, 1985; Solem, 1985; Löfstedt et al., 1994; Zhu et al., 1995; Kozlov et al., 1996; Bjostad et al., 1996; Jewett et al., 1996; Bergman et al., 2001, 2002). However, in some taxa in which the sternum V gland is present, studies have failed to show any long distance attraction of males to females (Duffield et al., 1977; Solem & Petersson, 1987; Kozlov & Zvereva, 1999). Furthermore, the gland function is generally unknown in males although the chemical composition of the gland secretion is known in many species (Duffield et al., 1977; Ansteeg & Dettner, 1991; Löfstedt et al., 1994; Bergmann, 2002; Bergman et al., 2002). The known gland functions in males are aggregation pheromone production in *Hydropsyche angustipennis* (Curtis) (Trichoptera: Hydropsychidae) (Löfstedt et al., 1994) and production of defensive substances in *Pycnopsyche scabripennis* (Rambur) (Trichoptera: Limnephilidae) (Duffield et al., 1977). The male gland secretions in several trichopteran species contain substances such as acetophenone, acetic acid, 2-methyl propanoic acid, hexanoic acid and octanoic acid (Ansteeg & Dettner, 1991; Löfstedt et al., 1994; Bergmann, 2002; Bergman et al., 2002). These substances are toxic and/or irritants (Ansteeg & Dettner, 1991; Mohsen et al., 1995; Lewis, 2000; Pohanish, 2002). As well as being present in males, these

types of substances are also known from females (Bergmann, 2002; Bergman et al., 2002). This suggests that a defensive function of the gland might be more widespread than is currently known.

COLLECTIONS

As part of my PhD project I collected both Trichoptera and Lepidoptera. I went on several major collecting trips. I collected nepticulids in Arizona, Utah, New Mexico and Texas in July and August 2005 (with T. Simonsen). I also collected nepticulids in Florida in June 2006 (with J. Dombroskie, D. Lawrie and T. Simonsen). I collected *Epimartyria auricrinella* Walsingham (Lepidoptera: Micropterigidae) in Québec in June 2007. I collected Apataniidae, Beraeidae, Brachycentridae, Calamoceratidae, Glossosomatidae, Helicopsychidae, Hydropsychidae, Hydroptilidae, Lepidostomatidae, Leptoceridae, Limnephilidae, Philopotamidae, Phryganeidae, Polycentropodidae, Rhyacophilidae and Sericostomatidae in Idaho, Utah, Nevada and California in July 2007 (with J. Dombroskie, D. Lawrie, L. Lumley, A. Roe, A. Rose and T. Simonsen). Apart from this I (with T. Simonsen) collected *Eriocrania cicatricella* (Zetterstedt) (Lepidoptera: Eriocraniidae) in Denmark in April 2005. I collected Hydropsychidae, Leptoceridae, Limnephilidae, Rhyacophilidae and Phryganeidae in Alberta (generally with T. Simonsen). I also attempted to collect *Eriocrania semipurpurella* (Stephens) in Alberta in April and May 2006 and 2007 (11 trips total). In addition to the specimens I collected, Felix Sperling, Jason Dombroskie and Thomas Simonsen collected Trichoptera for my project. I identified all specimens collected at least to family, and generally to genus or species. Specimens have been deposited in the Strickland Museum at the University of Alberta. A list of all the identified species is shown in Appendix 1.

SCOPE OF THE THESIS

In my thesis, I have aimed to produce a thorough description of the variations of the sternum V glands and associated structures in a representative selection of Trichoptera and Lepidoptera species (Figure 1-2). I use this information to

reconstruct the ancestral form of the gland and identify evolutionary trends as well as phylogenetically informative characters. I place the available knowledge of the chemistry and function of sternum V gland secretions in a phylogenetic context, and suggest ancestral gland compounds and ancestral gland function. I combine my morphological data with data on sternum V gland secretions from the literature, and identify correlations between characters, both between different morphological characters, between chemical compounds and between morphology and chemistry.

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FIGURE 1-1. Drawings showing position of gland openings. A: Female trichopteran with left wings removed. The arrow points to the position of the gland opening on sternum V. B: Middle of ventral abdomen of *Leptonema albovirens* (Walker) (Trichoptera: Hydropsychidae) showing the position of the paired gland openings and associated cuticular modifications. Go, gland opening; S II, sternum II; S III, sternum III; S IV, sternum IV; S V, sternum V; S VI, sternum VI.

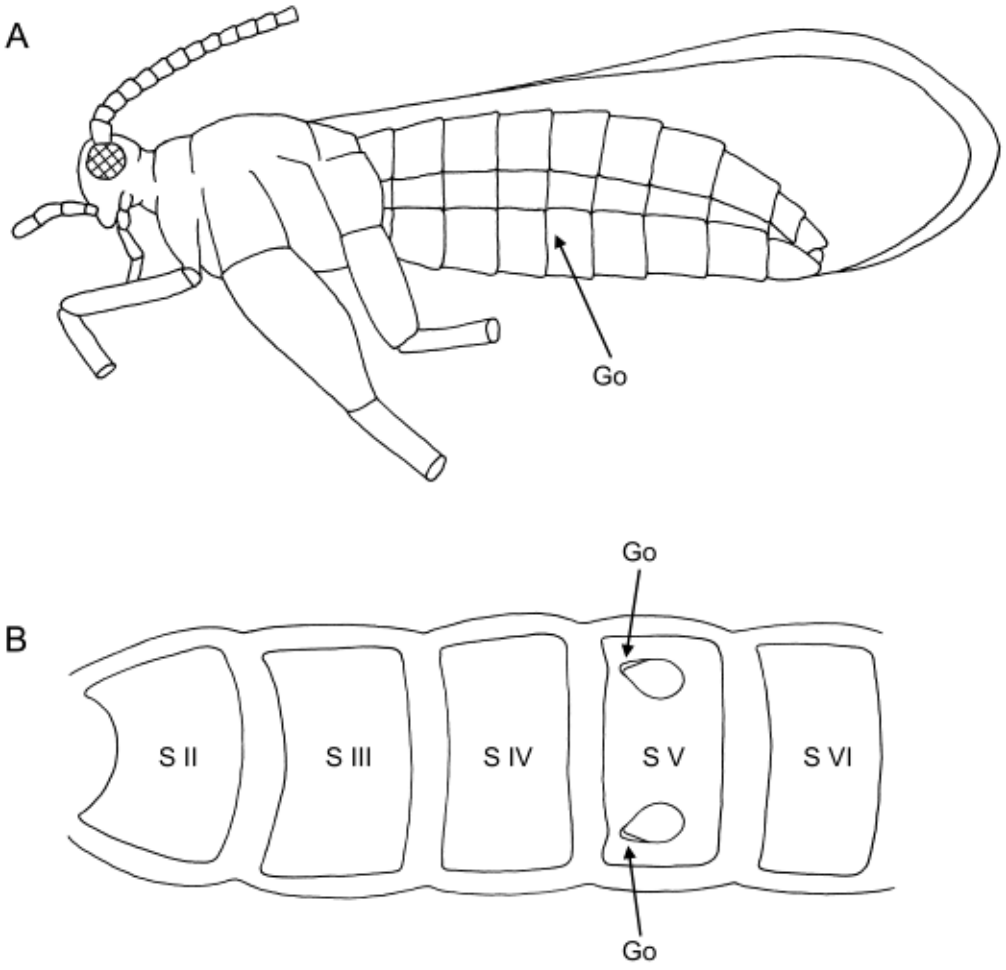
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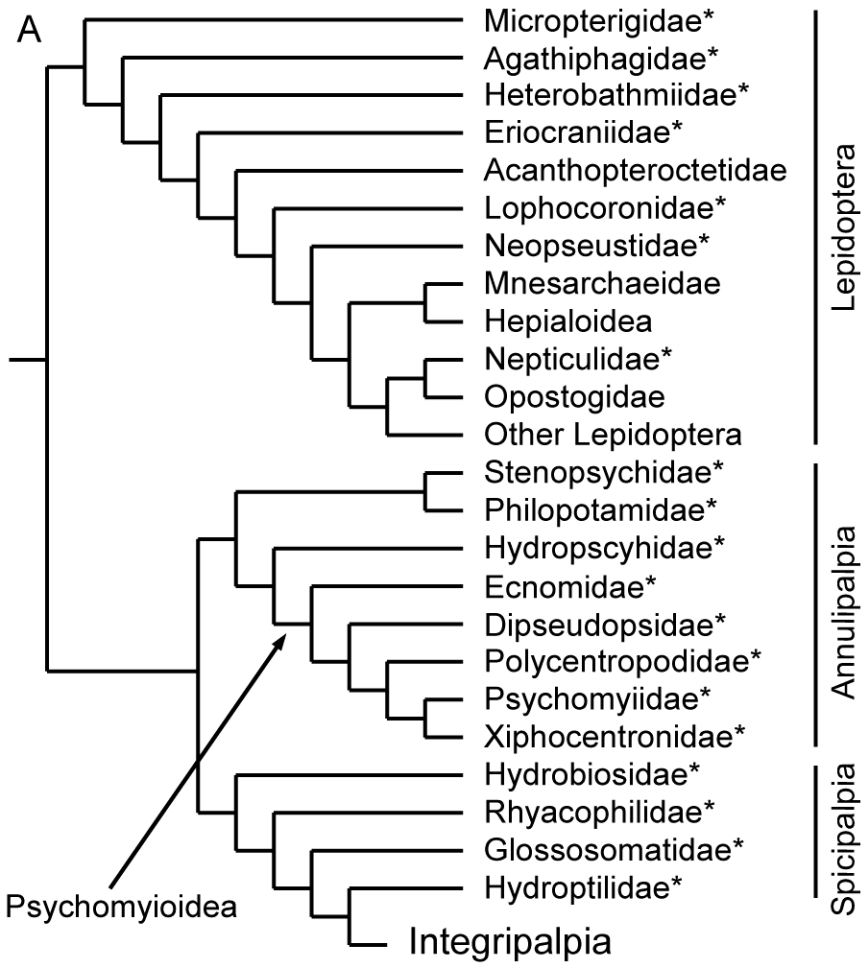
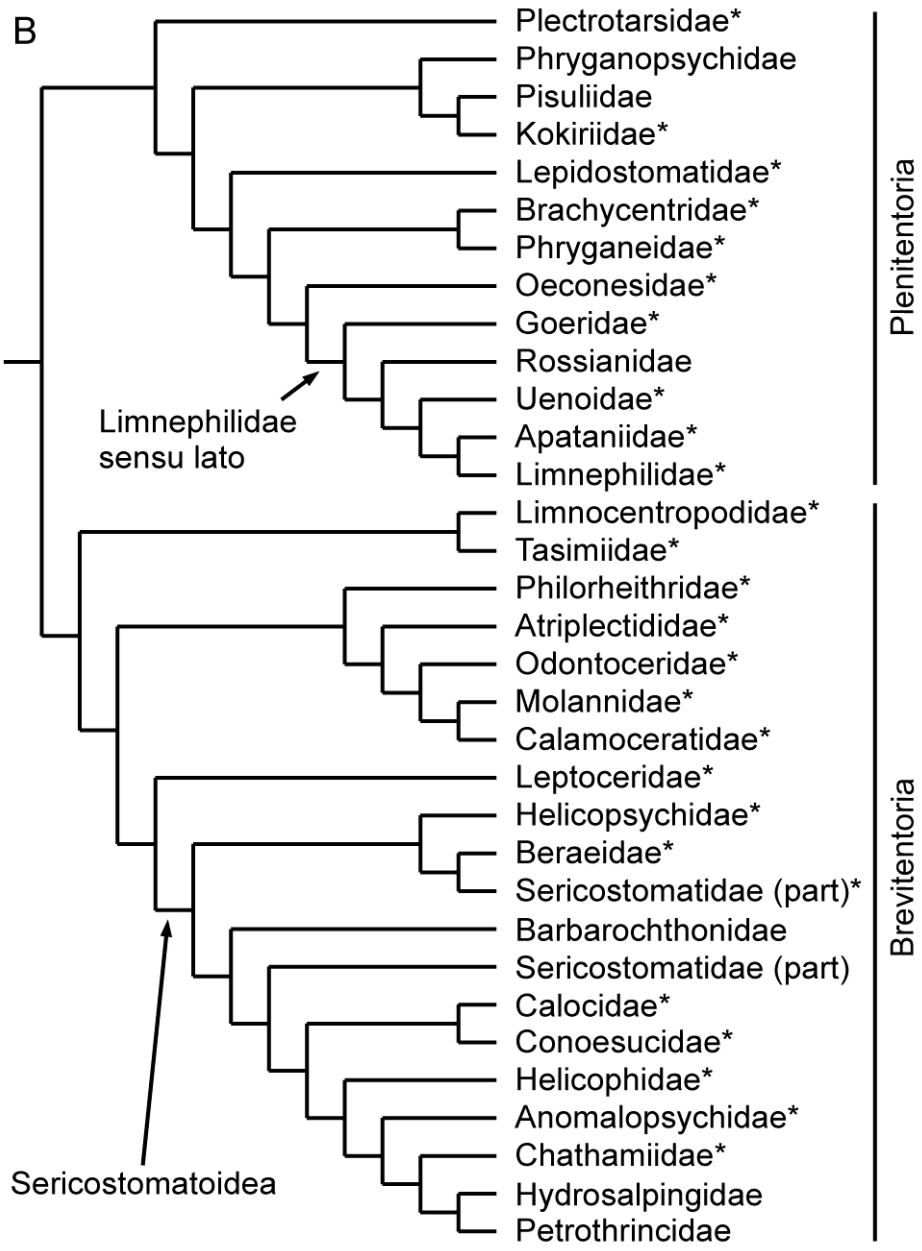
FIGURE 1-2. Tree showing family level relationships of Trichoptera and basal Lepidoptera. Families for which I have examined representatives as part of my PhD are marked with an asterisk. All taxa between order and family level that are referred to in the thesis are indicated. The lepidopteran part of the tree is based on Kristensen and Skalski (1998), and the trichopteran part is based on Holzenthal et al. (2007). The monotypic trichopteran family Antipodoeciidae (Integripalpia – Brevitentoria) is not included on the figure as it was neither included by Holzenthal et al. (2007) nor in the present study. A: Basal Lepidoptera, Annulipalpia and Spicipalpia. B: Integripalpia.


A
Micropterigidae*
Agathiphagidae*
Heterobathmiidae*
Eriocraniidae*
Acanthopteroctetidae
Lophocoronidae*
Neopseustidae*
Mnesarchaeidae
Hepialoidea
Nepticulidae*
Opostogidae
Other Lepidoptera
Stenopsychidae*
Philopotamidae*
Hydropscyhidae*
Ecnomidae*
Dipseudopsidae*
Polycentropodidae*
Psychomyiidae*
Xiphocentronidae*
Hydrobiosidae*
Rhyacophilidae*
Glossosomatidae*
Hydroptilidae*
Psychomyioidea
Integripalpia
Lepidoptera
Annulipalpia
Spicipalpia


B
Plectrotarsidae*
Phryganopsychidae
Pisuliidae
Kokiriidae*
Lepidostomatidae*
Brachycentridae*
Phryganeidae*
Oeconesidae*
Goeridae*
Rossianidae
Uenoidae*
Apataniidae*
Limnephilidae*
Limnephilidae
sensu lato
Plenitentoria
Limnocentropodidae*
Tasimiidae*
Philorheithridae*
Atriplectididae*
Odontoceridae*
Molannidae*
Calamoceratidae*
Leptoceridae*
Helicopsychidae*
Beraeidae*
Sericostomatidae (part)*
Barbarochthonidae
Sericostomatidae (part)
Calocidae*
Conoesucidae*
Helicophidae*
Anomalopsychidae*
Chathamiidae*
Hydrosalpingidae
Petrothrincidae
Sericostomatoidea
Brevitentoria

Chapter 2

Structure and phylogenetic significance of the sternum V glands in Trichoptera

INTRODUCTION

The sternum V glands, a pair of invaginations from sternum V found in Trichoptera and basal Lepidoptera, have been the subject of several studies, both morphological and functional. However, the large-scale studies (Nielsen, 1980; Ansteeg, 1989; Ivanov and Melnitsky, 1999, 2002) have focused mainly on large-scale morphology of the cuticular structures. Thus, detailed investigations that would enable meaningful comparisons of structures and functions, as well as full utilization of the phylogenetic potential of the sternum V gland, are still missing.

The gland is widespread in Trichoptera (known to occur in 25 of 45 families) and is also present in some of the most basal lepidopterans (Davis, 1975, 1978; Kristensen & Nielsen, 1979; Kristensen, 1984; Nielsen & Kristensen, 1996; Ivanov & Melnitsky, 2002; Wiggins, 2004). Thus, the gland is thought to be an autapomorphy for Amphiesmenoptera (Trichoptera + Lepidoptera) (Kristensen, 1981), one of the best supported superordinal groupings in the insects. In females, the gland usually produces sex pheromones (Löfstedt et al., 1994; Bergmann, 2002). While the gland is generally present in both sexes, the function of the gland in males is still largely unknown. Production of male aggregation pheromones has been demonstrated in a single species (Löfstedt et al., 1994) as has production of defensive substances by both sexes (Duffield et al., 1977).

Cuticular modifications

Phillpott (1925), in the first publication to recognise the sternum V gland as such, described a 'reticulated area' around the gland opening in *Hydrobiosella stenocera* Tillyard (Philopotamidae). Eltringham (1934) showed that the lateral

filaments (also called projections or protuberances) in *Diplectrona* (Hydropsychidae) and *Polycentropus* (Polycentropodidae) are associated with the sternum V gland, and described the 'reticulated cuticle' of the filament in *Diplectrona*. Razowski (1975) suggested that cuticular modifications associated with the gland might facilitate dispersion of gland products by providing an enlarged evaporative surface.

Nielsen (1980) was the first to study the sternum V gland in a larger number of species, and he described several types of cuticular modifications: 1) a protuberance with a conical basal part and finger-like distal part with the gland opening at the apex in females of Polycentropodidae; 2) a small protuberance with long setae in females of Hydroptilidae; 3) hexagonal cuticle covering sternum V in females of Apataniidae and Limnephilidae; and 4) a complete or partial sclerotized ridge which extends from the antecosta and around the gland opening in females of Beraeidae, Brachycentridae and Sericostomatidae. Ansteeg (1989) documented that the protuberance in Polycentropodidae had a groove, and that the gland opening was very close by, and thus suggested that the protuberance was used for the dispersal of gland products.

Ivanov and Melnitsky (1999) described the gland opening in polycentropodids as being at the base of the protuberance, contrary to Nielsen (1980). Ivanov and Melnitsky (2002) reported that there is varied development of protuberances associated with the gland opening in Annulipalpia and Spicipalpia, and that an absence of protuberances is a characteristic of Integripalpia.

Gland opening

Based on SEM studies of representatives of Beraeidae, Hydroptilidae, Limnephilidae, Polycentropodidae and Stenopsychidae, Ivanov and Melnitsky (2002) concluded that the sternum V gland opening is a perforated membrane. However, Hashimoto and Kobayashi (2009), likewise using SEM, reported that it is slit-like, as has also been reported in Lepidoptera (Kristensen & Nielsen, 1979; Kristensen, 1984; Nielsen & Kristensen, 1996).

Opening muscles

Nielsen (1980) reported the presence of gland-opening muscles[1]: one type in Hydroptilidae and a completely different type in Limnephilidae. In Hydroptilidae, it originates on the antecosta of sternum VI and inserts on the posterior side of the gland duct. In Limnephilidae, it originates on the antecosta of sternum V, proceeds laterally and inserts on the gland duct. Resh and Wood (1985) reported that a pair of muscles insert on the gland duct in *Gumaga nigricula* (McLachlan) (Sericostomatidae), and presumed that they controlled discharge from the reservoir; they did not report the point of origin. Ansteeg (1989) observed muscle fibres inserting on the gland duct in *Ecnomus tenellus* (Rambur) (Ecnomidae) and various limnephilids, but his preparations did not show the point of origin. Hashimoto and Kobayashi (2009) found a mass of muscle fibres inserting on the gland duct just inside the gland opening in *Eubasilissa regina* (McLachlan) (Phryganeidae). Their preparation did not show the point of origin, but they presumed the muscle to be homologous to the gland-opening muscle (which originates on the front edge of sternum VI) described by Kristensen (1984) in a micropterigid (Lepidoptera).

Gland reservoir: shape and musculature

The internal cuticular structures of the gland basically consist of a duct leading to a reservoir. Based on the shape of these structures, Ivanov and Melnitsky (1999, 2002) defined four types of the sternum V gland: 1) ampulliform glands: reservoir elongate, smooth transition to duct; 2) sacculous glands: reservoir near spherical, duct highly reduced; 3) kidney-shaped glands: reservoir reniform, duct connects centrally; 4) window or fenestral glands: reservoir and duct absent, secretory tissue directly below body wall. Type 1 is common in representatives of Annulipalpia and Spicipalpia, type 2 is common in representatives of Integripalpia, type 3 is present in representatives of Limnephilidae, Uenoidae and

[1] Nielsen (1980) clearly states that they function to open the gland duct (p. 89), even though he denoted them as 'obturator', closing, muscles.

Apataniidae and type 4 is present in some philopotamids (Ivanov & Melnitsky, 1999, 2002).

Ivanov and Melnitsky (2002) and Hashimoto and Kobayashi (2009) found muscle fibres surrounding the reservoir in representatives of Phryganeidae; additionally, Hashimoto and Kobayashi (2009) found the same in a stenopsychid. In both phryganeids and the stenopsychid, the muscle fibres occurred between the reservoir and the secretory cells (Ivanov & Melnitsky, 2002; Hashimoto & Kobayashi, 2009). Kristensen (1984) and Nielsen and Kristensen (1996) described muscle fibres surrounding the gland reservoir in representatives of Micropterigidae and Lophocoronidae (both Lepidoptera), but did not explicitly state whether they occurred between the reservoir and the secretory cells or surrounded both reservoir and secretory tissue.

Secretory tissue

Ivanov and Melnitsky (2002) showed the secretory cells as a layer surrounding the gland reservoir in all gland types except type 4. Hashimoto and Kobayashi (2009) found the secretory cells in *Stenopsyche marmorata* Navas (Stenopsychidae) to be grouped in nodules which then surround the reservoir, and the secretory cells in *Eubasilissa regina* (Phryganeidae) to be concentrated along the margins of the gland reservoir. In *Nemotaulius admorsus* (McLachlan) (Limnephilidae), the secretory tissue completely surrounds the gland reservoir (Hashimoto & Kobayashi, 2009). Eltringham (1931) described the secretory cells in *Agapetus fuscipes* Curtis, *A. cyrnensis* Mosely and *A. nimbulus* McLachlan (Glossosomatidae) as being organised in masses a little distance away from the reservoir with which they are connected by ductules. Eltringham (1934) described the secretory cells in *Diplectrona meridionalis* (Hagen) (Hydropsychidae) and *Polycentropus flavomaculatus* (Pictet) (Polycentropodidae) as organised in a reniform mass, separate from the reservoir with which they are connected by ductules. In *Ecclisopteryx guttulata* Pictet (Limnephilidae), Eltringham (1934) found that the secretory cells tightly surrounding the reservoir, without any ductules being visible. Ductules connecting to the gland reservoir were observed

by Ansteeg (1989) in representatives of Ecnomidae, Psychomyiidae, Polycentropodidae, Hydropsychidae, Rhyacophilidae, Glossosomatidae, Limnephilidae and Sericostomatidae. Based on these observations, Ansteeg (1989) concluded that the secretory cells of sternum V gland were type 3 as defined by Noirot and Quennedey (1974). Based on histological details, Hashimoto and Kobayashi (2009) reached the same conclusion. Melnitsky and Deev (2009) studied the ultrastructure of the secretory epithelium in *Rhyacophila obliterata* McLachlan (Rhyacophilidae) and *Chaetopteryx villosa* (Fabricius) (Limnephilidae), and described the secretory, ductule and epidermal cells, providing further details.

Potential source of characters

Features of the gland, especially the protuberances associated with the gland openings (lateral filaments in the literature), have been used as characters in keys (e.g. Mosely & Kimmins, 1953), and Schefter (1996, 2005) used presence/absence of protuberances as a character in reconstructing the phylogenies of Hydropsychidae and Hydropsychinae. Ansteeg (1989) suggested using presence/absence of the sternum V gland as a criterion for placing integripalpians in either Plenitentoria (gland present) or Brevitentoria (gland absent). Ivanov and Melnitsky (2002) suggested reniform glands as a synapomorphy for Limnephilidae, Uenoidae and Apataniidae. However, the potential of the sternum V gland as a source of phylogenetic characters has not been thoroughly investigated.

Present study

In the present study, I describe the morphological variation of the sternum V gland in Trichoptera, with a special focus on soft part morphology and cuticular microstructures. I investigate whether the cuticular structures associated with the gland opening are consistent with Razowski's (1975) idea that they should facilitate evaporation of gland products. I examine the gland opening to clarify whether it is a slit or a porous membrane. I survey the occurrence of both gland-

opening muscles and reservoir musculature. I investigate whether Ivanov and Melnitsky's (1999, 2002) gland types cover the variation in gland shapes in Trichoptera and whether I can confirm the distribution of gland types. I examine the organization of secretory cells to asses the variation in and distribution of types of organization of secretory cells. Finally, I map gland traits phylogenetically to identify characters that provide useful phylogenetic information at different levels and in different groups.

MATERIALS AND METHODS

Taxon sampling

I examined specimens of 88 species of Trichoptera in 38 families: all annulipalpian families, all spicipalpian families and all but nine integripalpian families. A list of all Trichopteran species examined is shown in Table 2-1.

Preparation

Most species were examined using both wholemounts and external SEMs. For some species I also used histology and/or internal SEMs. Generally, one specimen of each sex was used for each preparation. In cases in which a preparation had been of insufficient quality, I made replacement preparations if there were a sufficient number of specimens available. Results from different preparations as well as possible replacement preparations were checked against each other for individual differences. In general, individual differences were not found; however, it must be noted that in several cases all the studied specimens of a species were from a single population as possible differences between populations were not part of this study. Table 2-1 shows what preparations were used for representatives of each species.

I prepared wholemounts of the ventral mid-abdomen, lightly stained with dilute (in 70% ethanol) chlorazol black. Specimens were cleared in cedar oil and mounted in Canada balsam. Slides were studied using both brightfield and polarized light microscopy.

Specimens for external SEM were critical-point dried, mounted on SEM stubs and coated with gold. Most specimens for internal SEM were partially cleared in 10% KOH, and otherwise treated as specimens for external SEM. Internal SEMs for two species, *Phryganea cinerea* male and *Limnephilus harrimani* female were prepared from fresh specimens (killed just prior to dissection) dissected in 0.9% saline solution, otherwise they were treated as other SEM specimens.

Specimens for histology were fixed in 70-80% ethanol, the abdomen (for large animals where sternite V is more than ca. 0.7 mm long, only the ventral mid-abdomen) embedded in paraffin wax, sectioned at 8 μm, stained with Harris' hematoxylin and acidified eosin or Masson's trichrome stain (Harris' hematoxylin, Ponceau-acid fuchsin and acetic aniline blue) and mounted with DPX resin.

Drawings of wholemounts and histological sections were made using a camera lucida. Details of the musculature in wholemounts were observed using polarized light and added to the camera lucida drawings. Inked drawings were based on the camera lucida drawings, and, when necessary, further observations of the specimen. SEMs were consulted when drawing external cuticular structures (e.g. details of scaly structures around the gland opening). Inked drawings were photocopied and scanned (photocopying before scanning resulted in a crisper scan). Any remaining 'fuzziness' of the lines was manually removed in Adobe Photoshop®. Muscle fibres were shaded and cuticular ridges were crosshatched in Adobe Photoshop.

Character mapping

The phylogeny I used for mapping characters of the gland was largely based on the phylogeny produced by Holzenthal et al. (2007a), which is the most recent comprehensive trichopteran phylogeny. The phylogeny was adjusted with respect to: 1) the relationship between subfamilies of Hydropsychidae, here I followed Geraci (2007) who explored this question with a denser taxon sampling than Holzenthal et al. (2007a); 2) the placement of *Diplectrona zealandensis*

(Hydropsychidae, Diplectroninae) (not included by Holzenthal et al. (2007a)), here I followed Schefter (1996) and Geraci (2007) and placed *D. zealandensis* as sister to *Asmicridea* + *Smicrophylax* (Hydropsychidae, Smicrideinae). Otherwise, if I had no information to the contrary (such as placement of *D. zealandensis*), I presumed that taxonomic groups were monophyletic. Figure 2-1 shows the relationships between families in which the presence/absence of the sternum V gland has been examined in one or more species.

I constructed the character matrix and traced the characters using Mesquite 2.6 (build 486) (Maddison & Maddison, 2009). The scorings are based solely on my own observations. All characters were treated as unordered and ancestral states were reconstructed using parsimony. If two or more equally parsimonious reconstructions were possible, I chose to minimize the number of gains of a character. As this is not a phylogenetic analysis, but an exploration of potential characters, the same trait may be scored as more than one character, e.g. characters 1-3. The tree and character matrix used for mapping are included in Appendix 2.

The characters I traced were:

1) Presence/absence of gland: 0: absence; 1: presence (in either or both sexes)

2) Presence/absence of gland in females: 0: absence; 1: presence

3) Presence/absence of gland in males: 0: absence; 1: presence

4) Cuticular modifications with grooves demarcating borders between individual epidermal cells, binary: 0: absence; 1: presence

5) Cuticular modifications with grooves demarcating borders between individual epidermal cells, multistate: 0: absence; 1: scaly patch (e.g. Figures 2-2 B-E; 2-18 A); 2: polygons on protuberance (Figure 2-20); 3: hexagonal cuticle (e.g. Figure 2-23 A, B)

6) Bald area extending from front edge of sternite and encompassing gland opening: 0: absence; 1: presence (Figure 2-24 F-H)

7) Internal cuticular ridges extending from antecosta on both sides of gland opening and connecting in a smooth curve posterior to gland opening: 0: absence; 1: presence (Figures 2-15; 2-16; 2-26 G)

8) Presence/absence of protuberance: 0: absence; 1: presence (in either or both sexes), please refer to Figure 2-4 E for definition for protuberance versus bulge

9) Presence/absence of protuberance in females: 0: absence; 1: presence

10) Presence/absence of protuberance in males: 0: absence; 1: presence

11) Protuberance with groove extending at least from gland opening to apex with wavy cuticle along groove: 0: absence; 1: presence (Figure 2-18 E-H)

12) Protuberance with groove extending at least from gland opening to apex, protuberance covered with polygons separated by grooves: 0: absence; 1: presence (Figure 2-20)

13) Protuberance/bulge with setae: 0: absence; 1: presence (Figures 2-21 E, F; 2-22 A, C-E)

14) Short protuberance with very long setae: 0: absence; 1: presence (Figure 2-22 C)

15) Opening muscles: 0: absent while gland present (Figure 2-5 B, D, E); 1: present, originating on anterior edge of sternum VI (Figure 2-9 F); 2: present, originating mesad on cuticle of sternum V (e.g. Figure 2-13)

16) Gland reservoir shape: 0: none of the following (e.g. Figures 2-15 A; 2-26 F); 1: reniform (Figures 2-13 A; 2-26 C); 2: periform (e.g. Figures 2-2 A; 2-25 A); 3: ovoid or round (Figure 2-5 B); 4: elongate and compartmentalised (Figure 2-14 B)

17) Perforated patches on sternum IV in females (Figures 2-17 C, D; 2-18 C, D): 0: absent; 1: present and associated sternum V gland reservoir; 2: present and associated with separate fenestral glands (Figure 2-3 C)

18) Gland reservoir musculature: 0: absent (not observed) (e.g. Figure 2-13); 1: present (e.g. Figure 2-2)

19) Parallel gland reservoir musculature fibres: 0: absent; 1: present

20) Arrangement of secretory cells: 0: immediately adjacent to reservoir (e.g. Figure 2-8); 1: separate from reservoir (Figure 2-7)

Reconstruction of ancestral states depends heavily on the topology of the tree. I have chosen to mainly use the phylogeny produced by Holzenthal et al. (2007a) for the reasons stated above. Using a different tree would in some cases result in different reconstructions of ancestral states and thus possibly different conclusions about which characters could be useful at a specific level. However, most of the trichopteran families are regarded as good monophyletic groups, as are the two suborders Annulipalpia and Integripalpia (Morse, 1997; Holzenthal et al., 2007b). Other good groups are the two integripalpian infraorders, Plenitentoria and Brevitentoria, the superfamilies Psychomyioidea and Sericostomatoidea, and the group of families denoted Limnephilidae sensu lato (Frania & Wiggins, 1997; Morse, 1997; Ivanov, 2002; Holzenthal et al., 2007a, b). In general, my conclusions about which characters could be useful at which levels should remain reasonable, even if a slightly different tree is used.

OBSERVATIONS AND RESULTS

Presence/absence of glands

I found the gland to be present in representatives of 25 of the 38 included families. The gland is present in representatives of all families of Annulipalpia except Dipseudopsidae, and is generally present in both sexes (only females available for Stenopsychidae). The gland is present in representatives of all families of Spicipalpia, and is generally present in both sexes.

I studied representatives of ten families in Integripalpia – Plenitentoria. The gland is present in representatives of six families: Brachycentridae, Phryganeidae, Oeconesidae, Uenoidae, Apataniidae and Limnephilidae; and the gland is generally present in both sexes (only males available for Oeconesidae). The gland is absent in representatives of Plectrotarsidae (only males available), Kokiriidae (only males available), Lepidostomatidae and Goeridae.

I studied representatives of sixteen families in Integripalpia – Brevitentoria, and found the gland in representatives of eight families, although only in females in six of these. The gland is present in representatives of Limnocentropodidae (only females available), Molannidae (only in females), Beraeidae (only females available), Sericostomatidae, Conoesucidae (only in females), Helicophidae (only in females), Anomalopsychidae (only in females) and Chathamiidae (certainly present in females, possibly in males as well, see Discussion: Distribution of glands). The gland is absent in representatives of Tasimiidae, Philorheithridae, Atriplectididae, Odontoceridae, Calamoceratidae, Leptoceridae, Helicopsychidae and Calocidae (only males available of the latter).

A complete list of presence/absence of the gland for all Trichoptera species studied is available in Table 2-1. Figure 2-1 places the families in which the presence/absence of the sternum V gland has been examined in one or more species (present study plus data from the literature) in a phylogenetic context.

Cuticular modifications

Annulipalpia

A patch of scaly cuticle surrounding the gland opening (Figures 2-2 B-E; 2-3 A, B; 2-4 C, D; 2-5 D, E; 2-17 A, F; 2-18 A) is found in several annulipalpians: representatives of *Dolophilodes* and *Wormaldia* (both Philopotamidae), *Ecnomus tenellus* (Ecnomidae), *Xiphocentron haitiense* (Xiphocentronidae), *Lype diversa* and *Tinodes sigodanus* (both Psychomyiidae). The size of the individual 'scales' strongly suggests that each 'scale' is produced by one epidermal cell, with the grooves between the 'scales' demarcating the border between individual epidermal cells.

A protuberance with a conical or triangular base and slender distal part (Figures 2-5 A; 2-18 E; 2-19 A) is found in *Cyrnellus fraternus* and *Polycentropus cinereus* (both Polycentropodidae) with the gland opening at the base of the slender distal part. A groove with wavy sides runs from the gland opening to the apex in both, and also from the opening along the side of the base in *P. cinereus*.

A thumb-shaped protuberance is found in *Stenopsychodes mjoebergi* (Stenopsychidae) (Figure 2-2 A) and *Arctopsyche grandis* (Hydropsychidae: Arctopsychinae) (Figures 2-6 A, B; 2-19 B). A smaller protuberance is found in *Parapsyche elsis* (Hydropsychidae: Arctopsychinae) (Figure 2-19 C).

A protuberance with a bulbous base and a long slender distal part is found in *Diplectrona* sp. (Hydropsychidae: Diplectroninae) (Figures 2-7 A; 2-20 A), *Asmicridea edwardsi* (Figures 2-7 D; 2-20 D) and *Smicrophylax* sp. (Hydropsychidae: Smicrideinae). The gland opening is at the base of the distal part (Figure 2-20 A, B, D, E) and a groove runs from the opening to the apex which is densely covered with microtrichia (Figure 2-20 C, F). The entire protuberance is covered with a network of very small grooves, demarcating the borders between individual epidermal cells. *Diplectrona zealandensis* has a similar protuberance, except for lacking the basal bulb (Figure 2-7 C).

The gland opening in *Leptonema albovirens* (Hydropsychidae: Macronematinae) is placed at one end of a large ovoid area of ridged cuticle that

resembles a thumb print (Figures 2-6 C, D; 2-19 D-F). The ovoid area is much larger in the male.

In representatives of Hydropsychinae, the modifications associated with the gland opening range from a slightly raised bald area (Figure 2-19 G) over a bulge (Figure 2-19 H) to an elongate protuberance (Figure 2-8 E). Apart from representatives of *Cheumatopsyche*, the gland opening is also situated in a bald patch in *Chimarra aterrima, C. obscura* (Philopotamidae) (Figure 2-17 B) and *Austrotinodes panamensis* (Ecnomidae) (Figure 2-17 E).

Spicipalpia

The gland opening is situated at the end of a groove that leads to a ventral protuberance in female *Apsilochorema segitiga* (Hydrobiosidae) (Figure 2-21 A). Female *Atopsyche callosa* (Hydrobiosidae) also have a ventral protuberance on sternum V and the gland opening at the end of a groove, but the groove does not extend to the base of the protuberance. Male *A. callosa* have the gland opening at the apex of a protuberance (Figure 2-21 D). Female *Cailloma pumida* have the gland opening in a raised bald area.

The gland opening in the investigated representatives of Rhyacophilidae is situated on a bulge/protuberance devoid of microtrichia, but with some stout setae (Figure 2-21 E, F).

In male *Agapetus walkeri* (Glossosomatidae) the gland opening is situated inside a large involuted cuticular sac (Figure 2-10 D). The gland-opening muscle inserts on the sac, indicating that the sac is at least partially derived from the gland duct. In female *A. walkeri*, the gland opening is situated in a groove (Figure 2-10 C). Both male and female *Anagapetus debilis* (Glossosomatidae) have the gland opening situated on a triangular protuberance with reticulated cuticle (Figure 2-22 A). The reticulation consists of raised walls (Figure 2-22 B), not restricted to the borders between individual epidermal cells, and so is quite different from the scaly cuticle found in many annulipalpians. Female *Protoptila cana* (Glossosomatidae) have the gland opening situated in a triangular bald area,

with the posterior edge of the triangle defined by a transverse groove (Figure 2-22 C).

In Hydroptilinae sp. (Hydroptilidae) the gland opening is situated on a cuticular protuberance fitted with an enlarged three-flanged seta and two smaller setae that might be mechanoreceptors (Figure 2-22 E, F). All three setae are clearly different in both size and structure from the surrounding setae. In *Agraylea multipunctata* (Hydroptilidae: Hydroptilinae), a somewhat similar arrangement is found, the protuberance carries three setae, but apart from being elongated, they are not structurally different from those that cover the body. *Palaeagapetus guppyi* (Hydroptilidae: Ptilocolepinae) have the gland opening situated on a flat bald protuberance, the posterior edge of which continues as a transverse groove (Figure 2-22 D).

Integripalpia - Plenitentoria

A hexagonal pattern covering the entire sternum V with grooves between the hexagons (Figure 2-23 A) and centred on the gland opening is found in *Brachycentrus occidentalis* (Brachycentridae) (Figure 2-23 B), *Apatania zonella* (Apataniidae) (Figure 2-24 A), *Drusus annulatus* (Limnephilidae: Drusinae) and *Pseudostenophylax sparsus* (Limnephilidae: Pseudostenophylaxinae) (Figure 2-24 C). A similar pattern, but not covering the entire sternum V is found in *Hesperophylax* sp. (Limnephilidae: Limnephilinae). Several other limnephilines (*Anabolia bimaculata*, *Limnephilus externus*, *L. secludens* and *L. sericeus*) show a tendency towards hexagonal cuticle around the gland opening (Figure 2-24 D), but lack the grooves between the hexagons.

Any kind of protuberance is only found in *Oeconesus maori* (Oeconesidae) in which it is a bald thumb-shaped protuberance (Figure 2-23 E), and in *Pycnopsyche lepida*, in which it is a largely bald bulge (Figure 2-24 E), and *P. scabripennis* male (Limnephilidae: Limnephilinae).

Onocosmoecus unicolor (Limnephilidae: Dicosmoecinae) has both a bald area and some grooved, sculpted (but not hexagonal) cuticle associated with the gland opening (Figure 2-24 B).

The gland opening is situated in a bald area in all investigated phryganeids (Figure 2-23 C, D) and in *Neophylax concinnus* (Uenoidae) (Figure 2-23 F).

Integripalpia - Brevitentoria

When the sternum V gland is present, it is situated in a bald area that extends from the front edge of sternum V (Figure 2-24 F-H) in all studied species. A sclerotized ridge that extends posteriad from the antecosta on both sides of the gland opening loops around the gland opening (Figures 2-15; 2-16; 2-26 G).

Gland opening and duct

The gland opening is slit-like (e.g. Figure 2-24) in all investigated species; and in all species except *Psychomyia flavida* (Psychomyidae) it is situated on the anterior half of sternum V. In females of *P. flavida* (males not available), the gland opening is situated in the membranous cuticle between sternum IV and V (Figures 2-18 B; 2-25 E). The gland opening is curved, varying from a deep U-shaped curve (e.g. in *Diplectrona* sp. (Hydropsychidae)) (Figure 2-20 B) to a slight crescent found in some limnephilids (Figure 2-24 C). Again, *P. flavida* is the exception with a straight slit. The curve from the gland opening is continued in the U-shape of the gland duct (in cross section) just inside the gland opening (Figures 2-25 B; 2-26 D). The gland duct generally follows an approximately straight line or gentle curve between the opening and the reservoir. The exception is found in female hydrobiosids in which the gland duct proceeds anteriad from the gland opening, goes through a sharp bend and proceed anteriad before it widens into the gland reservoir (Figure 2-9 A, B, D).

Opening muscles

I found opening muscles to be nearly ubiquitous. Apart from absences likely due to artefacts, e.g. decomposition (see Table 2-1), a type of opening muscle is always present when the sternum V gland is present, except in representatives of Psychomyiidae. Two types of opening muscles are found. One type originates on the anterior edge of sternum VI and is found in Philopotamidae (Figures 2-2 B-D;

2-3 B, C), Rhyacophilidae (Figure 2-9 E, F), Glossosomatidae (Figure 2-10 A, B, D, E) and Hydroptilidae (Figure 2-11 A, B). Another type originates on the cuticle of sternum V, mesad of the gland opening (e.g. Figure 2-13), and is found in all other trichopterans with the sternum V gland, except Psychomyiidae. The second type is also found in *Wormaldia arizonensis* (Philopotamidae), the only species found to possess both types of opening muscle (Figure 2-2 B, C). As the first type is also found in Lepidoptera, I named it Lepidoptera-type. The second type is only found in Trichoptera; hence I named it Trichoptera-type. Both types of opening muscles insert on the gland duct just inside the gland opening, on the inside curve of the U-shaped gland duct.

The Trichoptera-type generally consists of 1-4 fan-shaped bundles with the base of the fan inserting on the gland duct (2-13). In some cases with only a single bundle, the bundle was elongate instead of fan-shaped. This configuration was found in *W. arizonensis*, and some hydropsychids: *Asmicridea edwardsii* (Figure 2-7 D) and *Smicrophylax* sp. A variation on this theme was found in *Diplectrona* sp. (Hydropsychidae) in which the opening muscle is entirely contained within the protuberance associated with the gland opening (Figure 2-7 A).

Gland reservoir: shape and musculature

Many annulipalpians have a periform reservoir with a smooth connection with the gland duct. At one extreme is, e.g., female *Chimarra obscura* (Figure 2-3 C) (Philopotamidae) in which duct and reservoir is completely contiguous, the other end of the spectrum is, e.g., female *Tinodes sigodanus* (Psychomyiidae) (Figure 2-5 D), in which there is a distinct duct, and a distinct reservoir, but the connection between the two is smooth. The spindle-shaped reservoirs found in some hydropsychids (Figure 2-8 C) can be viewed as a slender periform reservoir with a pointed apex. Reservoirs that are not periform or spindle-shaped have a more sharply demarcated border between the reservoir and the gland duct.

In addition to normally developed sternum V glands, most female philopotamids also have reservoirs and secretory cells associated with the

fenestrae, two areas of transparent cuticle on sternum IV (female *Wormaldia planae* do not have fenestrae) (Figure 2-3 C, D). SEM showed that the fenestrae were perforated (Figure 2-17 C, D), and also revealed perforated cuticle associated with the gland reservoirs in female Psychomyiidae (Figures 2-18 C, D; 2-25 E).

Representatives of Rhyacophilidae, Glossosomatidae and Hydroptilidae have elongate reservoirs, often with a quite smooth transition between the reservoir and the gland duct (Figures 2-9 E, F; 2-10 A-E; 2-11 A, B; 2-25 F). All examined female hydrobiosids have more ovoid reservoirs (Figure 2-9 A, B, D) and male *Atopsyche callosa* have a spindle-shaped reservoir with an abrupt connection to the gland duct (Figure 2-9 C).

Many integripalpians have ovoid reservoirs. Another common reservoir shape is reniform with the duct connecting in the middle of the inside curve (Figure 2-26 C). This is found in *Neophylax concinnus* (Uenoidae) (Figure 2-13 A, B), possibly female *Apatania zonella* (Apataniidae), most limnephilids (Figure 2-13 C, E-G, 2-26 C, E) and *Molanna flavicornis* (Molannidae) (Figure 2-15 B). Male *Drusus annulatus* (Limnephilidae) have a reniform reservoir, but the duct connects at one end (Figure 2-13 D).

Gland reservoir musculature is widespread. I found it in representatives of all annulipalpian families with the sternum V gland except Polycentropodidae (Figures 2-2; 2-3; 2-4 B, D; 2-5 B-D; 2-6 A, B, D; 2-7; 2-8 A, B, D, E), in representatives for all spicipalpian families (Figures 2-9 A-D, F; 2-10; 2-11 A, B), and in representatives for Phryganeidae (Figures 2-11 E, G; 2-12 C; 2-26 A, B), Oeconesidae (Figure 2-12 E), Sericostomatidae (Figure 2-15 D, E), Helicophidae (Figure 2-16 B), Chathamiidae (Figure 2-15 C) and Anomalopsychidae (Figure 2-15 D). It is notably absent in representatives of Limnephilidae (Figures 2-13 C-H; 2-14, A, B). Generally, the muscle fibres surround only the cuticular reservoir, but in some cases they surround both reservoir and secretory tissue. The latter is found in *Psychomyia flavida* (Psychomyiidae), *Arctopsyche grandis* (Hydropsychidae), *Anagapetus debilis* and *Protoptila cana*, but not *Agapetus walkeri* (all Glossosomatidae), Hydroptilinae sp. and *Agraylea multipunctata*, but

not *Palaeagapetus guppyi* (all Hydroptilidae), and *Austrocentrus griseus* (Helicophidae). An intermediate condition, in which some muscle fibres surround only the reservoir and other fibres surround both reservoir and secretory tissue, is found in several species: *Dolophilodes pallidipes*, *Wormaldia gabriella* female, *W. planae* (all Philopotamidae), *Parapsyche elsis*, *Cheumatopsyche campyla*, *C. speciosa*, *Hydropsyche cockerelli*, *H. confusa*, *H. occidentalis* male (all Hydropsychidae), *Agrypnia straminea*, *Yphria californica* male (both Phryganeidae), *Gumaga griseola* (Sericostomatidae), *Philanisus plebeius* (Chathamiidae). A brief description of the musculature associated with the gland reservoir is given for each species in Table 2-1.

Secretory tissue

The most widespread arrangement of the secretory cells is as a layer around the gland reservoir (e.g., Figure 2-13) and sometimes the secretory cells are grouped in lobes or nodules around the reservoir (e.g., Figures 2-15 E; 2-16 D). Notable exceptions are: *Polycentrus cinereus* (Polycentropodidae) (Figure 2-5 A) and *Diplectrona* sp. (Hydropsychidae: Diplectroninae) (Figure 2-7 A, B), in which the secretory cells are grouped in a loose conglomerate adjacent to the reservoir. *Diplectrona zealandensis* (Figure 2-7 C) (Hydropsychidae: Diplectroninae) *Asmicridea edwardsii* (Figure 2-7 D, E) and *Smicrophylax* sp. (Hydropsychidae: Smicrideinae) in which the secretory cells are grouped in a rounded conglomerate separate from the reservoir. Female *Agapetus walkeri* (Figure 2-10 C) (Glossosomatidae) in which the secretory cells are loosely arranged around the reservoir (secretory cells not visible in male). In all the species mentioned above, except *P. cinereus*, the ductules that connect the secretory cells to the reservoir were visible in wholemount preparations.

Internal SEMs of specimens cleared in KOH showed cuticular ductules that connect to the gland reservoir (Figure 2-25 C) in *Psychomyia flavida* (Psychomyiidae), *Arctopsyche grandis* (Hydropsychidae), *Anagapetus debilis* (Glossosomatidae), *Brachycentrus occidentalis* (Brachycentridae), *Onocosmoecus unicolor* (Limnephilidae) and *Gumaga griseola* (Sericostomatidae). Furthermore,

the same treatment showed ductules that connect to the fenestral reservoir in *Chimarra obscura* (Philopotamidae). The ductules are 0.25-0.6 μm in diameter and widen at the apex. The widening can have different configurations: it is funnel-shaped in *A. grandis* (Figure 2-25 D), but is a cluster of even tinier ductules in *G. griseola* (Figure 2-26 H).

Character mapping

Character 1 (presence/absence of gland) distinguishes the dipseudopsid species (gland absent) from other annulipalpians, except *Philopotamus montanus* (Philopotamidae). Absence of gland in *Goera calcarata* (Goeridae) distinguishes it from other limnephilids sensu lato.

Comparison of characters 2 (presence/absence in females) and 3 (presence/absence in males) illustrates that in representatives of Brevitentoria, glands are often only present in females. Character 3 distingushes *Gumaga griseola* (Sericostomatidae) (glands present in males) from other brevitentorians, although male glands are possibly present in *Philanismus plebeius* (Chathamiidae), and I did not have any limnocentropodid males available for examination.

Character 4 shows that grooves between individual epidermal cells occur in species throughout Annulipalpia and Plenitentoria (Figure 2-27). However, there are three distinct forms of the pattern and character 5 illustrates this. A scaly patch is characteristic for philopotamids with reversal in *Chimarra* (not applicable in *P. montanus* as the gland is absent). It is also characteristic for representatives of Xiphocentronidae + Psychomyiidae (not present in *Psychomyia flavida* in which the gland opening is situated in the membranous cuticle between sternum IV and V), but it is paralleled in *Ecnomus tenellus* (Ecnomidae). The polygons on the protuberance are only found in representatives of Diplectroninae and Smicrideinae (both Hydropsychidae), and the hexagonal pattern is only found in representatives of Apataniidae + Limnephilidae and in *Brachycentrus occidentalis* (Brachycentridae). However, many limnephilids do not have sternum V covered with the hexagonal pattern.

Character 6 (bald area from front edge of sternite) is characteristic of all brevitentorians that possess the sternum V gland. However, it is also present in *Austrotinodes panamensis* (Ecnomidae) and *Oeconesus maori* (Oeconesidae) (Figure 2-27).

Character 7 (internal ridges extending from antecosta and connecting posterior to gland opening) is, together with character 6, characteristic of all brevitentorians that possess the sternum V gland (Figure 2-27). However, ridges extending from the antecosta on one or both sides of the gland opening, but not connecting, are present in female, but not male *Brachycentrus occidentalis* (Brachycentridae) (Figure 2-11 C, D), some phryganeids (Figures 2-11 E, F; 2-12 A, B), in *Oeconesus maori* (Oeconesidae) (Figure 2-12 D) and in *Austrotinodes panamensis* (Ecnomidae) (Figure 2-4 A, B).

Character 8 illustrates that some form of protuberance occurs in species scattered throughout Trichoptera, except in representatives of Brevitentoria (Figure 2-27). Comparing characters 9 and 10, the presence/absence of protuberance in females and males, respectively, reveals that protuberances are more common in males. However, there are several different configurations of protuberances, e.g., character 11-14.

Character 11 (protuberance with groove with wavy sides) is unique to representatives of Polycentropodidae. Character 12 (protuberance with groove and polygons) is restricted to representatives of Dipletroninae and Smicrideinae (both Hydropsychidae). A protuberance or bulge with setae (character 13) only occurs in representatives of Rhyacophilidae, Glossosomatidae and Hydroptilidae, and a short protuberance with very long setae (character 14) only in representatives of Hydroptilinae.

Character 15 illustrates how widespread opening muscles are (Figure 2-27). Lack of opening muscles with gland present is unique to psychomyiids. The character also illustrates the disjunct distribution of the two types of opening muscles. The opening muscles that originate on the anterior edge of sternum VI are found in representatives of Philopotamidae, a unique condition in Annulipalpia, and in representatives of three families in the spicipalpian grade:

Rhyacophilidae, Glossosomatidae and Hydroptilidae, but not in representatives of Hydrobiosidae.

Character 16 reveals that reniform reservoirs are only found in representatives of Integripalpia, and that reniform is the most common reservoir shape in representatives of Limnephilidae sensu lato (Figure 2-27). Periform reservoirs occur in representatives of Annulipalpia and Spicipalpia. Ovoid or round reservoirs occur in species throughout Trichoptera, but are more common in representatives of Spicipalpia and Integripalpia. Elongate and compartmentalised reservoirs (Figure 2-14 B) are restricted to representatives of *Pycnopsyche* (Limnephilidae), but are not present in female *P. lepida* (Figure 2-14 A). There are also some reservoirs in species throughout Trichoptera that do not fit in any of these shape categories.

Character 17 shows that patches of perforated cuticle on sternum IV in females (Figures 2-17 C, D; 2-18 C, D) occur in two families: associated with separate fenestral glands in philopotamids (Figure 2-3 C, D) with a loss in *Wormaldia planae*, and with the regular gland reservoir in psychomyiids (Figures 2-5 C; 2-25 E; 2-27).

Character 18 illustrates both the widespread presence of gland reservoir musculature, and that it is not found in representatives of Limnephilidae sensu lato (Figure 2-27). However, there are several other taxa in which I did not observe any muscle fibres associated with the gland reservoir. The muscle fibres around the reservoir are in a few cases parallel to each other (Character 19). This occurs in a few single species, but also distinguishes representatives of Smicrideinae from other hydropsychids, including *Diplectrona zealandensis*, and it occurs in all the examined hydrobiosids.

Generally the secretory tissue is closely associated with the reservoir, but in a few cases it is separate from the reservoir (character 20). The latter arrangement in is found in *Polycentropus cinereus* (Polycentropodidae) (data not available for *Cyrnellus fraternus*), in representatives of Diplectroninae and Smicrideinae (both Hydropsychidae) as well as in *Agapetus walkeri* (Glossosomatidae).

DISCUSSION

Distribution of glands

I investigated representatives of 38 trichopteran families and found the sternum V gland in representatives of 25 of those. Prior to this study, presence of the gland had not been investigated in representatives of Dipseudopsidae, Xiphocentronidae (both Annulipalpia), Plectrotarsidae (Integripalpia – Plenitentoria), Limnocentropodidae, Tasimiidae, Atriplectididae, Helicopsychidae, Anomalopsychidae and not in females of Calamoceratidae (all Integripalpia – Brevitentoria). Combined with the results of Ivanov and Melnitsky (2002) the sternum V gland is now known to be present in representatives of 28 families, and presence or absence of the gland have been investigated in representatives of 40 out of 45 families. These families and the presence/absence data are placed in a phylogenetic context in Figure 2-1.

In general, my observations regarding presence/absence of the sternum V gland agree with those of Ivanov and Melnitsky (1999, 2002). Exceptions are discussed below.

Ivanov and Melnitsky (1999) did not find the sternum V gland in any of the males of *Dolophilodes*, *Philopotamus* or *Wormaldia* (all Philopotamidae) they studied, although they did note the presence of 'callus' on sternum V. I confirm the absence of sternum V glands in male *P. montanus*, but clearly demonstrate that the gland is present in males of *Dolophilodes* (two species) and *Wormaldia* (four species). Furthermore, I found that the gland opening is associated with a patch of scaly cuticle (Figures 2-2 B-E; 2-3 A, B; 2-17 A), often denoted 'callus' in the literature. I studied different species two genera than did Ivanov and Melnitsky (1999). However, I conclude that sternum V glands are most likely present in the male *Dolophilodes* and *Wormaldia* studied by Ivanov and Melnitsky, and that the observed 'calli' mark the gland openings.

The sternum V gland is generally present in both sexes in Hydrobiosidae (present study; Ivanov & Melnitsky, 1999, 2002), but absent in male *Cailloma pumida*.

I found evidence that the sternum V gland might be present in male *Philanisus plebeius* (Chathamiidae), although the abdomen of the only specimen available had unfortunately been cleared in KOH. Brightfield microscopy revealed a structure that resembles a gland sac, and SEM showed a bald area that extends from the front edge of the sternum (associated with the gland opening in other brevitentorians). Unfortunately, the posterior edge of sternum IV partially obscured the critical area, so the presence or absence of a gland opening could not be confirmed. Ivanov and Melnitsky (2002) stated that the gland is absent in a male Chathamiidae, but did not indicate in which species.

The known distribution of the gland is placed in a phylogenetic context in Figure 2-1. Although the gland has apparently been lost many times, no clade above the family level is characterized by a complete absence of the sternum V gland. However, higher clades do appear to differ in the tendency to lose the sternum V gland. In Annulipalpia, the gland is absent in representatives of Dipseudopsidae, otherwise it is only found to be absent sporadically (e.g. male *Philopotamus montanus* (Philopotamidae)). In Integripalpia, loss of the gland is more widespread, especially in Brevitentoria. In Brevitentoria, the gland is only present in representatives of nine of sixteen investigated families, and only in representatives of Sericostomatidae and possibly Chathamiidae is it present in both sexes (males of Limnocentropodidae not studied). Thus, in addition to a tendency to lose the sternum V gland completely, there is a strong tendency to lose it in males only.

Cuticular modifications

Differences to earlier studies

The present study found that the gland opening in polycentropodids is neither at the apex of the protuberance as suggested by Nielsen (1980), nor at the base as suggested by Ivanov and Melnitsky (1999). Instead the gland opening is at the base of the apical part of the protuberance. The reason for Nielsen (1980) to suggest that the opening was at the apex of the protuberance might be that the groove running from the gland orifice to the apex looks quite similar to the gland

duct when studied under a light microscope. Ivanov and Melnitsky (2002, figures 3 and 5) show the gland opening in *Holocentropus* is in the same position as in *Polycentropus* and *Cyrnellus* in the present study, but the authors still state that the gland opening is at the base of the protuberance. A possible explanation is that Ivanov and Melnitsky (1999, 2002) only regard the apical part of the protuberance as actually protruding. However, Figures 2-18 B and 2-19 A show very clearly that the basal, conical/triangular part of the protuberance protrudes from the body wall and that the gland opening is situated approximately equidistant from the apex and the base of the protuberance.

I demonstrated that the gland duct in *Agapetus walkeri* (Glossosomatidae) is expanded into a large involuted cuticular sac. The involuted sac was described by Eltringham (1931) in *A. fuscipes*, *A. cyrnensis* and *A. nimbulus*, but he did not observe that the gland duct is connected to the sac, and thus he concluded that its function was independent of the sternum V gland. Ansteeg (1989) observed that the gland duct opened into the involuted sac, but as did Eltringham (1931), he presumed that the sac was of tracheal origin.

Evaporative structures

Razowski (1975) suggested that cuticular structures associated with the gland opening facilitate evaporation of gland products. That does seem likely for several of the cuticular modifications found in this study.

The scaly patch associated with the gland opening in many annulipalpians (see Table 2-1) provides grooves between each 'scale' and capillary forces would pull gland products through the grooves.

The protuberance in *Polycentropus cinereus* (Polycentropodidae) have a groove that runs from the base to the tip of the protuberance with the gland opening situated about halfway. The gland opening is not directly in the groove, but contraction of the gland-opening muscle would both open the gland orifice and deepen the slight groove that connects the gland orifice (Figure 2-18 G) to the groove running the length of the protuberance. Released gland products would be pulled along the groove by capillary forces. The wavy structure of the cuticle

along the groove provides an enlarged surface area for evaporation and the troughs are deep enough to transport the gland product by capillary forces. Thus I agree with Ansteeg (1989) that the polycentropid type of protuberance likely functions in the dispersal of gland products. *Cyrnellus fraternus* (Polycentropodidae) have a similar structure, restricted to the distal part of the protuberance, which probably functions in the same way.

The protuberance found in smicridines and diplectronines (both Hydropsychidae) employ the functional elements of both the scaly patches (grooves delimiting individual epidermal cells) and the protuberance found in polycentropodids by having a longitudinal groove from the gland opening to the apex of the protuberance. The groove running from the gland opening to the tip would pull released gland product to the tip of the protuberance by capillary forces, and the smaller grooves between the epidermal cells would pull the gland product all around the protuberance. Furthermore, the tip of the protuberance is covered with microtrichia that give it a brush-like appearance and further increase the evaporative surface (Figure 2-20 C, F). *Diplectrona zealandensis* lacks the basal, bulbous, part of the protuberance found in *Diplectrona* sp. and the smicridines, but it has the groove leading from the gland opening to the tip of the protuberance and the smaller grooves, so it probably functions in much the same way.

Leptonema albovirens (Hydropsychidae, Macronematinae) has an area of grooved or ridged cuticle associated with the gland opening, which is not seen in any of the other species examined in this study. In the female, the area is not very large and likely to be pulled into the body when the gland-opening muscles are contracted. However, it does provide a slightly increased evaporative surface compared to completely smooth cuticle. Furthermore, as it is pulled into the body when the gland duct is opened, it becomes covered with gland product, and this enlarged gland product covered surface would be exposed just after the gland duct has closed. In the male, this area is much larger (ca. four times as long, thus area ca. sixteen times larger), and with grooves running from the gland opening it provides an effective evaporative structure.

Female *Apsilochorema segitiga* (Hydrobiosidae) have the gland opening situated at the end of a groove that leads to a ventral protuberance (Figure 2-21 A). Furthermore, the pattern of microtrichia around the gland opening is modified so that any liquid exuded from the gland opening would be directed towards and along the groove (Figure 2-21 B). Once gland secretions have reached the groove, gravity might also play a role. The tip of the ventral protuberance is furnished with stout setae that are, at least some of the time, dragged along the substrate as they show wear-marks (Figure 2-21 A, C). Thus, if gland products make it all the way to the protuberance, a pheromone trail might be laid down on the substrate. Indeed, behavioural observations by Ivanov (1993) indicate that some female Trichoptera do exactly that. The duration of a pheromone trail would depend upon the amount laid down and the volatility of the pheromone compounds. However, Ivanov (1993) described the male as following at most 5 cm behind the female, thus the trail would not have to last more than a few seconds.

In male *Agapetus walkeri* (Glossosomatidae), the gland duct, instead of opening to the outside, expands into a large involuted cuticular sac, thus gland secretion would be released between the folds of the sac, not directly to the outside. The sac resembles an eversible structure, and eversion would expose a large surface covered in gland products. The gland-opening muscle attach to the distal part of the sac (Figure 2-10 D), and would be able to pull the sac back after eversion. Ross (1956) used the involuted sac to define subgenus *Agapetus*, and based on the variations he saw, proposed that it originated as an invagination from the transverse groove. As the gland opening in female *A. walkeri* is situated in a transverse groove, it is entirely possible that the involution is in part derived from the transverse groove. However, the insertion of the gland-opening muscle on the distal part of the sac suggests that the involution is at least in part derived from the gland duct.

Anagapetus debilis (Glossosomatidae) have the gland opening situated on triangular protuberances with a network of tiny ridges. This does provide an enlarged surface area, but distribution of the gland products over it would require the animals to actively distribute the gland products. Duffield et al. (1977)

demonstrated that *Pycnopsyche scabripennis* (Limnephilidae) use their legs to distribute gland products over a larger area, but there have been no such observations in representatives of *Anagapetus*.

In both Hydroptilinae sp. and *Agraylea multipunctata* the gland opening is situated at the base of 1-3 very long setae; these likely provide a large evaporative surface.

The hexagonal pattern with grooves between the hexagons covering all or large parts of sternum V found in *Brachycentrus occidentalis* (Brachycentridae), *Apatania zonella* (Apataniidae), *Drusus annulatus* (Limnephilidae: Drusinae), *Pseudostenophylax sparsus* (Limnephilidae: Pseudostenophylaxinae) and *Hesperophylax* sp. (Limnephilidae: Limnephilinae) provides an enlarged surface area. The grooves are centred on the gland opening and thus released gland products would be pulled along the grooves by capillary forces.

Onocosmoecus unicolor (Limnephilidae, Dicosmoecinae) have the gland opening situated adjacent to a patch of sculptured cuticle with grooves and a dense cover of microtrichia. These combine to form an enlarged evaporative surface and gland products will be dispersed in the grooves by capillary forces.

Looking at the cuticular structures associated with the gland opening, there are a variety of structural modifications that would serve both to enlarge the surface area and to distribute the gland products over a large area. Some of these may have evolved for other purposes, but for some of the more elaborate structures (e.g. in polycentropodids, diplectronines, smicridines (the two latter Hydropsychidae) and male *Agapetus (Agapetus)*(Glossosomatidae)) dispersion of liquids, in this case gland products, seems the most likely function. Thus Razowski's (1975) hypothesis about the function of the cuticular modifications associated with the gland opening appears to be correct in many cases.

However, in contrast to the species described above, the gland opening in many other species is not visibly associated with structures to dispense pheromones. This is the case in all the species of Brevitentoria (Integripalpia) included in the present study: here the gland opening is situated in a bald patch of cuticle while the rest of sternum V is covered with microtrichia. In

brevitentorians, the gland is generally lost in males and often lost in both sexes, indicating that the sternum V gland is of decreased importance. This corroborates earlier suggestions that courtship behaviour has become simplified during trichopteran evolution (Solem & Petersson, 1987).

Gland opening and duct

I found the gland opening to be a slit in all investigated species. This agrees with Hashimoto and Kobayashi (2009) as well as observations in Lepidoptera (Kristensen & Nielsen, 1979; Kristensen, 1984; Nielsen & Kristensen, 1996; Chapter 3). No evidence was found for the perforations reported by Ivanov and Melnitsky (2002), even at magnifications at which the pores (1.5 μm in diameter) should have been impossible to overlook.

Opening muscles

Opening muscles are widespread in Trichoptera; they are always associated with the sternum V gland, except in psychomyiids. This was highly unexpected as gland-opening muscles had previously only been identified in representatives of Ecnomidae, Hydroptilidae, Limnephilidae, Phryganeidae and Sericostomatidae (Nielsen, 1980; Resh & Wood, 1985; Ansteeg, 1989; Hashimoto & Kobayashi, 2009). Eltringham (1934) did depict the gland-opening muscle in *Diplectrona meridionalis* (Hagen) (Hydropsychidae), but did not recognise it as a gland-opening muscle.

Furthermore, I confirmed Nielsen's (1980) finding that two completely different types of gland-opening muscles are present. The most widespread type originates on the cuticle of sternum V mesad of the gland opening, while the other, less common type, originates on the front edge of sternum VI. The latter is only found in representatives of Rhyacophilidae, Glossosomatidae, Hydroptilidae and Philopotamidae in Trichoptera. As it originates in the same position as the gland-opening muscle described in Lepidoptera (Kristensen, 1984; Nielsen & Kristensen, 1996; Chapters 3, 5) they are likely homologous. However, the gland-opening muscle found by Hashimoto and Kobayashi (2009) in *Eubasilissa regina*

(Phryganeidae) is probably not homologous to its Lepidoptera counterpart: the gland-opening muscles in the phryganeids (and all other integripalpians) included in the present study originate on the cuticle of sternum V. For a more detailed discussion of the gland-opening muscles, including homology and number of origins, please see Chapter 5.

Gland reservoir: shape and musculature

Ivanov and Melnitsky (1999, 2002) defined four gland types: ampulliform, sacculous, reniform and fenestral, with the latter only found in philopotamids. While I found separate glands associated with the fenestra in female philopotamids, I found them to be present alongside normally developed sternum V glands, and to have a different structure (Figure 2-3 C, D). Ansteeg (1989) observed separate glandular structures on sternum IV in female *Wormaldia copiosa* (McLachlan), but did not seem to connect this to the presence of the fenestra in female philopotamids. Ivanov and Melnitsky (1999, 2002) described the fenestral glands as lacking a reservoir, instead consisting of secretory tissue directly below the cuticle. Contrary to this, my investigations revealed that the fenestral glands do have a reservoir and that the secretory tissue surrounds the reservoir and secretes into this. Please see Chapter 5 for a detailed discussion of both the origin and functional implications of the fenestral gland structure.

I use the terms periform, ovoid, round, reniform as well as others to describe gland reservoir shape. Mapping of cases in which I scored periform, ovoid or round, reniform and other shape showed that periform gland reservoirs are common in representatives of Annulipalpia, and to a large degree overlap with Ivanov and Melnitsky's (1999, 2002) type 1, ampulliform glands. I found reniform glands in representatives of Uenoidae, Apataniidae and Limnephilidae, but other shapes are also found in this group. I also found reniform glands in *Molanna flavicornis* (Molannidae). Ivanov and Melnitsky (2002) suggested that reniform glands are a synapomorphy for Uenoidae, Apataniidae and Limnephilidae. However, the present study shows that the reniform shape is paralleled elsewhere, and that several limnephilids do not have reniform glands.

Ovoid or round gland reservoirs are found in species throughout Trichoptera, and especially in representatives of Sericostomatoidea (although it is often an irregular ovoid).

Apart from the fenestral glands in female Philopotamidae, which are very distinctive (Figure 2-3 C, D), I did not find enough differences based on gland shape to group the gland into different types. Also, apart from the fenestral glands there are several intermediate forms, e.g., ovoid periform, see Table 2-1.

I found that muscle fibres surrounding the gland reservoir are widespread in Trichoptera: in representatives for all annulipalpian families with the sternum V gland except Polycentropodidae; in representatives for all spicipalpapian families; and in representative for some integripalpian families (Phryganeidae, Oeconesidae, Sericostomatidae, Helicophidae, Anomalopsychidae and Chathamiidae). I did not find such muscle fibres in any representatives of Uenoidae, Apataniidae or Limnephilidae. As reservoir musculature was previously only known from representatives of Stenopsychidae and Phryganeidae (Ivanov & Melnitsky, 2002; Hashimoto & Kobayashi, 2009), it can certainly be overlooked, but I investigated several species of Limnephilidae, including representatives for all subfamilies, thus I am reasonably certain that the gland reservoir musculature is indeed absent from at least Limnephilidae.

In most cases the reservoir musculature encloses only the reservoir as observed by Ivanov and Melnitsky (2002) and Hashimoto and Kobayashi (2009), but in some cases it encloses both reservoir and secretory tissue. While I rarely found that all or the majority of muscle fibres enclose both reservoir and secretory tissue, it is much more common that just a few fibres also enclose some secretory tissue while the majority of fibres enclose only the reservoir. Possibly muscle fibres surrounding the only the reservoir are a more efficient arrangement than muscle fibres surrounding both the reservoir and the secretory tissue for emptying the gland reservoir. Muscle fibres surrounding both the reservoir and the secretory tissue might not be able to completely compress the reservoir due to the secretory tissue functioning as a hydrostatic skeleton around the reservoir during muscle contrations.

There is some co-occurrence between reniform reservoirs and absence (or at least non-observance) of gland reservoir musculature. I did not find reservoir musculature in any of the species with reniform reservoirs. However, not all species without (observed) gland musculature had reniform reservoirs. Whether this is just coincidence or if there is some functional significance is unclear.

There is a distinctive 'sunburst' musculature associated with the fenestral glands in female Philopotamidae (Figure 2-3 C, D) and with the gland reservoir in female Psychomyiidae (Figure 2-5 B-D). The 'sunburst' musculature originates on the cuticle and inserts on the fenestral (in philopotamids) or the gland reservoir (in psychomyiids). The 'sunburst' musculature is associated with perforated cuticle (Figures 2-17 C, D; 2-18 C, D) in both cases. Please refer to Chapter 5 for a detailed discussion.

Secretory tissue

My findings confirmed Eltringham's (1931, 1934) results with respect to the arrangement of secretory tissue in representatives of *Agapetus* (Glossosomatidae), *Diplectrona* (Hydropsychidae, Diplectroninae) and *Polycentropus* (Polycentropodidae). Furthermore, I found a very similar arrangement in the investigated Smicrideinae species (Hydropsychidae). However, having the secretory cells some distance from the reservoir is otherwise unusual; in all other investigated species the secretory tissue is closely associated with the reservoir, either a layer as described by Ivanov and Melnitsky (2002), or a number of lobes or nodules as described by Hashimoto and Kobayashi (2009).

Hashimoto and Kobayashi (2009) suggested that the secretory cells are type 3 as defined by Noirot and Quennedey (1974) as they are separate from the lining of the reservoir. My observations of muscle fibres running between the secretory cells and the reservoir, the most widespread organisation of reservoir musculature, support this. Furthermore, like Eltringham (1931, 1934), I observed ductules visible in a light microscope connecting the secretory cells with the reservoir (in *Agapetus walkeri* (Glossosomatidae) and the investigated species of Diplectroninae and Smicrideinae (both Hydropsychidae)). Internal SEMs of KOH

treated specimens showed cuticular ductules otherwise obscured by soft tissue in *Chimarra obscura* (Philopotamidae), *Psychomyia flavida* (Psychomyiidae), *Arctopsyche grandis* (Hydropsychidae), *Anagapetus debilis* (Glossosomatidae), *Brachycentrus occidentalis* (Brachycentridae), *Onocosmoecus unicolor* (Limnephilidae) and *Gumaga griseola* (Sericostomatidae). Cuticular ductules were also observed by Ansteeg (1989) in representatives of Ecnomidae, Psychomyiidae, Polycentropodidae, Hydropsychidae, Rhyacophilidae, Glossosomatidae, Limnephilidae and Sericostomatidae. Based on this, he concluded that the secretory cells are type 3. Ivanov and Deev's (2009) ultrastructural studies showed the presence of ductule cells in representatives of Rhyacophilidae and Limnephilidae, and they thus supported the conclusion of earlier studies that the secretory cells are type 3. My observations support this, and with the present study concluded there is now evidence for the presence of type 3 secretory cells in representatives of the majority of Trichoptera families with the sternum V gland.

Possible phylogenetic characters

Presence/absence of glands

The sternum V gland is an autapomorphy for Amphiesmenoptera (Trichoptera + Lepidoptera) (Kristensen, 1981); its presence in Trichoptera is thus a plesiomorphy. Furthermore, in Trichoptera as a whole, absence of the gland is a highly homoplastic character. However, within some groups of trichopterans, absence of the gland is likely to be a useful character.

The dipseudopsid species lack the gland, and within Annulipalpia this condition is only paralleled in male *Philopotamus montanus*. Thus absence of the sternum V gland is a possible autapomorphy for Dipseudopsidae.

Although presence of the gland is much more widespread in representatives of Plenitentoria than in representatives of Brevitentoria, Ansteeg's (1989) suggestion of using presence/absence as a criterion for placing integripalpians in either Plenitentoria or Brevitentoria is too simplistic. However, within these groups presence/absence may still be a useful character.

In Plenitentoria, the sternum V gland is present in most limnephilids sensu lato, but absent in *Goera calcarata*, *Goera pilosa* (Fabricius) and *Silo pallipes* (Fabricius) (Ivanov & Melnitsky, 1999; present study). Thus absence of the gland is a possible autapomorphy for Goerinae or even Goeridae.

In Brevitentoria presence of the gland in females only might offer support for the clade containing Calocidae, Conoesucidae, Helicophidae, Anomalopsychidae and Chathamiidae (data from Ivanov & Melnitsky, 2002; present study). However, the gland might be present in male Chathamiidae. Furthermore, no representatives of either Hydrosalpingidae or Petrothrincidae, which together constitutes the sister clade to Chathamiidae, have been investigated, nor were any representatives of the two "sericostomatid" genera (*Myotrichia* and *Parasericostoma*) and Barbarochthonidae that Holzenthal et al. (2007a) placed as the closest relatives of the clade containing Calocidae, Conoesucidae, Helicophidae, Anomalopsychidae and Chathamiidae. In any case, the gland being present only in females is paralleled in Molannidae, Beraeidae and possibly Limnocentropodidae (data from Ivanov & Melnitsky, 2002; present study). Thus presence or absence of the gland is unlikely to be a useful character within Brevitentoria.

Cuticular modifications

A scaly patch with grooves between individual epidermal cells is present in several annulipalpians, including all philopotamids with the gland, except representatives of *Chimarra*. Thus absence of the patch could be an autapomorphy for *Chimarra* or Chimarrinae. Presence of the same trait is a likely synapomorphy for Xiphocentronidae and Psychomyiidae, although not present in *Psychomyia flavida* (gland opening uniquely situated in the membrane between sternum IV and V). The polygons that cover the protuberance in representatives of Diplectroninae and Smicrideinae (both Hydropsychidae) do not define a clade, and neither do the hexagons that appear here and there in representatives of Plenitentoria.

The presence of a bald area that extends from the front edge of the sternite encompassing the gland opening, and internal ridges that extend from the antecosta and around the gland opening are both characteristic of Brevitentoria. Although each is paralleled elsewhere, the bald area in *Austrotinodes panamensis* (Ecnomidae) and *Oeconesus maori* (Oeconesidae), the ridge in *Brachycentrus maculatus* (Fourcroy) (Brachycentridae) according to Nielsen (1980) and partially in some phryganeids, *Brachycentrus occidentalis* and *A. panamensis*, the combination is unique to Brevitentoria and is a possible autapomorphy.

The simple presence/absence of a protuberance appears too homoplastic to be useful, although the presence of protuberances is an autapomorphy for Polycentropodidae compared to Ecnomidae, Dipseudopsidae, Xiphocentronidae and Psychomyiidae. It might also be an autapomorphy for Oeconesidae. However, more specific definitions of the kind of protuberance appear to yield several good characters. A protuberance with a groove that extends from the gland opening to the apex with 'wavy' cuticle along the groove is a likely autapomorphy for Polycentropodinae or even Polycentropodidae as it is known in representatives of *Cyrnellus* (present study), *Holocentropus* (Ivanov & Melnitsky, 2002), *Plectrocnemia* (Ansteeg, 1989), and *Polycentropus* (Ansteeg, 1989; present study). A polygon-covered protuberance with a groove that extends from the gland opening to the apex is only found in representatives of Diplectroninae and Smicrideinae (Both Hydropsychidae). A bulge or protuberance with setae is only found in representatives of Hydroptilidae, Glossosomatidae and Rhyacophilidae. These three families are not considered to form a monophyletic group (Morse, 1997; Holzenthal, 2007a). However, one of the most parsimonious reconstructions is that it originated in Spicipalpia + Integripalpia after the branch leading to Hydrobiosidae diverged, and was then lost in stem group Integripalpia as well as in some glossosomatids (Figure 2-27 B). So despite being characteristic of a paraphyletic group, the character might still be homologous in representatives of the three families. A short protuberance with one or several very long setae is only found in representatives of Hydroptilinae, and is apparently present in all members of the subfamily (Marshall, 1979). Thus it constitutes a good

autapomorphy for the subfamily. Variation in the number of setae and shape of the individual setae might to be useful characters within the subfamily.

Opening muscles

Unfortunately neither of the two different kinds of opening muscles (one originating on the anterior edge of sternum VI, Lepidoptera-type, one mesad on sternum V, Trichoptera-type) is restricted to monophyletic groups (Figure 2-27 A). However, absence of gland-opening muscles while possessing the sternum V gland is a unique autapomorphy for Psychomyiidae. Furthermore, within Annulipalpia, the presence of Lepidoptera-type opening muscles is an autapomorphy for Philopotamidae. The presence of Lepidoptera-type opening muscles in representatives of three families of Spicipalpia (Rhyacophilidae, Glossosomatidae and Hydroptilidae) is particularly intriguing. This type of opening muscle is also present in Lepidoptera (see Chapter 5 for a discussion of homology), and the placement of the four spicipalpian families is one of the more prominent problems in an overall phylogeny of Trichoptera (Morse, 1997). The presence of Lepidoptera-type opening muscle in these three families could indicate that they form a monophyletic group, or that they belong at the very base of Trichoptera. Since this character is also present in Philopotamidae, it is homoplastic to some degree, but might still shed some light on the correct placement of the spicipalpian families.

Gland reservoir shape and musculature

As suggested by Ivanov and Melnitsky (2002), reniform glands can be regarded as a synapomorphy for Uenoidae, Apataniidae and Limnephilidae, although it has been reversed in members of all families and is paralleled in representatives of *Molanna flavicornis* (Molannidae). Generally, other reservoir shapes seem too homoplastic to contain much information, but elongate, compartmentalized reservoirs might an be autapomorphy for *Pycnopsyche* (Limnephilidae), although reversed in female *P. lepida*.

Absence of reservoir musculature is a possible autapomorphy for Limnephilidae sensu lato. However, this character should be treated with caution, as the musculature can be hard to see, and, if the specimens are not well preserved, it can be lost to decomposition. In any case, the gland musculature was also missing even in well preserved specimens of other taxa (e.g. *Molanna flavicornis*).

Configuration of reservoir musculature is less prone to mis-scoring, but the ability to score the character still depends on well preserved specimens. Parallel muscle fibres associated with the reservoir is a likely autapomorphy for Smicrideinae excl. *Diplectrona zealandensis* and unique within Hydropsychidae + Psychomyioidea. Within Philopotamidae it might be an autapomorphy for *Chimarra*, although paralleled in male *Wormaldia planae*. Furthermore, it might be an autapomorphy for Hydrobiosidae, although paralleled in *Rhyacophila arnaudi* (Ryacophilidae), *Agapetus walkeri* (Glossosomatidae) and partially in *Oeconesus maori* (Oeconesidae) as well as the annulipalpian taxa mentioned.

Fenestrae/perforated patches

Perforated patches associated with separate fenestral glands (Figures 2-3 C, D; 2-17 C, D) in females are a unique autapomorphy for Philopotamidae. Perforated patches associated with the regular gland reservoir (Figures 2-5 C; 2-18 C, D) are a unique autapomorphy for Psychomyiidae within Trichoptera, but this trait has been paralleled in Lepidoptera (Chapters 3, 5). Johanson and Espeland (2010) placed the presumed psychomyiid *Zelandoptila moselyi* Tillyard within Ecnomidae based on molecular data. As the ecnomids included in the present study did not have perforated patches associated with the gland reservoir, lack of these in *Zelandoptila* would be consistent with Johanson and Espeland's (2010) placement, whereas presence of these patches would support the placement in Psychomyiidae.

Arrangement of secretory tissue

Separation of the secretory tissue from the reservoir is a likely autapomorphy for Polycentropodinae or Polycentropodidae. Although only known directly from *Polycentropus* (Eltringham 1934; present study), Ansteeg (1989) remarked that the ductules were longer than in other trichopterans in representatives of *Plectrocnemia*, *Polycentropus* and *Cyrnus* (no other polycentropodid genera studied). The separation of secretory tissue and reservoir is also present in representatives of Diplectroninae and Smicrideinae (both Hydropsychidae) (further discussed below) and in *Agapetus walkeri* (Glossosomatidae), although in the latter the secretory tissue still surrounds the reservoir, just with some space between secretory tissue and reservoir.

Possible characters not mapped

There are other possible characters that were not mapped on the phylogeny as they were only present in one included species. These include the involuted and likely eversible sac in male *Agapetus walkeri* which is present in males in all members of subgenus *Agapetus*, and variously developed, according to Ross (1956). Thus the sac would be an autapomorphy for the subgenus, and the degree of development might provide a useful character within the subgenus. The ovoid ridged area associated with the gland opening in *Leptonema albovirens* is one development of the 'clear, oval, boss-like structures' characteristic of the genus (Flint et al., 1987). Depending on its presence in other macronematines it might be an autapomorphy for the genus. It is variously developed within the genus, so it might supply valuable characters for reconstructing the phylogeny of the genus as well, e.g. it is distinctly protruding in species group *davisi* (Flint et al., 1987).

Suggested applications

Polycentropodidae and Dipseudopsidae

Over the years there has been disagreement over which taxa to include in Dipseudopsidae, and which in Polycentropodidae. Based on morphological and behavioural characters, the two families are often regarded as sister taxa (Frania &

Wiggins, 1997; Li et al., 2001), but Ivanov (2002) did not consider them particularly closely related. Malicky (1973) included Polycentropodinae, Pseudoneureclipsinae and Hyalopsychinae in Polycentropodidae, and only the genera *Dipseudopsis* and *Limnoecetis* in Dipseudopsidae. Wells and Cartwright (1993) transferred Hyalopsychinae to Dipseudopsidae, and Li et al. (2001) transferred Pseudoneureclipsinae to Dipseudopsidae. Malicky (1991) placed the genus *Kambaitipsyche* in Polycentropodidae (as subfamily Kambaitipsychinae). Thus Polycentropodidae are presently considered to consist of Polycentropodinae and Kambaitipsychinae, while Dipseudopsidae consist of Dipseudopsinae, Pseudoneureclipsinae and Hyalopsychinae (Holzenthal et al., 2007b). However, recent molecular results (Holzenthal et al., 2007a) have placed Pseudoneureclipsinae and Polycentropodinae as sister groups, while Hyalopsychinae and Dipseudopsinae were sister groups (Kambaitipsychinae not included). In this analysis, Polycentropodinae + Pseudoneureclipsinae were more closely related to Xiphocentronidae and Psychomyiidae than to Dipseudopsinae + Hyalopsychinae. To add to the complexity, Johanson and Espeland's (2010) molecular analyses placed *Pseudoneuroclepsis* as sister to or within Ecnomidae, separate from the dipseudopsid and polycentropodid taxa included in the analyses.

The polycentropine taxa have a highly distinctive configuration of the sternum V gland (protuberance with groove with wavy edges, glandular tissue separate from reservoir) (Eltringham, 1934; Ansteeg, 1989; Ivanov & Melnitsky, 2002; present study). The dipseudopsid taxa included in the present study completely lack the sternum V gland, and the ecnomid taxa have a 'standard' sternum V gland. Thus characters from the sternum V gland might help in sorting out which taxa belong in Polycentropodidae and which in Dipseudopsidae, or possibly Ecnomidae, and with the subsequent definition and recognition of these families.

According to Malicky (1973) Polycentropodidae (including Pseudoneureclipsinae and Hyalopsychinae) always have a thread-like protuberance on sternum V. This would make lack of sternum V glands an autapomorphy for Dipseudopsinae only. However, *Phylocentropus placidus*

(Hyalopsychinae) do not have a sternum V gland or an associated protuberance, thus lack of sternum V glands is at least an autapomorphy for Dipseudopsinae + Hyalopsychinae, supporting Holzenthal et al.'s (2007a) result. Unfortunately, I did not have any specimens of either Pseudoneureclipsinae or Kambaitipsychinae available for study. However, the polycentropodine type of protuberance is easily visible in a dissection microscope, so workers on these groups should be able to detect the presence/absence of this structure easily.

Hydropsychidae

Schefter (1996) used presence/absence of a protuberance associated with the gland opening in a phylogeny of Hydropsychidae. Within Hydropsychidae she scored the protuberance ("filament-like lobe") as present in Diplectroninae, Smicrideinae except *Smicridea* (sugenus *Smicridea*), some *Hydropsyche* as well as a hypothetical ancestor (based on presence in Polycentropodidae).

The present study demonstrates that the protuberances in representatives of Diplectroninae and Smicrideinae are exceedingly similar, and very distinctive compared to all other amphiesmenopterans. While they share an important functional characteristic (a groove that extends from opening to apex) with the protuberances in representatives of Polycentropodidae, they are easily distinguishable. The protuberances in *Hydropsyche* are much smaller, do not have a groove that extends from the gland opening to the apex and are not covered in polygons each following the outline of individual epidermal cells. While protuberances in some representatives of *Hydropsyche* do have a slightly scaly appearance, these scaly structures are not large enough to correspond to epidermal cells, and were never observed to extend into teeth or microtrichia.

Schefter (1996) scored the "filament-like lobe" as absent in *Smicridea* (sugenus *Smicridea*). However, according to Flint (1983, 1989) the protuberances in representatives of subgenus *Smicridea* extend to the posterior edge of sternum V, and are sometimes more than twice as long as sternum V (*Smicridea anticura* Flint & Denning), thus it seems to be present in at least some representatives of the subgenus.

With this in mind, the protuberance in representatives of Diplectroninae and Smicrideinae should not be scored as the same character (state) as either the one in some *Hydropsyche* or the one in polycentropodids, but at least as a unique character state. Within Hydropsychidae, representatives of Diplectroninae and Smicrideinae are further distinguished by having the secretory tissue separate from the reservoir. All this suggests a close relationship between Diplectroninae and Smicridinae. These two subfamilies have been suggested as sister groups by Mosely (1933), and, under some conditions, come out as sister groups based on molecular characters (Geraci, 2007, fig. 1.13 a, b).

There are several similarities of the sternum V gland and associated structures between representatives of Polycentropodidae, Diplectroninae and Smicrideinae. All have a protuberance with a groove that extends from the gland opening to the apex, and have the secretory tissue separate from the glandular tissue. Whether these similarities are homologies or simply convergence is unclear at present.

Conclusion

In summary, the sternum V gland supplies characters that are phylogenetically informative at several levels. The gland itself is an autapomorphy for the superorder Amphiesmenoptera, the characteristic bald patch that extends from the front edge of sternum V combined with the cuticular ridge supports the infraorder Brevitentoria. Multiple characters support families or subfamilies, but the sternum V gland can also be informative at the species level. Thus, properly investigated and interpreted, the sternum V gland would often be a valuable addition to the structures supplying characters (e.g. mouthparts, wings and genitalia) when doing phylogenetic studies of Trichoptera.

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TABLE 2-1. List of all species examined showing the treatments employed for each species and description of morphology (presence/absence of the sternum V gland, short descriptions of any cuticular modifications associated with the gland opening, the type of opening muscles present, the shape of the gland reservoir, presence/absence and a short description of reservoir musculature and a short description of the arrangement of secretory cells). '?' denotes that a structure could either 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. F, female; M, male; Lep-type, Lepidoptera-type; Trich-type, Trichoptera-type.

Taxa	Treatments	Gland	Gland opening/ Evaporative structures	Opening muscle	Reservoir shape	Reservoir musculature	Arrangement of secretory cells
Annulipalpia							
Stenopsychidae							
Stenopsychodes mjoebergi Ulmer F	wholemount	present	on thumb-shaped protuberance	Trich-type, 1 elongate bundle	ovoid periform	some fibres across reservoir, excl. secretory tissue, on cuticle side	secretory tissue not discernible
Philopotamidae							
Chimarra aterrima Hagen F + M	external SEM	present	in tiny bald patch	?	?	?	?
C. obscura (Walker) F + M	wholemount, ext. SEM, histology, + int. SEM of F	present	in tiny bald patch	Lep-type	F: elongate periform M: short periform	F: parallel fibres around reservoir excl. secretory tissue M: not discernible	F: around reservoir M: ?
Dolophilodes sp. F	wholemount	present	in protruding, scaly patch	?[a]	round periform	network of fibres around gland sac excl. secretory tissue	thick layer around reservoir

D. novusamericanus (Ling) F + M	wholemount	present	F: in large, scaly hyaline patch M: on scaly protoberance	F: ?[a] M: Lep-type	F: round periform M: periform	F: network of fibres around reservoir, most excl. secretory tissue M: network of fibres around reservoir, excl. secretory tissue	F: around reservoir M: thick layer around reservoir w. extra lobe laterally (close to opening)
D. pallidipes Banks M	wholemount	present	on large flat scaly protoberance	Lep-type	ovoid periform	network of fibres around gland sac, most excl. secretory tissue	thick layer/lobes around reservoir
Philopotamus montanus (Donovan) M	wholemount	absent	n/a	n/a	n/a	n/a	n/a
Wormaldia arizonensis (Ling) F + M	wholemount, ext. SEM	present	in scaly patch	Lep-type + Trich-type, 1 elongate bundle	F: elongate periform M: periform	fibres around reservoir F: incl. secretory tissue M: excl. secretory tissue	around reservoir
W. gabriella (Banks) F + M	wholemount	present	F: in large scaly patch w. groove extending towards midline M: on transverse elongate scaly protuberance	Lep-type	F: irregular ovoid M: ovoid	F: network of fibres around gland sac, most excl. secretory tissue M: a few fibres around gland sac excl. secretory tissue	around reservoir
W. planae (Ross & King) F + M	wholemount, ext. SEM	present	in scaly patch	Lep-type	F: ovoid M: elongate ovoid to periform	well developed, fibres around reservoir, most excl. secretory tissue. Fibres parallel in M	layer around reservoir, relatively thicker in male
W. occidea (Ross) F + M	wholemount	present	in large scaly patch, in M patches connect in a band across midline	?[a]	F: ovoid periform M: ?	?	F: around reservoir M: ?

Ecnomidae							
Austrotinodes panamensis Flint F + M	wholemount, + ext. SEM of F	present	in bald area extending from front edge of sternite. Folds leading from opening to microtrichia outside bald area	F: Trich-type, 1 bundle M: ?	elongate periform	F: not discernible M: very well developed, fibres parallel to long axis of reservoir, excl. secretory tissue	F: layer around reservoir M: layer around distal part of reservoir
Ecnomus tenellus (Rambur) F + M	wholemount	present	in scaly patch	?[a]	F: ovoid M: ?	?	?
Dipseudopsidae							
Dipseudopsis capensis Lestage F	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Phylocentropus placidus (Banks) F + M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Xiphocentronidae							
Xiphocentron haitiense (Banks) F + M	wholemount, ext. SEM	present	in scaly patch	Trich-type, 1 bundle	F: elongate ovoid to periform M: round periform	F: not discernible M: some muscle fibres around reservoir excl. secretory tissue	F: layer around reservoir M: possibly secretory cells around anterior part of reservoir
Psychomyiidae							
Lype diversa (Banks) F + M	wholemount	present	in protruding, scaly patch	absent	round ovoid	?	not discernible
Psychomyia flavida Hagen F	wholemount, ext. & int. SEM, histology	present	in membranous area between sternites	absent	round	muscle fibres across reservoir incl. secretory tissue + 'sunburst'	layer around reservoir
Tinodes sigodanus (Ross & Merkley) F + M	wholemount, ext. SEM, histology	present	in scaly patch	absent	ovoid periform	F: muscle fibres around reservoir excl. secretory tissue + 'sunburst' M: not discernible	F: layer around reservoir M: thick layer around reservoir

Polycentropodidae							
Cyrnellus fraternus (Banks) F	external SEM	present	on long, grooved protuberance with conical base	?	?	?	?
Polycentropus cinereus Hagen F + M	wholemount, ext. SEM	present	on long, grooved protuberance with conical base	Trich-type, 1 bundle	ovoid	not discernible	loose conglomerate adjacent to reservoir
Hydropsychidae							
Arctopsyche grandis (Banks) F + M	wholemount, ext. SEM, + histology, int. SEM of F	present	on bald, thumb-shaped protuberance	Trich-type, 1 bundle	elongate periform to elongate ovoid	fibres around reservoir incl. secretory tissue, more in M	layer around reservoir, in M thicker on anterior and median side
Parapsyche elsis Milne F + M	wholemount, ext. SEM	present	on small bald protuberance w. some wrinkles poss. leading out of bald area	Trich-type F: 2 bundles M: 1 bundle	periform	fibres around reservoir incl. some secretory tissue	F: layer around anterior/distal part of reservoir M: thick layer around reservoir
Diplectrona sp. (N. Carolina) F + M	ext. SEM, + wholemount, histology of F	present	on long, grooved protuberance with basal bulb	F: Trich-type, 1 bundle, originates at base of bulb M: ?	F: apparently ovoid (highly folded) M: ?	F: fibres on reservoir on side away from cuticle (excl. secretory tissue) M: ?	F: in loose conglomerate separate from reservoir M: ?
D. zealandensis Mosely M	wholemount	present	on long, grooved protuberance	Trich-type, 1 bundle	periform	fibres on reservoir on side away from cuticle (excl. secretory tissue)	in rounded conglomerate separate from reservoir
Cheumatopsyche campyla Ross F + M	wholemount	present	in small, bald, slightly raised area	Trich-type F: 2 parallel bundles M: 1 bundle	F: ovoid periform M: ovoid	F: fibres around reservoir, most excl. secretory tissue, poss. originating on cuticle M: fibres around reservoir most excl. secretory tissue	F: layer/lobes around reservoir M: thick layer/lobes around reservoir

C. speciosa (Banks) F + M	wholemount, ext. SEM	present	in small, bald, slightly raised area	Trich-type, 1 bundle	F: elongate ovoid M: spindle-shaped periform	F: fibres around reservoir, most excl. secretory tissue, poss. originating on cuticle M: fibres around reservoir most excl. secretory tissue	layer around reservoir
Hydropsyche bronta Ross F + M	wholemount	present	F: on small bulge M: at base of elongate protuberance	Trich-type F: 3 bundles M: 2 bundles	F: periform M: ovoid	F: not discernible M: ?	F: ? M: layer around reservoir
H. cockerelli Banks M (syn. *H. jewetti*)	wholemount	present	at base of elongate protuberance	Trich-type, 1-2 bundles	elongate	fibres around reservoir, most excluding secretory tissue	layer around reservoir, layer poss. bilayered
H. confusa (Walker) M	wholemount	present	on small protuberance	Trich-type, 1 bundle	periform	fibres around reservoir, most excluding secretory tissue	nodules around reservoir
H. occidentalis Banks F + M	wholemount	present	on small bulge/ protuberance	Trich-type, 1 bundle	F: spindle-shaped periform M: ovoid periform	F: poss. few fibres along reservoir excl. secretory tissue M: few fibres around reservoir, most excl. secretory tissue	F: probably nodules along/around reservoir M: layer/nodules around reservoir
H. oslari Banks F + M	wholemount	present	F: on small bulge/ protuberance M: on elongate protuberance	F: ?[b] M: Trich-type, 1 bundle	F: ?[b] M: elongate	F: ?[b] M: fibres around reservoir excl. secretory tissue	F: ?[b] M: layer around reservoir
H. placoda Ross F + M	wholemount, ext. SEM	present	on small bulge	Trich-type, 2 bundles	F: ovoid periform M: periform	fibres around reservoir excl. secretory tissue	F: layer/nodules around reservoir M: lobes/layer around reservoir

H. tana Ross F + M	wholemount	present	F: on small bulge M: on elongate protuberance	Trich-type, 1 bundle	F: spindle-shaped periform M: ?[c]	F: fibres around reservoir excl. secretory tissue M: ?[c]	F: layer/nodules around apical part of reservoir M: ?[c]
Leptonema albovirens (Walker) F + M	wholemount, ext. SEM	present	at anterior end of ovoid area with textured cuticle, area much larger in M	Trich-type F: 2 bundles M: 1 bundle	F: periform M: ballooning periform, very large	F: dense network around reservoir excl. secretory tissue M: ?	F: nodules around reservoir, most on side away from cuticle M: ?
Asmicridea edwardsii (McLachland) F + M	wholemount, ext. SEM, histology	present	on long, grooved protuberance with slender basal bulb	Trich-type, 1 elongate bundle	periform	parallel fibres around reservoir on side away from cuticle (excl. secretory tissue)	in rounded conglomerate separate from reservoir
Smicrophylax sp. F	wholemount, ext. SEM, histology	present	on long, grooved protuberance with slender basal bulb	Trich-type, 1 elongate bundle	periform	parallel fibres around reservoir on side away from cuticle (excl. secretory tissue)	in rounded conglomerate separate from reservoir
Spicipalpia							
Hydrobiosidae							
Apsilochorema segitiga Weaver & Huisman F	wholemount, ext. SEM	present	in groove connecting to ventral protuberance	Trich-type, 1 bundle	ovoid	parallel fibres around reservoir excl. secretory tissue	?
Atopsyche callosa Navas F + M	wholemount, ext. SEM	present	F: on bulge continued as a groove M: on protuberance	Trich-type, 1 elongate bundle	F: round ovoid, seemingly sclerotised M: spindle-shaped	parallel fibres around reservoir excl. secretory tissue	F: layer around reservoir M: lobes of secretory tissue around apical part of reservoir
Cailloma pumida Ross F + M	wholemount, + ext. SEM of F	F: present M: absent	F: in raised bald area M: n/a	F: ? M: n/a	F: ovoid periform M: n/a	F: parallel fibres around reservoir excl. secretory tissue M: n/a	F: layer around reservoir M: n/a

Rhyacophilidae							
Himalopsyche phryganea (Ross) F + M	wholemount, + ext. SEM of M	present	on bulge w. some setae	F: ? M: Lep-type	F: extremely elongate M: very elongate ovoid	F: fibres around basal half of reservoir, excl. secretory tissue M: fibres around reservoir, excl. secretory tissue + fibres running parallel along duct	F: thick layer around reservoir M: layer around reservoir
Rhyacophila arnaudi Denning F + M	wholemount, ext. SEM, histology	present	on bulge/small protuberance w. some setae, protuberance more pronounced in M	Lep-type	F: elongate periform M: elongate ovoid	F: absent M: near parallel fibres around reservoir excl. secretory tissue	F: layer around reservoir M: ?
Glossosomatidae							
Agapetus walkeri (Betten & Mosely) F + M	wholemount	present	F: in groove M: inside large eversible sac	F: ? M: Lep-type, inserts on eversible sac	F: elongate periform M: elongate ovoid to periform	F: parallel fibres along reservoir excl. secretory tissue M: parallel fibres around reservoir excl. secretory tissue	F: cells loosely arranged around reservoir w. visible ductules M: ?
Anagapetus debilis (Ross) F + M	wholemount, ext. SEM, + int. SEM of F	present	on triangular protuberance with reticulated cuticle and short setae	Lep-type	elongate	F: well developed cris-crossing fibres around reservoir + secretory tissue M: some mostly parallel fibres around reservoir + secretory tissue	F: layer around reservoir M: very thick layer around reservoir, esp. at apex

Protoptila cana Flint F + M	wholemount, + ext. SEM of F	present	F: on triangular bald area at either end of a transverse groove, bald area with a few short setae M: adjacent to transverse groove	Lep-type	F: elongate M: elongate ovoid to elongate periform	well developed fibres around reservoir + secretory tissue	F: very thick layer around reservoir M: thick layer around reservoir
Hydroptilidae							
Hydroptilinae sp. F + M	wholemount, ext. SEM	present	on short protuberance with one long and two very short setae	Lep-type	elongate	well developed network of fibres around reservoir + secretory tissue	thick layer around reservoir
Agraylea multipunctata Curtis F + M	wholemount, ext. SEM	present	on short protuberance with three long setae	Lep-type	F: elongate M: elongate ovoid	fibres around reservoir + secretory tissue	thick layer around reservoir
Palaeagapetus guppyi Schmid F + M	wholemount, ext. SEM	present	on flat protuberance with short setae, posterior edge continued as groove	Lep-type	elongate ovoid	fibres around reservoir excl. secretory tissue, more in M	?
Integripalpia - Plenitentoria							
Plectrotarsidae							
Plectrotarsus tasmanicus Mosely M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Kokiriidae							
Pangullia faziana Navas M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Lepidostomatidae							
Lepidostoma pluviale (Milne) F + M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Brachycentridae							
Brachycentrus occidentalis Banks F + M	wholemount, ext. SEM, + int. SEM of F	present	all of S V covered with hexagons, grooves in between centred on opening	Trich-type, 1 well-developed bundle	round ovoid, long axis diagonal	absent	thick layer around reservoir

Phryganeidae							
Agrypnia straminea Hagen M	wholemount, ext. SEM	present	in small bald area	Trich-type, 3 bundles	irregular ovoid, long axis diagonal	well developed, most excl. secretory tissue	thick layer around reservoir
Phryganea cinerea Walker F + M	wholemount, + ext. & int. SEM of M	present	in bald area, raised and sclerotised in F	Trich-type, 3-4 bundles	round ovoid F: long axis transverse	F: absent M: very well developed, excl. secretory tissue	thick layer around reservoir
Yphria californica (Banks) F + M	wholemount, ext. SEM	present	in bald area, slightly raised in M	Trich-type, 1-2 bundles	F: elongate ovoid M: elongate ovoid w. apical 'finger' pointed anteriad Both: long axis diagonal	F: absent M: well developed, some excl. secretory tissue	F: thick layer around reservoir, more around apex M: concentrated around 'finger' part of reservoir
Oeconesidae							
Oeconesus maori McLachlan M	wholemount, ext. SEM	present	on bald thumb-shaped protuberance	Trich-type, 1 very wide bundle	round w. 'shelf' externally towards middle	extremely well developed, surrounding reservoir excl. secretory tissue, some connecting to 'shelf'	thick layer around reservoir except posteriorly
Goeridae							
Goera calcarata Banks F + M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Uenoidae							
Neophylax concinnus McLachlan F + M	wholemount, ext. SEM	present	in small bald area, area larger in F	Trich-type, 1 bundle	reniform, long axis longitudinal	not discernible	thick layer around reservoir
Neothremma alicia Dodds & Hisaw F + M	wholemount	present	no particular modifications	?	ovoid long axis: F: longitudinal M: transverse	?	F: ? M: thick layer around reservoir except posteriorly

Apataniidae							
Apatania zonella (Zetterstedt) F + M	wholemount, ext. SEM	present	all of S V covered with hexagons, grooves in between centred on opening	Trich-type, 1 bundle	F: likely reniform M: rounded	not discernible	F: around reservoir, poss. arranged in nodules M: ?
Limnephilidae							
Onocosmoecus unicolor (Banks) F + M	wholemount, ext. SEM, + int. SEM of F	present	adjacent to small sculptured patch	Trich-type F: 1 bundle M: 2-3 bundles	round reniform	absent	thin layer around reservoir
Drusus annulatus (Stephens) F + M	wholemount	present	all of S V covered with hexagons, grooves in between centred on opening	Trich-type, 1 well developed bundle	reniform M: gland duct connection at one end	absent	layer around reservoir
Anabolia bimaculata (Walker) M	wholemount, ext. SEM	present	tendency to hexagonal cuticle around opening, but no grooves	Trich-type, 1 well developed bundle	irregular heart shaped, gland duct connecting on one side	absent	very thick layer around median and dorsal sides of reservoir
Hesperophylax sp. M	wholemount	present	part of S V covered with hexagons, grooves in between centred on opening	Trich-type, 1 well developed bundle	elongate reniform	absent	thick layer around reservoir
Limnephilus externus Hagen F + M	wholemount	present	tendency to hexagonal cuticle around opening, but no grooves	Trich-type, 1 well developed bundle	F: compressed reniform M: irregular ovoid	absent	F: thick layer around reservoir M: very thick layer around reservoir
L. harrimani Banks F	internal SEM	present	?	?	reniform to compressed reniform	?	layer around reservoir
L. secludens Banks F + M	wholemount, ext. SEM, + histology of M	present	tendency to hexagonal cuticle around opening, but no grooves	Trich-type, 1 well developed bundle	reniform	absent	thin layer around reserovir, thinner in M

L. sericeus (Say) F	wholemount	present	tendency to hexagonal cuticle on anterior part of S V, but no grooves	Trich-type, 1 bundle	elongate reniform	absent	thin layer around reserovir
Pycnopsyche lepida (Hagen) F + M	wholemount, ext. SEM	present	on side of protuberance, p. larger in M, some microtrichia on p. in both	Trich-type F: 3-4 bundles M: 2 bundles	F: elongate ovoid M: elongate, tendency to compartments	absent	F: very thick layer around reservoir M: thick layer around reservoir
P. scabripennis (Rambur) F + M	wholemount, + histology of F	present	F: at end of ridge from side of S V M: on side of protuberance with 'shark tooth'	Trich-type, 1 bundle bigger in M	elongate, tendency to compartments	absent	thick layer around reservoir
Pseudostenophylax sparsus (Banks) (ssp. *uniformis* (Betten)) F + M	wholemount, ext. SEM	present	all of S V covered with hexagons, grooves in between centred on opening	Trich-type F: 2 bundles M: 1 well developed bundle	reniform	absent	F: very thick layer around reservoir M: extremely thick layer around reservoir
Integripalpia - Brevitentoria							
Limnocentropodidae							
Limnocentropus grandis Banks F	wholemount, ext. + int. SEM	present	in bald area extending from front edge	Trich-type, 1 bundle	irregular, long axis longitudinal, duct attaching on median side	not discernible	thick layer around reservoir
Tasimiidae							
Trichovespula macrocera Schmid F + M	wholemount, + ext. SEM of M	absent	n/a	n/a	n/a	n/a	n/a
Philorheithridae							
Psilopsyche molinai Navas F + M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a

Atriplectididae							
Atriplectides dubius Mosely F + M	wholemount, + ext. SEM of M	absent	n/a	n/a	n/a	n/a	n/a
Odontoceridae							
Marilia flexuosa Ulmer F + M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Molannidae							
Molanna flavicornis Banks F + M	wholemount, + ext. SEM of F	F: present M: absent	F: in bald area extending from front edge M: n/a	F: Trich-type, 1 bundle M: n/a	F: modified reniform M: n/a	F: absent M: n/a	F: thick layer around reservoir M: n/a
Calamoceratidae							
Anisocentropus bicoloratus (Martynov) F + M	wholemount	absent	n/a	n/a	n/a	n/a	n/a
Phylloicus aeneus (Hagen) F + M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Leptoceridae							
Triaenodes reuteri McLachlan F + M syn. *Triaenodes griseus* syn. *Ylodes griseus*	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Helicopsychidae							
Helicopsyche borealis (Hagen) F + M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Beraeidae							
Beraea pullata (Curtis) F	wholemount, ext. SEM	present	in bald area extending from front edge	?	ovoid, long axis longitudinal, duct attaching at one end	?	layer around reservoir
Sericostomatidae							
Gumaga griseola (McLachlan) F + M	wholemount, ext. SEM, + int. SEM of F	present	in bald area extending from front edge	Trich-type F: 1 bundle + single fibre M: 1 bundle	F: ovoid, long axis longitudinal M: round	F: some around reservoir and parts of secretory tissue M: some around reservoir	F: thick layer/lobes around reservoir M: in lobes around reservoir

Calocidae							
Caloca saneva (Mosely) M	wholemount, ext. SEM	absent	n/a	n/a	n/a	n/a	n/a
Conoesucidae							
Olinga feredayi (McLachlan) F + M	wholemount, ext. SEM	F: present M: absent	F: in bald area extending from front edge M: n/a	F: Trich-type, 1 bundle + reverse fibres M: n/a	F: ovoid, long axis transverse M: n/a	F: not discernible M: n/a	F: thin layer around reservoir M: n/a
Helicophidae							
Austrocentrus griseus Schmid F + M	wholemount, ext. SEM	F: present M: absent	F: in bald area extending from front edge M: n/a	F: Trich-type, 1 bundle M: n/a	F: ovoid, long axis transverse M: n/a	F: couple of fibres around reservoir + secretory tissue M: n/a	F: layer around reservoir M: n/a
Anomalopsychidae							
Contulma talamanca Holzenthal & Flint F + M	wholemount, ext. SEM	F: present M: absent	F: in bald area extending from front edge M: n/a	F: Trich-type, 1 bundle M: n/a	F: irregular ovoid, long axis transverse M: n/a	F: well developed, longitudinal fibres around reservoir excl. secretory tissue M: n/a	F: lobes around reservoir, more developed anteriorly M: n/a
Chathamiidae							
Philanisus plebeius Walker F + M	wholemount, + ext. SEM of M	F: present M: possibly present	in bald area extending from front edge	F: Trich-type, 1 bundle + reverse bundle M: ?[d]	F: ovoid, long axis diagonal, duct connecting on one side M: seemingly irregular, long axis transverse	F: well developed, mostly longitudinal fibres around reservoir, most excl. secretory tissue M: ?[d]	F: lobes around reservoir M: ?[d]

[a] ventrolongitudinal musculature missing as well, so absence is likely due to decomposition
[b] internal structures lost in dissection
[c] reservoir lost in dissection
[d] specimen KOH treated, all soft parts dissolved

FIGURE 2-1. Tree showing all families in which gland presence/absence has been investigated in at least one species. The tree is based on the phylogeny by Holzenthal et al. (2007a), and the presence/absence data combine information from the present study with information from the literature. Families marked with '*' shows that I have utilised data from the literature. (F?) denotes families in which no females have been investigated. (M?) denotes families in which no males have been investigated. (F) denotes families in which the gland is only present in females in investigated species. Stenopsychidae: Presence/absence data for males from Ivanov and Melnitsky (2002) and Hashimoto and Kobayashi (2009). Phryganopsychidae (only females investigated), Pisuliidae, Oeconesidae (females), Beraeidae (males), Calocidae (females): Presence/absence data from Ivanov and Melnitsky (2002).

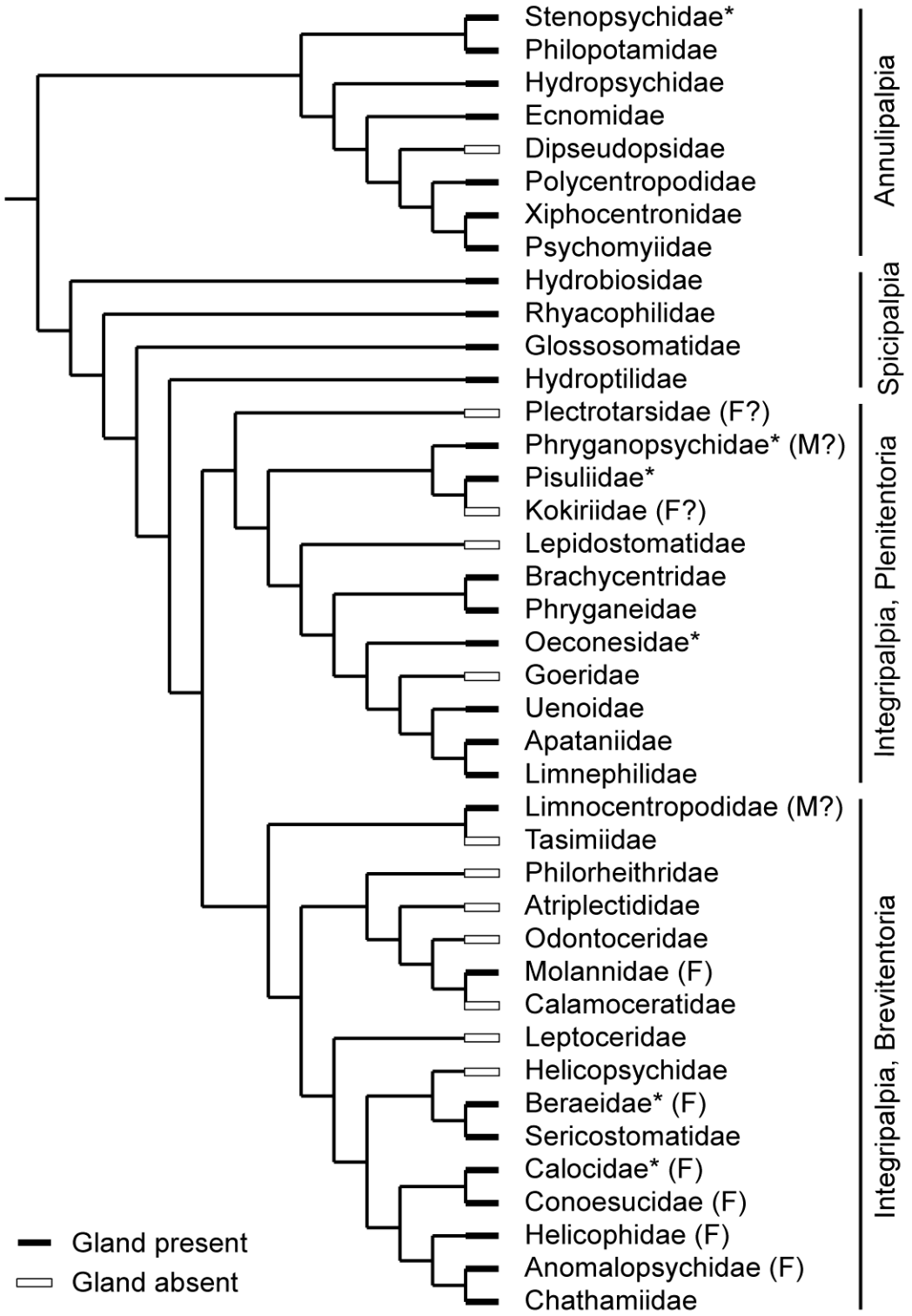

Stenopsychidae*
Philopotamidae
Hydropsychidae
Ecnomidae
Dipseudopsidae
Polycentropodidae
Xiphocentronidae
Psychomyiidae
Hydrobiosidae
Rhyacophilidae
Glossosomatidae
Hydroptilidae
Plectrotarsidae (F?)
Phryganopsychidae* (M?)
Pisuliidae*
Kokiriidae (F?)
Lepidostomatidae
Brachycentridae
Phryganeidae
Oeconesidae*
Goeridae
Uenoidae
Apataniidae
Limnephilidae
Limnocentropodidae (M?)
Tasimiidae
Philorheithridae
Atriplectididae
Odontoceridae
Molannidae (F)
Calamoceratidae
Leptoceridae
Helicopsychidae
Beraeidae* (F)
Sericostomatidae
Calocidae* (F)
Conoesucidae (F)
Helicophidae (F)
Anomalopsychidae (F)
Chathamiidae
Annulipalpia
Spicipalpia
Integripalpia, Plenitentoria
Integripalpia, Brevitentoria
Gland present
Gland absent

FIGURE 2-2. Drawings based on wholemounts. The structures are viewed through the cuticle, anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A: *Stenopsychodes mjoebergi* female (Stenopsychidae), Trichoptera-type opening muscle originating mesad on cuticle of sternum V and inserting on the gland duct wall just inside gland opening. The secretory tissue was not discernible, possibly due to decomposition. The muscle fibres around the reservoir are only found on the side towards the cuticle. B-E: Philopotamidae, note scaly patches around gland opening. B and C: *Wormaldia arizonensis* female and male respectively, note the presence of both Lepidoptera-type and Trichoptera-type opening muscles. D and E: *W. planae* female and male respectively, only Lepidoptera-type opening muscle present. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle; L-t, Lepidoptera-type opening muscle. Scale bars: A = 100 μm; B-E = 20 μm.

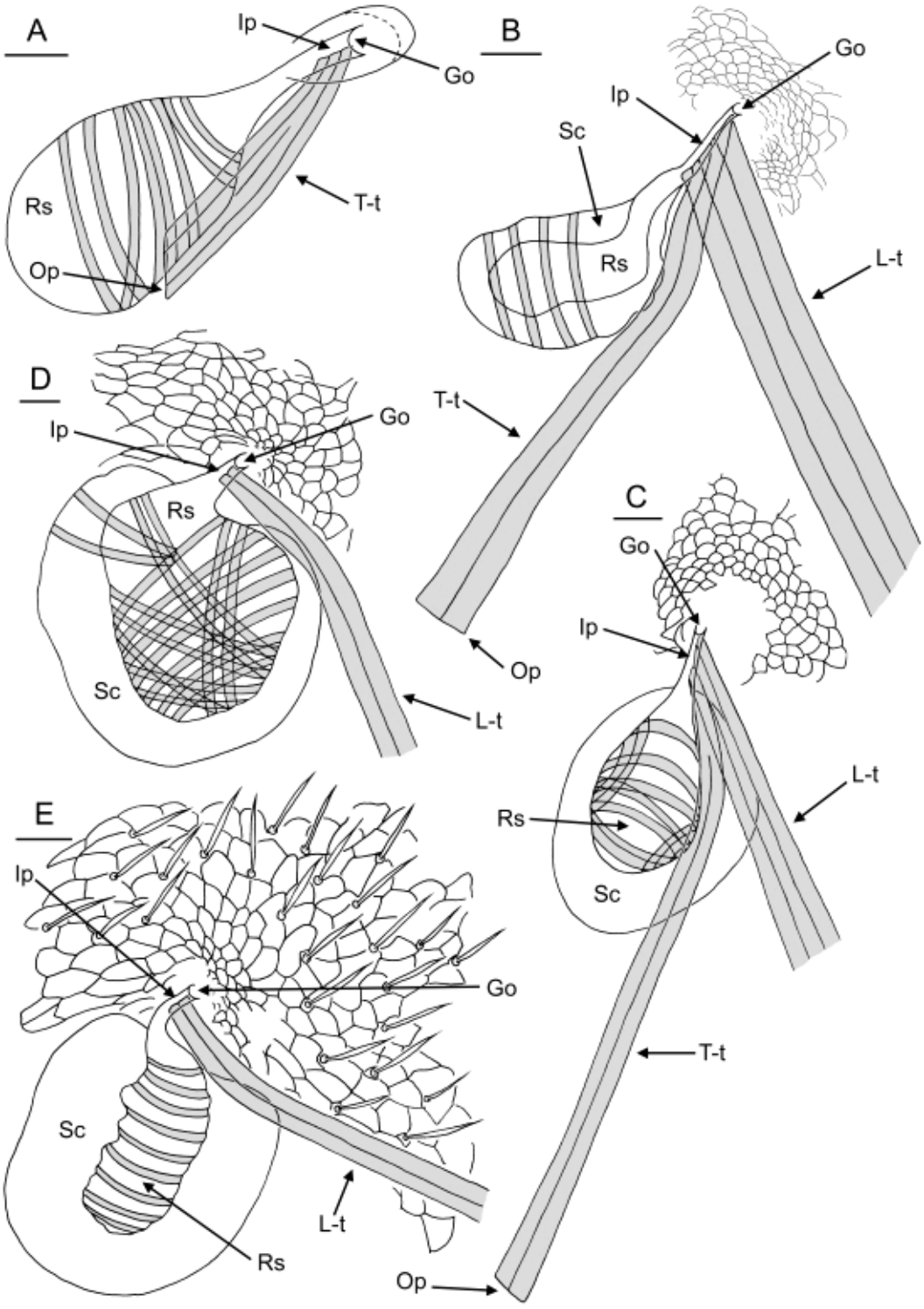

A
Ip
Go
Rs
T-t
Op
B
Go
Ip
Sc
Rs
L-t
T-t
Op
D
Ip
Go
Rs
Sc
L-t
C
Go
Ip
L-t
Rs
Sc
T-t
E
Ip
Go
Sc
L-t
Rs
Op

FIGURE 2-3. Drawings based on wholemounts (A-C) or histological sections (D). The drawings based on wholemounts are viewed through the cuticle, anterior is to the left with down being mesad; thus the drawings show the left gland. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is only shown in D where cross hatching indicates non-transparent cuticle. A-D: Philopotamidae. A and B: *Dolophilodes novusamericanus* female and male respectively, note large scaly patch in female. The gland-opening muscle was missing in the female, but the ventrolongitudinal musculature was missing as well, so the absence is likely due to decomposition. C and D: *Chimarra obscura* female. C: Co-occurring normal sternum V gland and fenestral gland, note the sunburst-like arrangement of muscle fibres associated with the fenestral gland. D: Cross section of fenestral gland with position of fenestra (transparent cuticle) indicated, also note the muscle fibres (part of the 'sunburst') that originate on the cuticle and insert on the reservoir. S IV, sternum IV; S V, sternum V; Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; L-t, Lepidoptera-type opening muscle; Rs, gland reservoir; Sc, secretory cells; SVg, sternum V gland; Fg, fenestral gland; Tc, transparent (and perforated) cuticle. Scale bars: A-D = 20 μm.

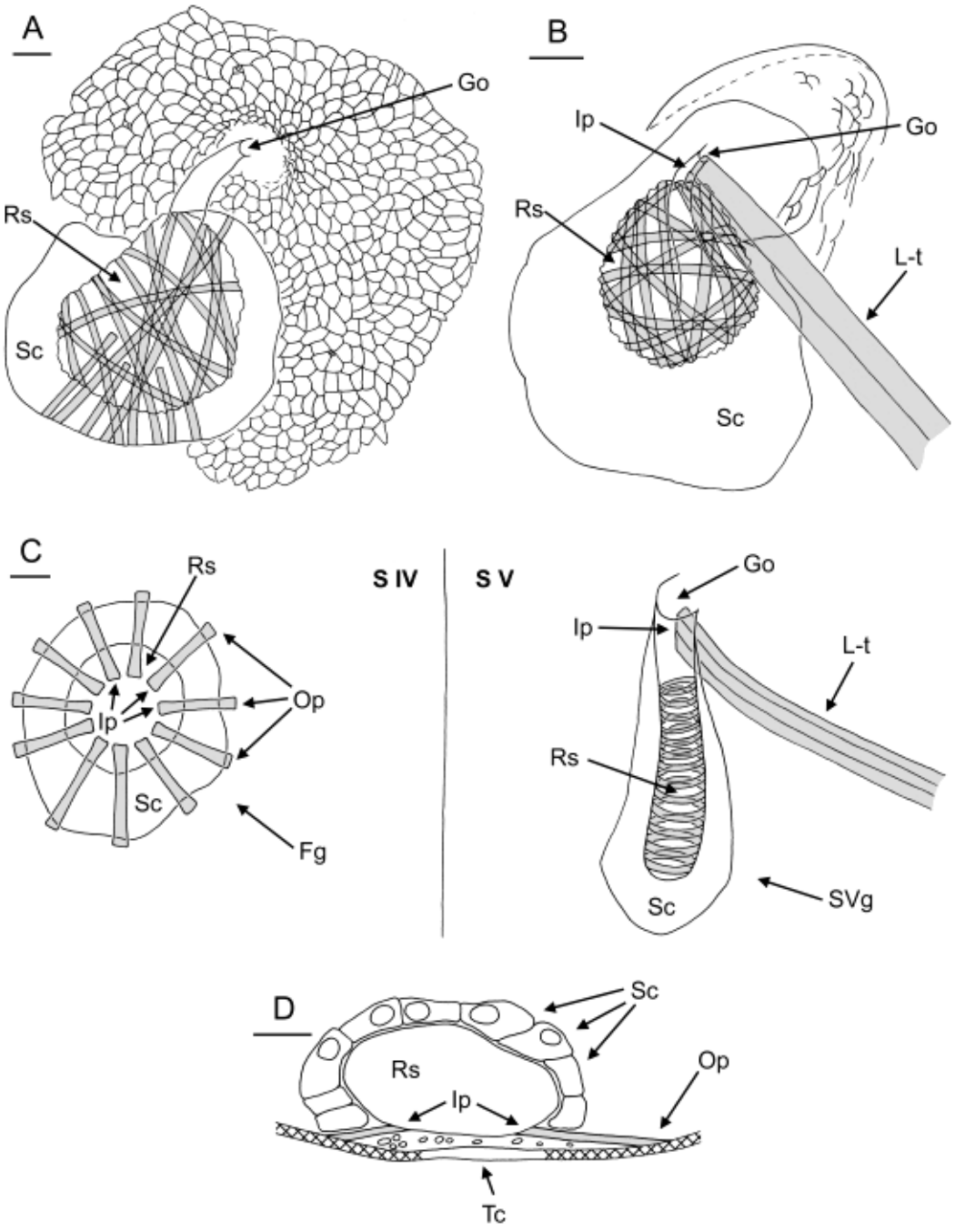

A
Go
Rs
Sc
B
Ip
Go
Rs
L-t
Sc
C
Rs
Op
Ip
Sc
Fg
S IV
S V
Go
Ip
L-t
Rs
Sc
SVg
D
Sc
Rs
Op
Ip
Tc

FIGURE 2-4. Drawings based on wholemounts. The structures are viewed through the cuticle, anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. Cross hatching shows the presence and position of thickened cuticular ridges. A and B: *Austrotinodes panamensis* (Ecnomidae) female and male respectively. The gland-opening muscle was missing in the male, most likely due to decomposition. C and D: *Xiphocentron haitiense* (Xiphocentronidae) female and male respectively, note scaly patches around gland opening. The secretory tissue in the male was not discernible, possibly due to decomposition. E: Illustration of a bulge versus a protuberance. I classified 1 as a bulge and 2 and 3 as protuberances. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. Scale bars: A-C = 20 μm; D = 10 μm.

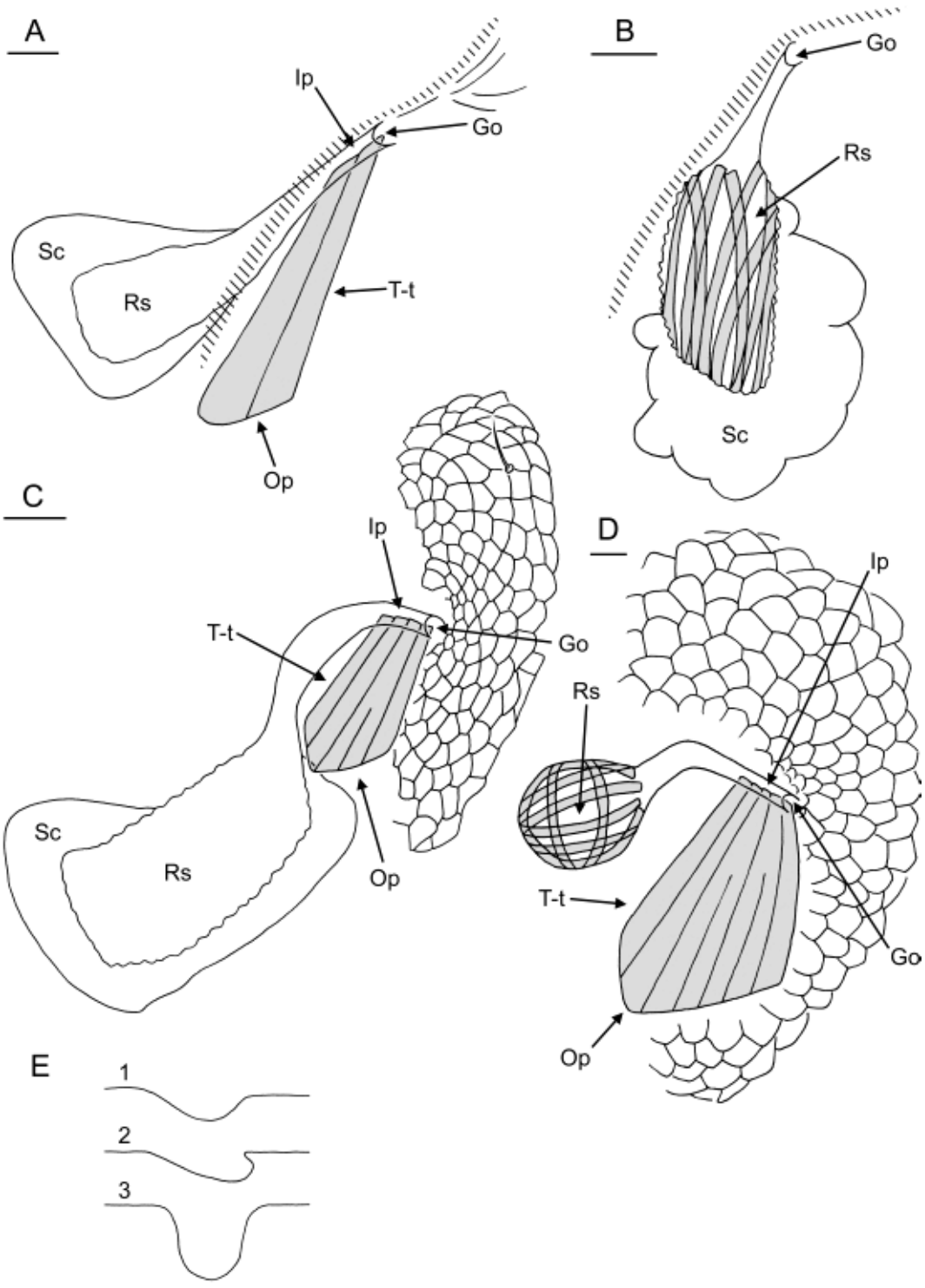

A
Ip
Go
Sc
Rs
T-t
Op
B
Go
Rs
Sc
C
Ip
T-t
Go
Sc
Rs
Op
D
Ip
Rs
T-t
Go
Op
E
1
2
3

FIGURE 2-5. Drawings based on wholemounts (A, B, D, E) or histological sections (C). The drawings based on wholemounts are viewed through the cuticle, anterior is to the left with down being mesad; thus the drawings show the left gland. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is not indicated. A: *Polycentropus cinereus* (Polycentropodidae) male. B-E: Psychomyiidae, note the complete lack of gland-opening muscles. B and C: *Psychomyia flavida* female. B: The gland opening is placed in the membranous area between sternum IV and V, not on sternum V, also note the sunburst-like arrangement of muscle fibres associated with the reservoir. C: The very thin cuticle underneath part of the secretory tissue is perforated (Figures 2-18 C, D; 2-25 E), also note the muscle fibres (part of the 'sunburst') that originate on the cuticle and insert on the gland reservoir. D and E: *Tinodes sigodanus* female and male respectively. D: Note the sunburst-like arrangement of muscle fibres associated with the reservoir. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle; A SV, anterior edge of sternum V; P SIV, posterior edge of sternum IV. Scale bars: A = 50 μm; B-E = 20 μm.

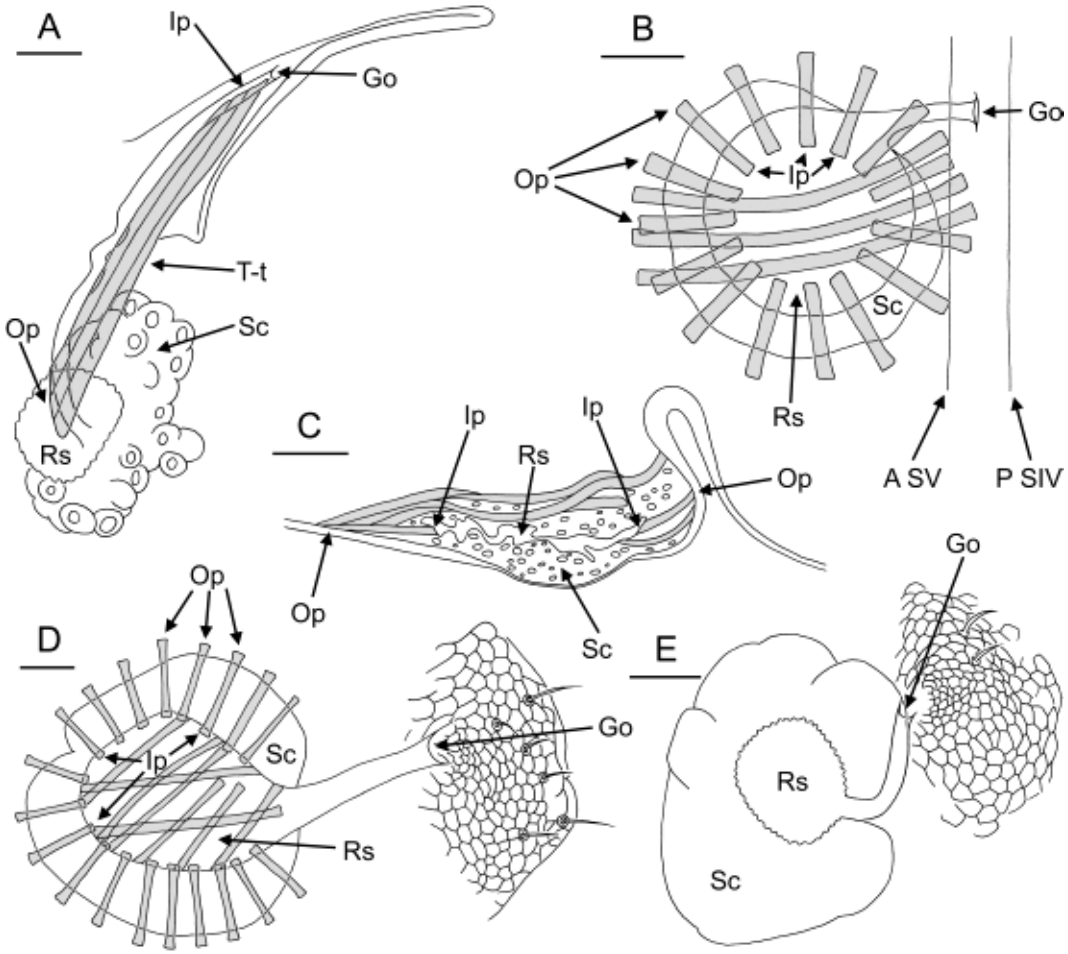

A
Ip
Go
T-t
Op
Sc
Rs
B
Go
Op
Ip
Sc
Rs
A SV
P SIV
C
Ip
Ip
Rs
Op
Op
Sc
D
Op
Sc
Ip
Rs
Go
E
Go
Rs
Sc

FIGURE 2-6. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A-D: Hydropsychidae. A and B: *Arctopsyche grandis* (Arctopsychinae) female and male respectively. C and D: *Leptonema albovirens* (Macronematinae) male and female respectively. Note the extremely large ballon-like reservoir in the male. The secretory tissue in the male was not discernible, possibly due to decomposition. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. Scale bars: A-D = 50 μm.

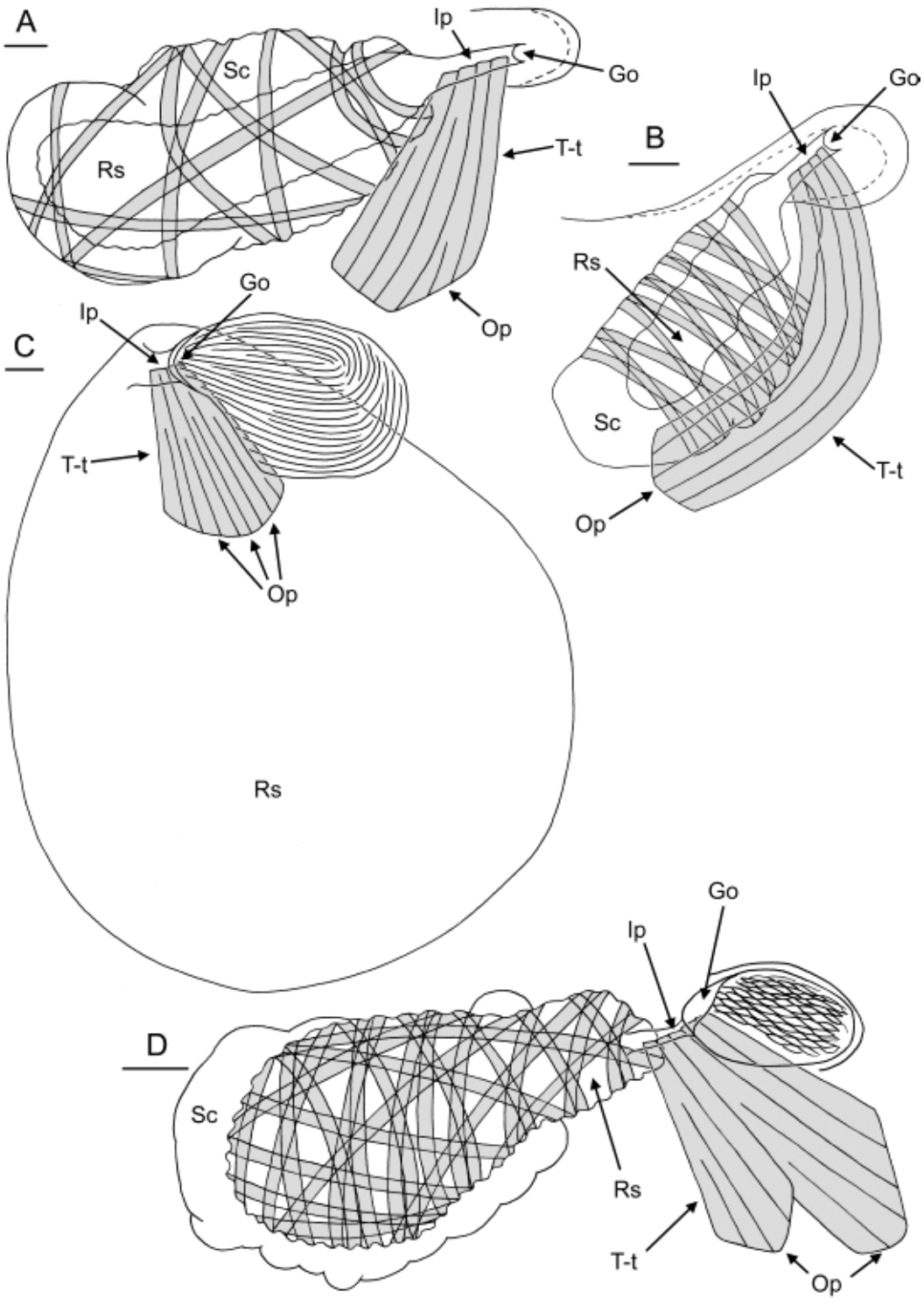

A
Ip
Go
Sc
Rs
T-t
Op
B
Ip
Go
Rs
Sc
T-t
Op
C
Go
Ip
T-t
Op
Rs
D
Go
Ip
Sc
Rs
T-t
Op

FIGURE 2-7. Drawings based on wholemounts (A, C, D) or histological sections (B, E). The drawings based on wholemounts are viewed through the cuticle and anterior is to the left with down being mesad; thus the drawings show the left gland. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is not indicated. A-E: Hydropsychidae. A-C: Diplectroninae. A and B: *Diplectrona* sp. female, note the presence of long visible ductules connecting the secretory cells with the reservoir. A: Note that the opening muscle is entirely contained within the protuberance. B: Cross section through gland reservoir, reservoir musculature and secretory cells. C: *Diplectrona zealandensis* male, note the visible ductules connecting the secretory cells with the reservoir. D and E: *Asmicridea edwardsii* (Smicrideinae) male, the female is very similar. Note the visible ductules connecting the secretory cells with the reservoir. E: Cross section through gland reservoir, reservoir musculature, gland-opening muscle and secretory cells. 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; T-t, Trichoptera-type opening muscle.

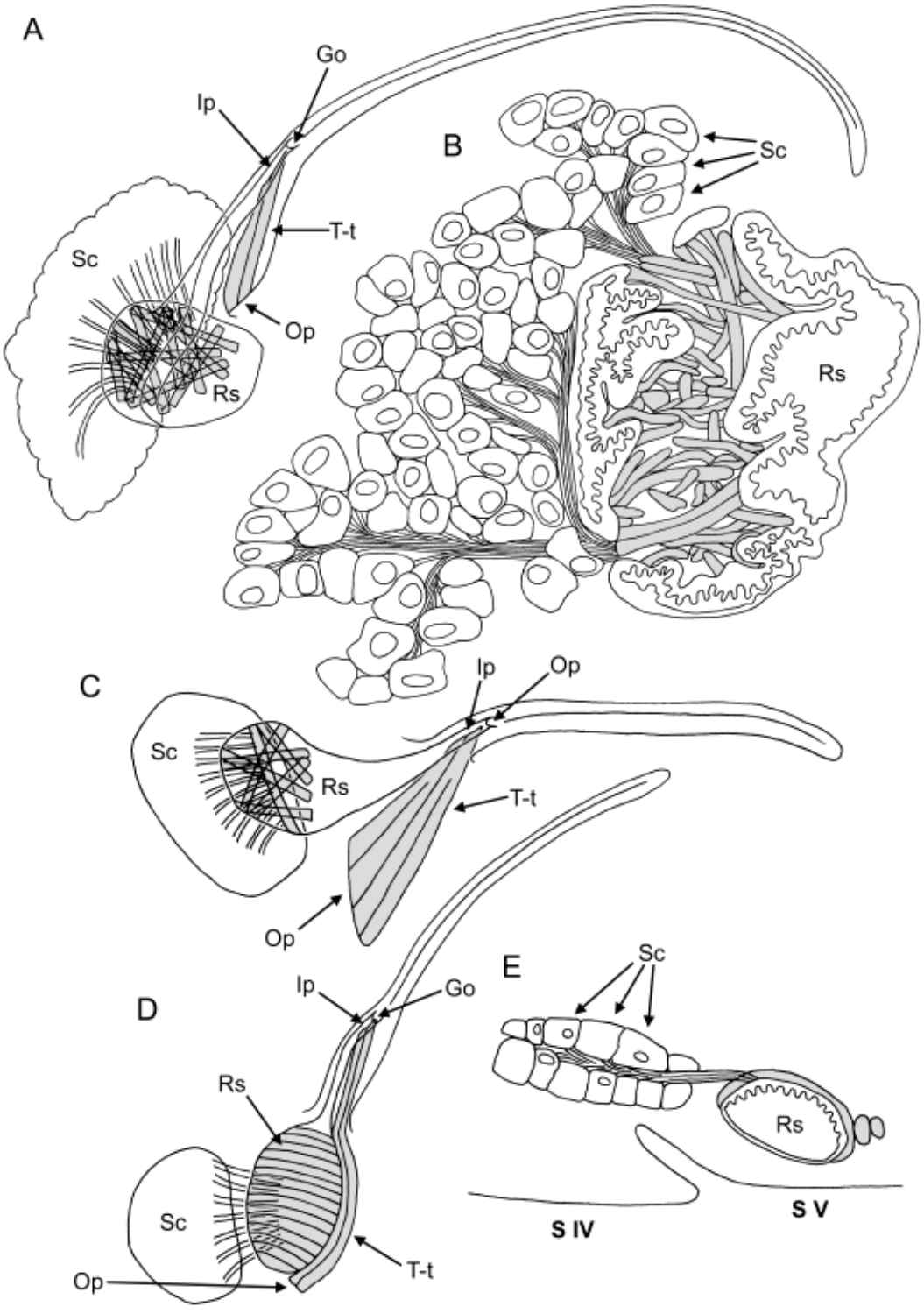
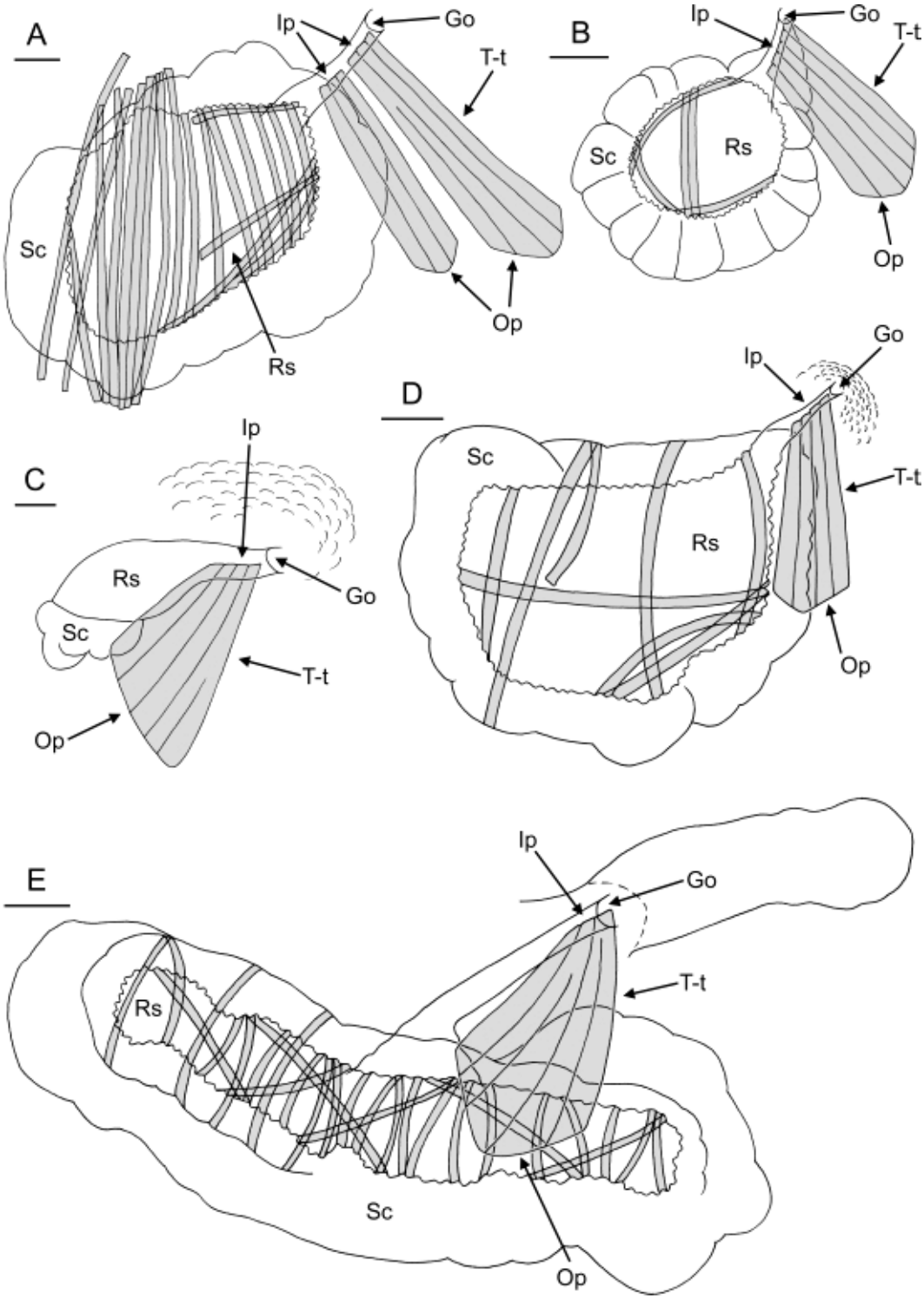

A
Go
Ip
T-t
Sc
Op
Rs
B
Sc
Rs
C
Ip
Op
Sc
Rs
T-t
Op
D
Ip
Go
Rs
Sc
T-t
Op
E
Sc
Rs
S IV
S V

FIGURE 2-8. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A-E: Hydropsychidae, Hydropsychinae. A and B: *Cheumatopsyche campyla* female and male respectively. *C. speciosa* is similar. C and D: *Hydropscyhe occidentalis* female and male respectively. Female *H. tana* is very similar to female *H. occidentalis* and female *H. bronta* is somewhat similar. Female and male *H. placoda*, male *H. confusa*, and internal structures of male *H. bronta* are similar to male *H. occidentalis*. E: *H. cockerelli* male. Male *H. oslari* and the protuberance in male *H. bronta* are similar to male *H. cockerelli*. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. Scale bars: A-C = 20 μm; D, E = 50 μm.


A
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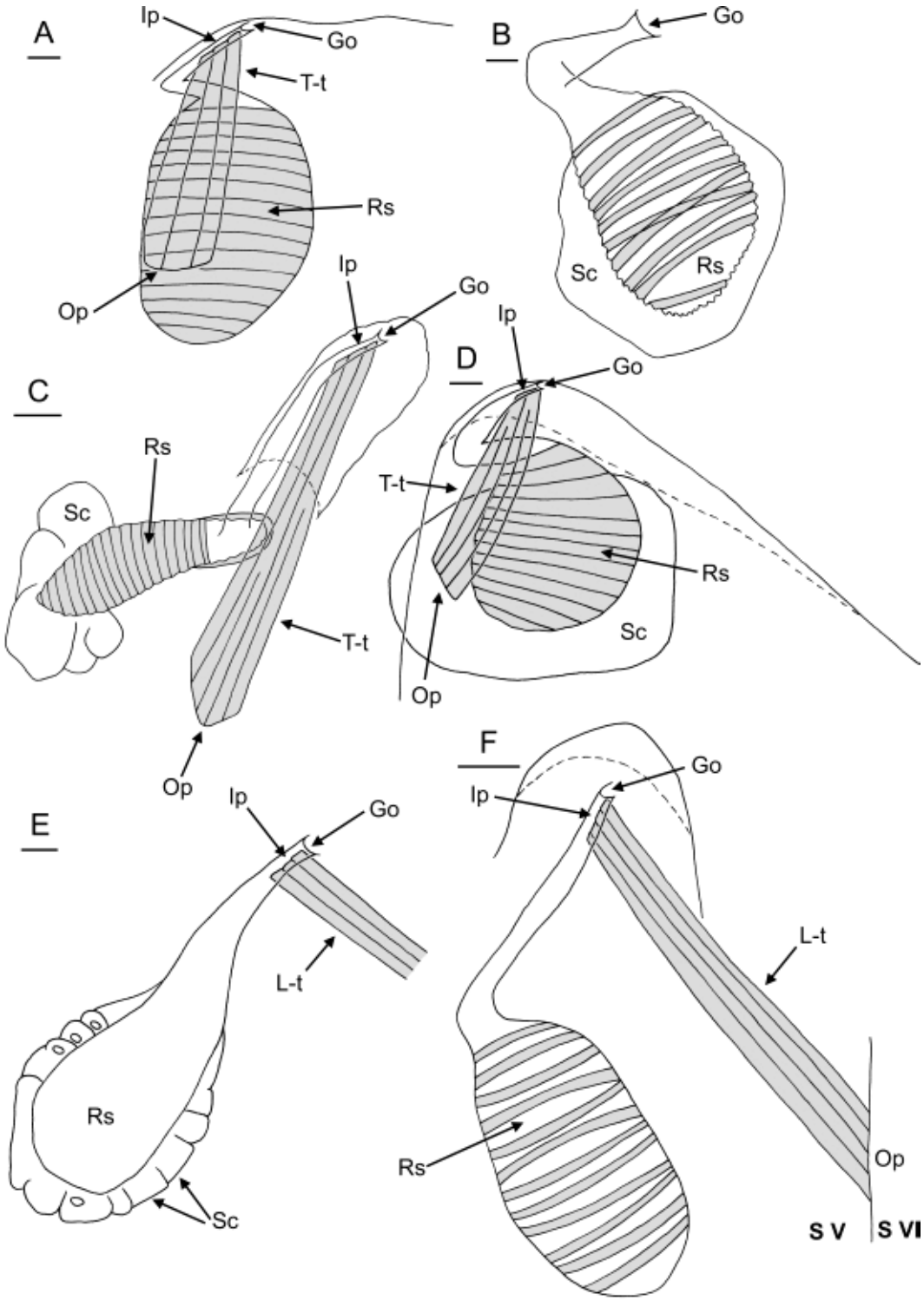
Go
T-t
Sc
Rs
Op
B
Ip
Go
T-t
Sc
Rs
Op
C
Ip
Rs
Go
Sc
T-t
Op
D
Ip
Go
Sc
T-t
Rs
Op
E
Ip
Go
T-t
Rs
Sc
Op

FIGURE 2-9. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A-D: Hydrobiosidae. A: *Apsilochorema segitiga* female, note the sharp bend in the gland duct. The secretory tissue was not discernible, possibly due to decomposition. B: *Cailloma pumida* female, note the sharp bend in the gland duct. The gland-opening muscle was missing, most likely due to decomposition. C and D: *Atopsyche callosa* male and female respectively. Note the sharp bend in the gland duct in the female. E and F: *Rhyacophila arnaudi* (Rhyacophilidae) female and male respectively. Note that the Lepidoptera-type opening muscle originates medioanteriad on sternum VI and inserts on the gland duct wall just inside gland opening. The secretory tissue in the male was not discernible, possibly due to decomposition. S V, sternum V; S VI, sternum VI; Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle; L-t, Lepidoptera-type opening muscle. Scale bars: A-D = 20 μm; E, F = 50 μm.


A
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B
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C
Ip
Go
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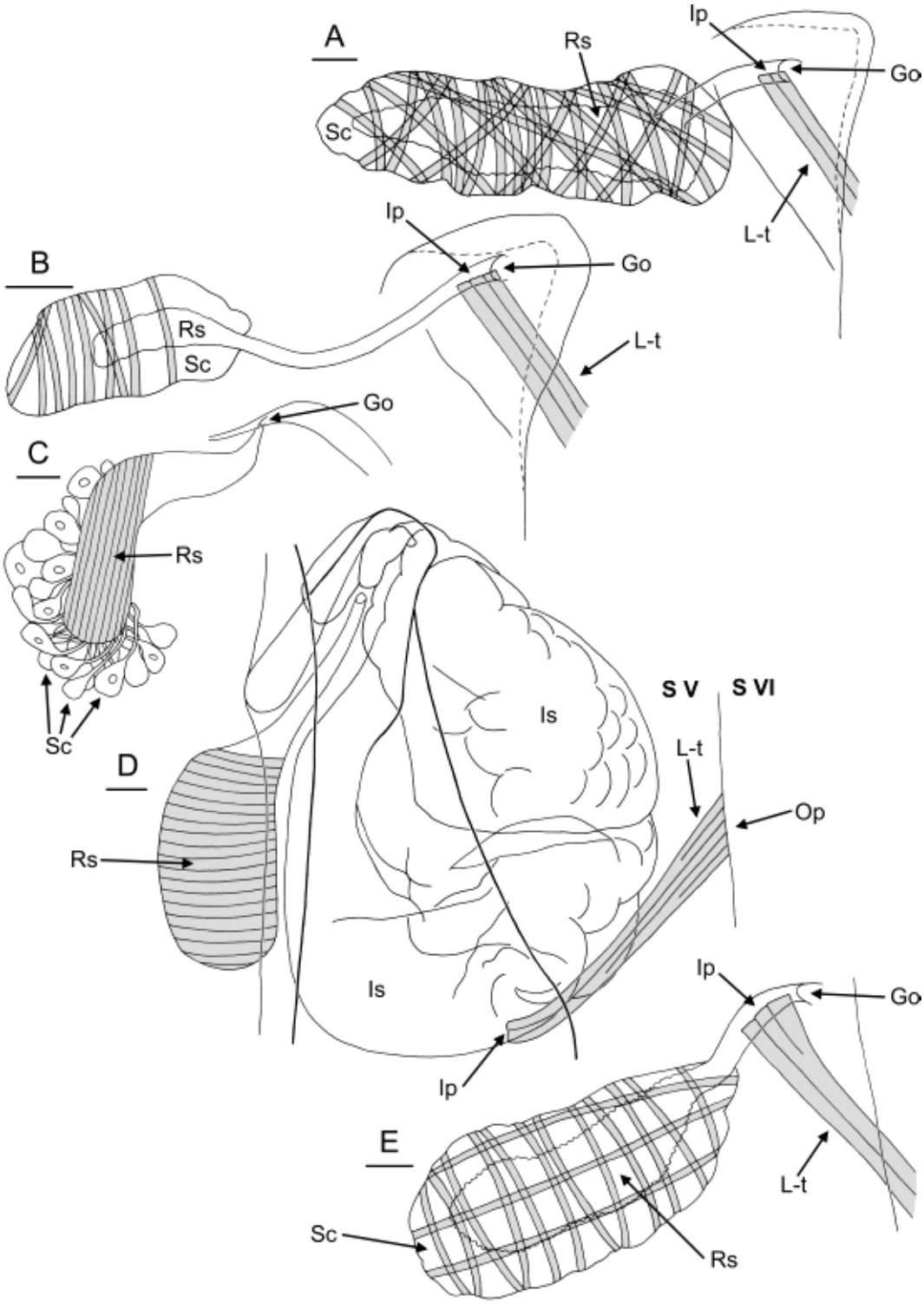
Sc
T-t
Op
D
Ip
Go
T-t
Rs
Sc
Op
E
Ip
Go
L-t
Rs
Sc
F
Go
Ip
L-t
Rs
Op
S V
S VI

FIGURE 2-10. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A-E: Glossosomatidae. A and B: *Anagapetus debilis* female and male respectively. C and D: *Agapetus walkeri* female and male respectively. Note the loosely arranged secretory cells in the female, the lack of secretory cells in the male is most likely an artefact as is the missing gland-opening muscle in the female. Note that the gland-opening muscle in the male inserts on the large involuted sac and originates at the front edge of sternum VI. Thicker black lines in the male indicate places where the cuticle bends away from the surface of the animal. E: *Protoptila cana* male, the female is similar. S V, sternum V; S VI, sternum VI; Go, gland opening; Ip, insertion point for muscle; Is, involuted sac; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; L-t, Lepidoptera-type opening muscle. Scale bars: A, B = 50 μm; C-E = 20 μm.


A
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Sc
L-t
B
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L-t
C
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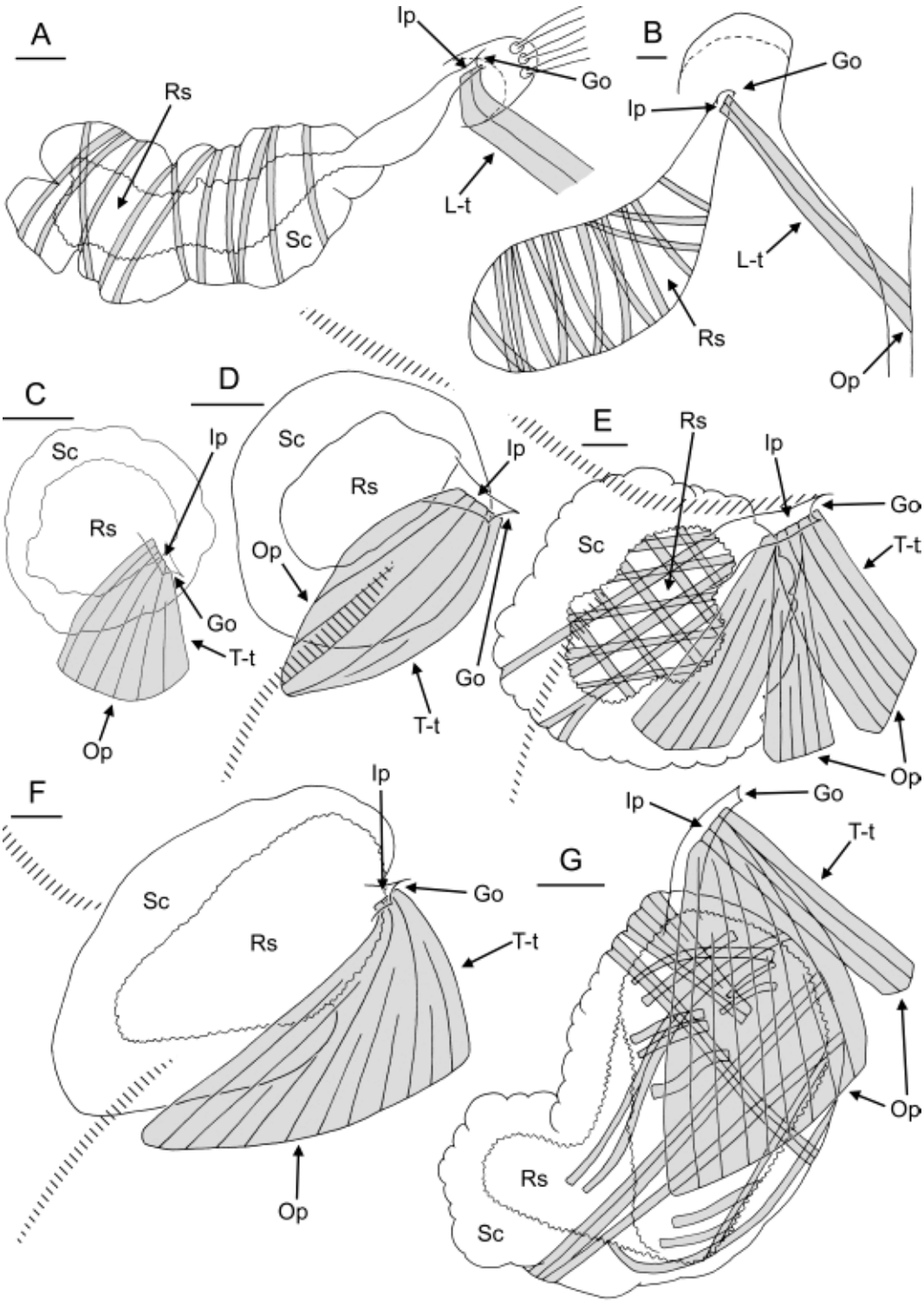
Rs
Sc
D
S V
S VI
Is
L-t
Op
Rs
Is
Ip
Ip
Go
E
L-t
Sc
Rs

FIGURE 2-11. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. Cross hatching shows the presence and position of thickened cuticular ridges. A: *Agraylea multipunctata* (Hydroptilidae) female, male very similar. B: *Palaeagaptus guppyi* (Hydroptilidae) male, female very similar. The secretory tissue was not discernible, possibly due to decomposition. C and D: *Brachycentrus occidentalis* (Brachycentridae) female and male respectively. E-F: Phryganeidae. E: *Agrypnia straminea* male. F and G: *Yphria californica* female and male respectively. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. L-t, Lepidoptera-type opening muscle. Scale bars: A, B = 20 μm; C-G = 50 μm.


A
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L-t
Sc
B
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Ip
L-t
Rs
Op
C
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Ip
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Go
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D
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Ip
Rs
Op
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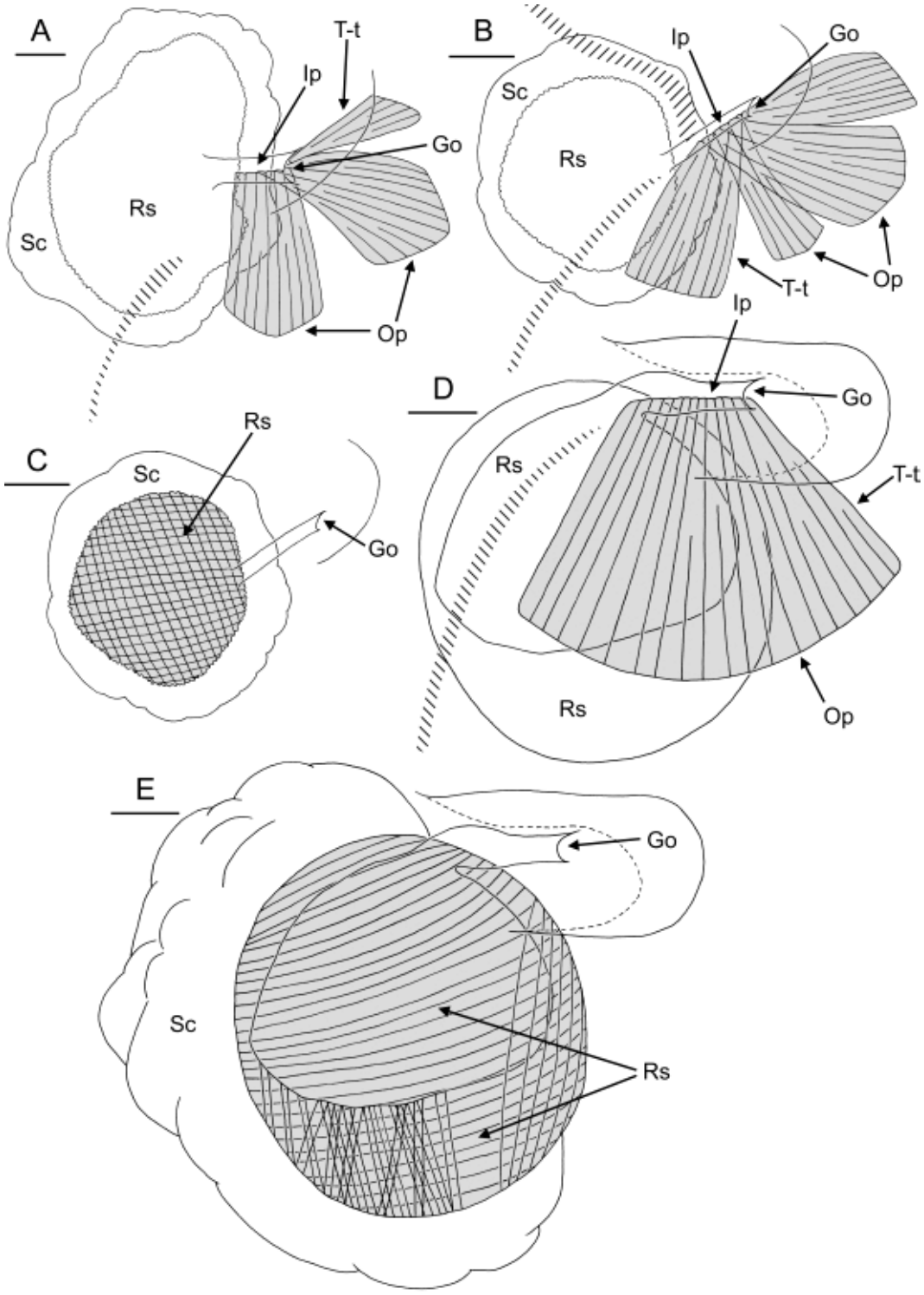
E
Rs
Ip
Go
Sc
T-t
Op
F
Ip
Sc
Go
Rs
T-t
Op
G
Ip
Go
T-t
Op
Rs
Sc

FIGURE 2-12. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. Cross hatching shows the presence and position of thickened cuticular ridges. A-C: *Phryganea cinerea* (Phryganeidae). A: Female. B: Male, showing gland-opening muscle and cuticular ridges. C: Male, showing gland reservoir musculature. D and E: *Oeconesus maori* (Oeconesidae) male. The muscle fibres nearly parallel to the long axis of the abdomen are on the side of the reservoir facing away from the body wall, the other muscle fibres are on the side facing the body wall. D: Gland-opening muscle and cuticular ridges. E: Gland reservoir musculature and secretory cells. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. Scale bars: A-C = 100 μm; D, E = 50 μm.


A
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Go
Rs
Sc
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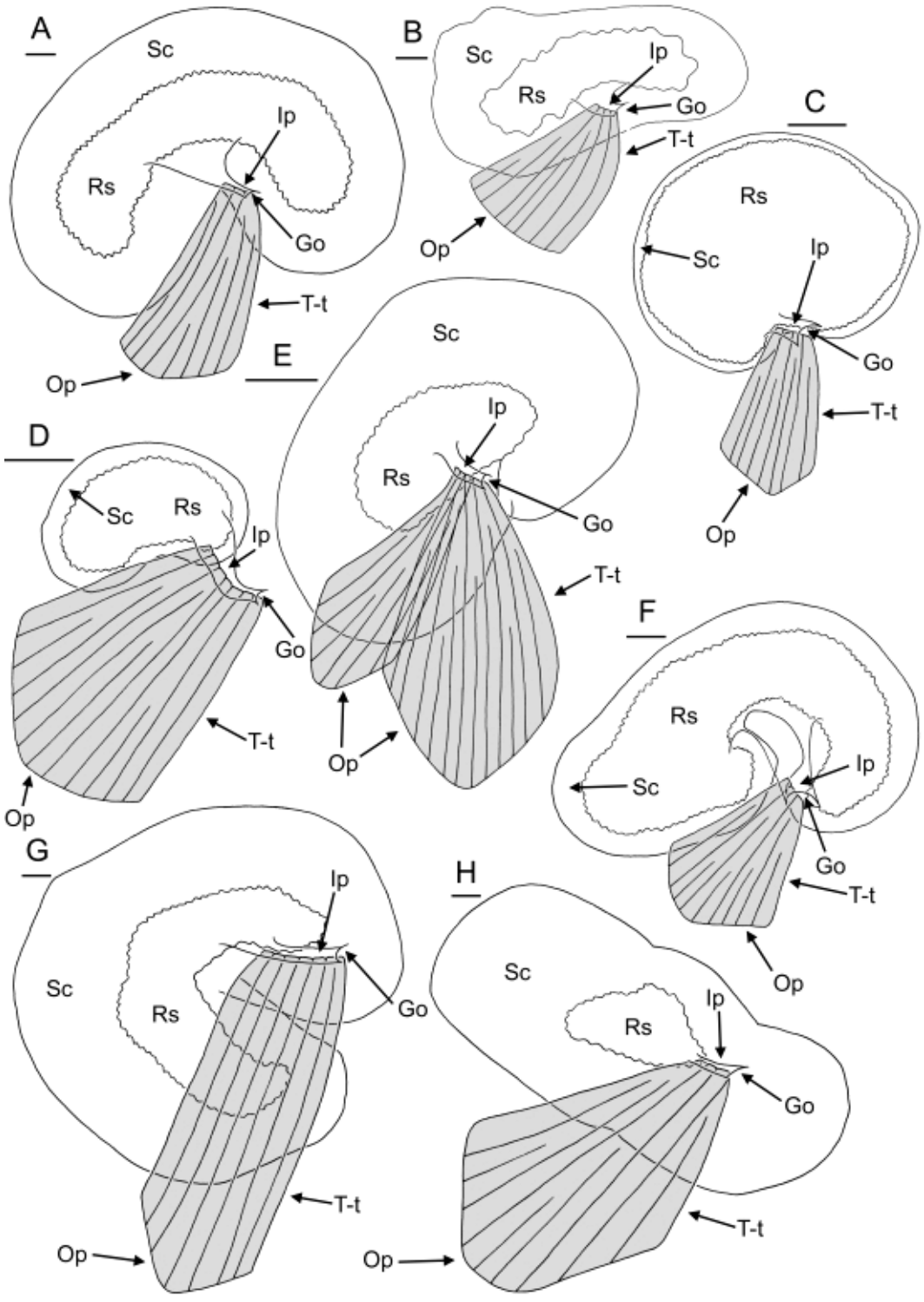
Go
Sc
Rs
T-t
Op
C
Rs
Sc
Go
D
Ip
Go
Rs
T-t
Rs
Op
E
Go
Sc
Rs

FIGURE 2-13. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A-H: Limnephilidae sensu lato, note the complete absence of gland reservoir musculature. A and B: *Neophylax concinnus* (Uenoidae) female and male respectively. Female has very typical reniform gland. C-H: Limnephilidae. C: *Onocosmoecus unicolor* (Dicosmoecinae) female, male similar. D: *Drusus annulatus* (Drusinae) male, aberrant reniform gland with gland duct at one end. Female has normal reniform gland. E: *Pseudostenophylax sparsus* (Pseudostenophylacinae) female, male similar. F-H: Limnephilinae. F: *Limnephilus secludens* female, male similar. Female *L. sericeus* similar. G and H: *L. externus* female and male respectively. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. Scale bars: A, B, G, F = 20 μm; C = 100 μm; D-F = 50 μm.


A
Sc
Ip
Rs
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T-t
Op
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Rs
Go
T-t
Op
C
Rs
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Op
D
Sc
Rs
Ip
Go
T-t
Op
E
Sc
Ip
Rs
Go
T-t
Op
F
Rs
Sc
Ip
Go
T-t
Op
G
Ip
Sc
Rs
Go
T-t
Op
H
Sc
Ip
Rs
Go
T-t
Op

FIGURE 2-14. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A and B: *Pycnopsyche lepida* (Limnephilidae: Limnephilinae) female and male respectively. Note the partial division of the gland reservoir into compartments in the male. The internal structures in female and male *P. scabripennis* are very similar to those in male *P. Lepida*. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. Scale bars: A, B = 20 μm.

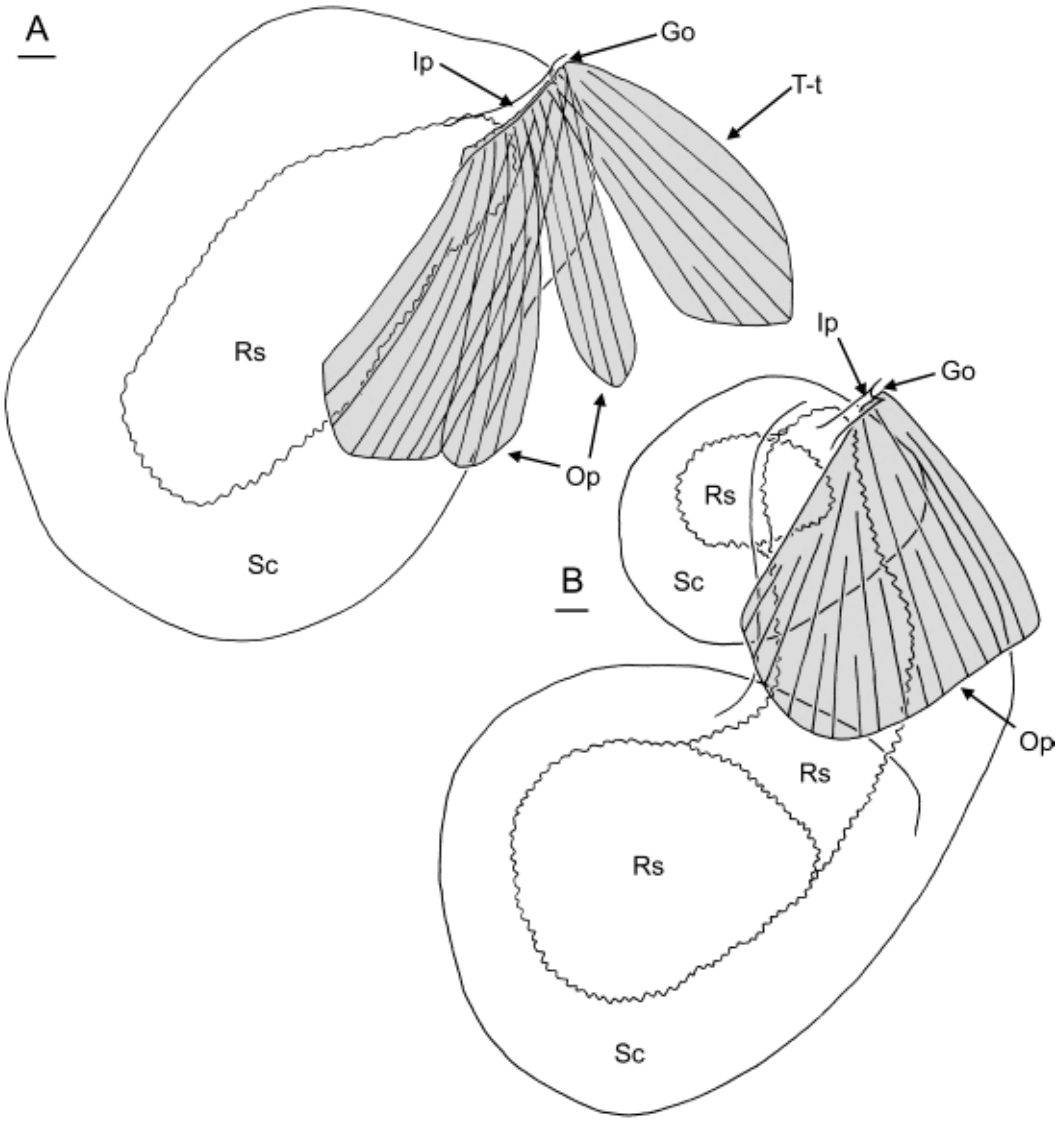

A
Go
Ip
T-t
Rs
Op
Sc
B
Ip
Go
Rs
Sc
Op
Rs
Rs
Sc

FIGURE 2-15. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. Cross hatching shows the presence and position of thickened cuticular ridges. A: *Limnocentropus grandis* (Limnocentropodidae) female. B: *Molanna flavicornis* (Molannidae) female. C: *Beraea pullata* (Beraeidae) female. D and E: *Gumaga griseola* (Sericostomatidae) female and male respectively. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle. Scale bars: A, D, E = 20 μm; B = 50 μm; C = 10 μm.

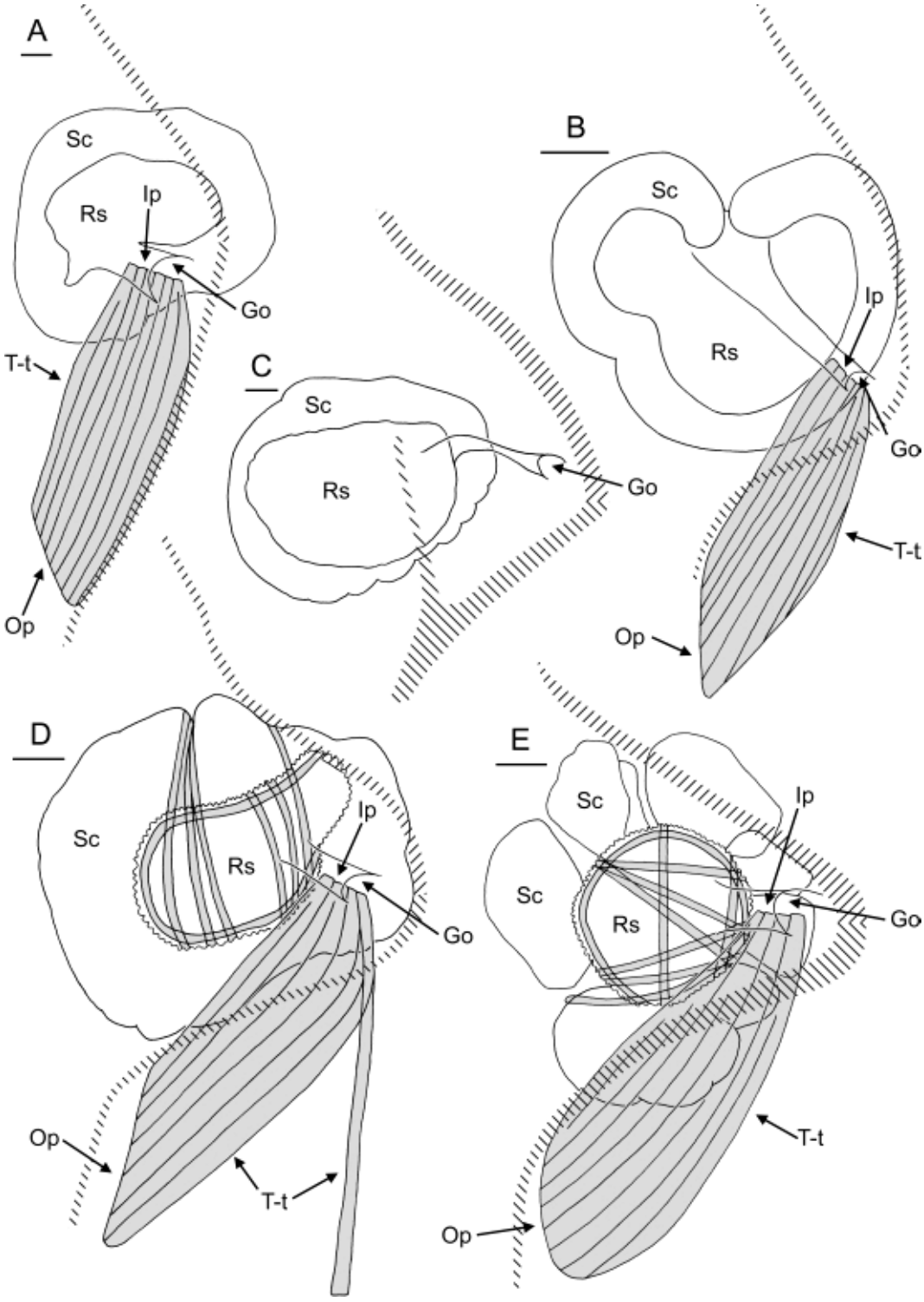

A
Sc
Rs
Ip
Go
T-t
Op
B
Sc
Rs
Ip
Go
T-t
Op
C
Sc
Rs
Go
D
Sc
Rs
Ip
Go
Op
T-t
E
Sc
Sc
Rs
Ip
Go
T-t
Op

FIGURE 2-16. Drawings based on wholemounts. The structures are viewed through the cuticle and anterior is to the left with down being mesad; thus all drawings show the left gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. Cross hatching shows the presence and position of thickened cuticular ridges. A: *Olinga feredayi* (Conoesucidae) female. Note the single muscle fibre (RT-t) originating laterad on cuticle of sternum V and inserting on the gland duct. B: *Austrocentrus griseus* (Helicophidae) female. C: *Philanisus plebeius* (Chathamiidae) female. Note the bundle of muscle fibres (RT-t) originating laterad on cuticle of sternum V and inserting on the gland duct. D: *Contulma talamanca* (Anomalopsychidae) female. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; T-t, Trichoptera-type opening muscle; RT-t, reverse Trichoptera-type opening muscle. Scale bars: A-B, D = 20 μm; C = 15 μm.

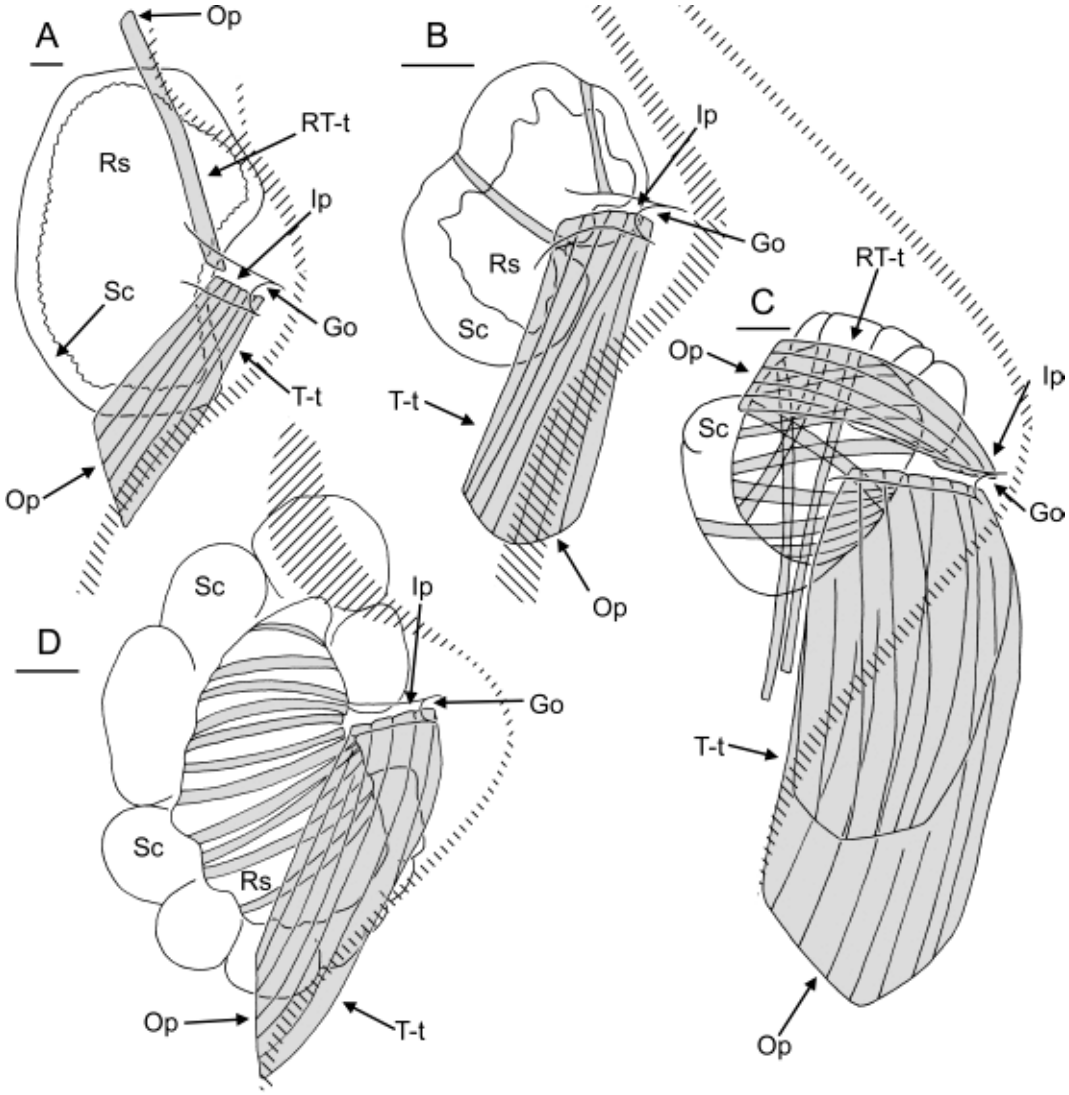

A
Op
RT-t
Rs
Ip
Sc
Go
T-t
Op
B
Ip
Go
Rs
Sc
T-t
Op
C
RT-t
Op
Ip
Sc
Go
T-t
Op
D
Sc
Ip
Go
Sc
Rs
Op
T-t

FIGURE 2-17. SEMs of external structures. A-D: Philopotamidae. A: *Wormaldia planae* female, male very similar, gland opening and surrounding scaly patch. B-D: *Chimarra obscura* female. B: Gland opening in tiny bald patch, male very similar. C: Overview of fenestra. D: Close-up of same. E: *Austrotinodes panamensis* (Ecnomidae) female, gland opening and surrounding bald area. F: *Xiphocentron haitiense* (Xiphocentronidae) male, note how the individual 'scales' of the scaly patch overlaps, female similar, with less overlap of 'scales'. Go, gland opening. Scale bars: A-C, E, F = 10 μm; D = 1 μm.

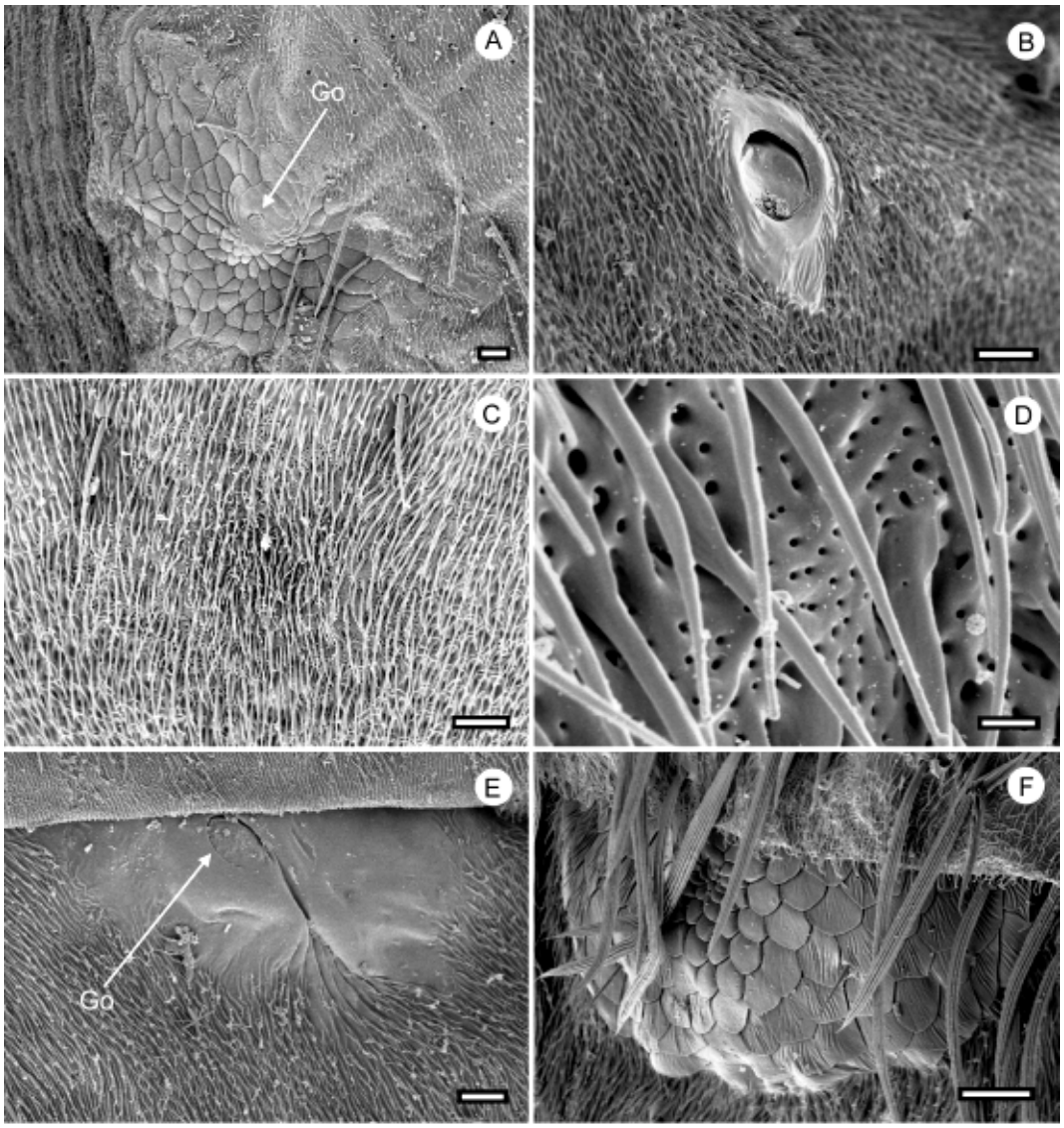

A
Go
B
C
D
E
Go
F

FIGURE 2-18. SEMs of external structures. A-D: Psychomyiidae. A: *Tinodes sigodanus* male, female very similar, gland opening and surrounding scaly patch. B-D: *Psychomyia flavida* female. B: Gland opening, note that it is situated in the membranous area between sternites IV and V, not on sternite V. C: Overview of perforated patch, the clear distinctive surface structure of the perforated patch was only found in this species. D: Close-up of same. E-H: *Polycentropus cinereus* (Polycentropodidae), male and female very similar. E: Male, protuberance on sternum V, note the groove running the length of the protuberance. F: Male, close-up of groove at base of protuberance, note the wavy cuticle along the groove. G: Female, close-up of gland opening, note the slight groove connecting the gland orifice to the groove running the length of the protuberance. H: Female, close-up of apex of protuberance, again note the wavy cuticle along the groove. Go, gland opening. Scale bars: A-C, E, H = 10 μm; D, F, G = 1 μm.

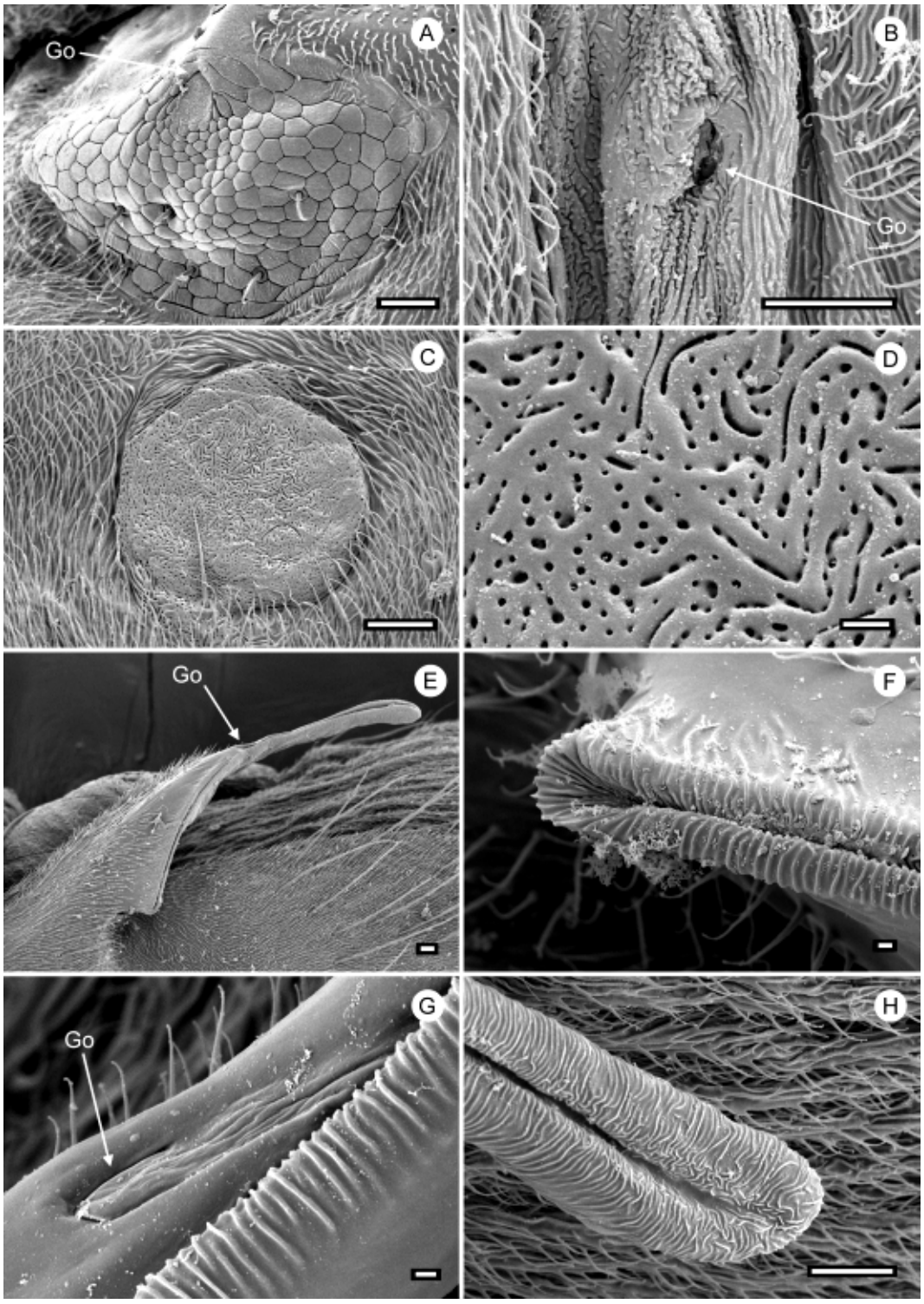
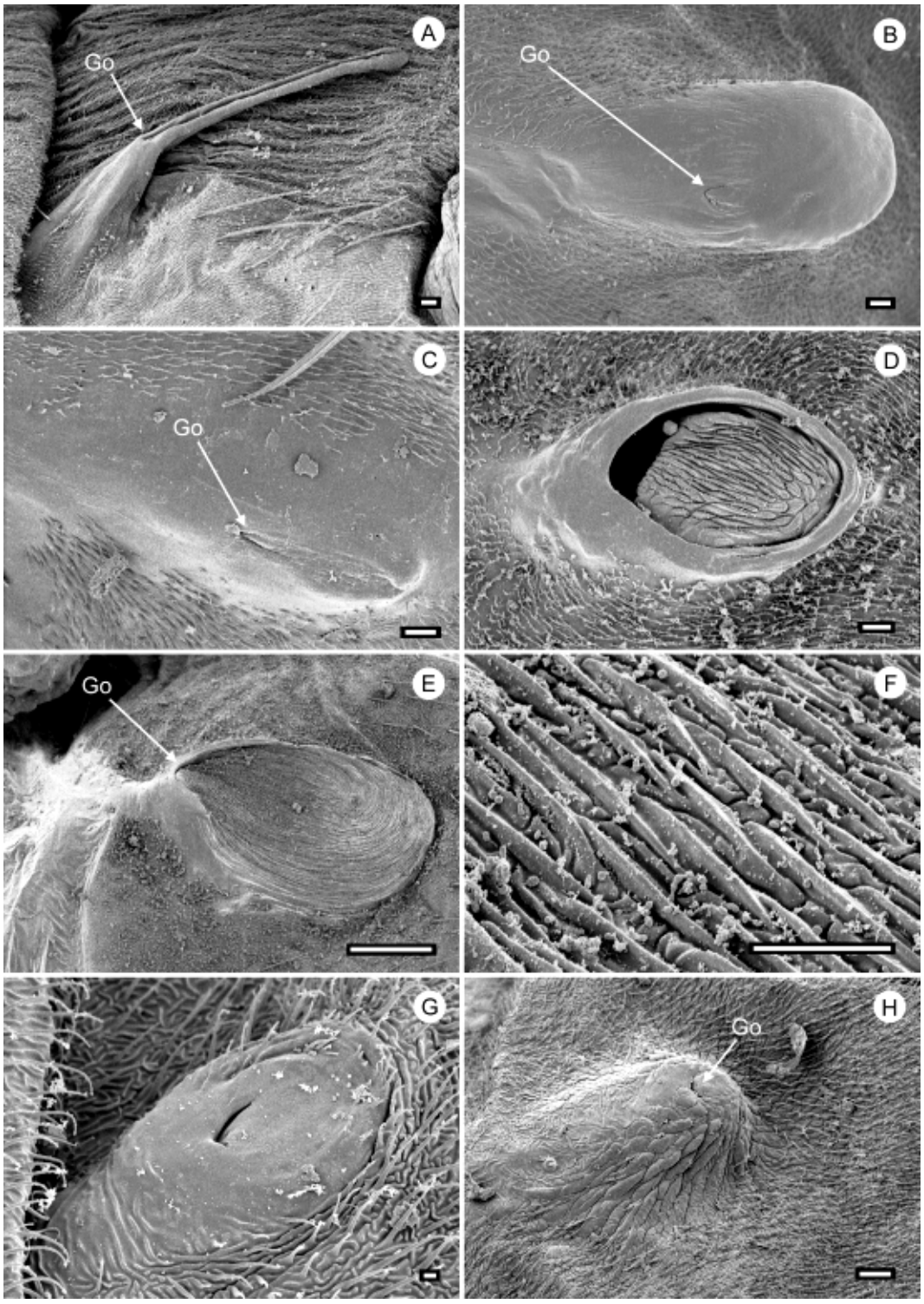

A
Go
B
Go
C
D
E
Go
F
G
Go
H

FIGURE 2-19. SEMs of external structures. A: *Cyrnellus fraternus* (Polycentropodidae) female, protuberance on sternum V, note the groove running from the gland opening to the apex. B-H: Hydropsychidae. B, C: Arctopsychinae. B: *Arctopsyche grandis* female, male very similar, gland opening on bald protuberance. C: *Parapsyche elsis* male, female very similar, gland opening on small bald protuberance. D-F: *Leptonema albovirens* (Macronematinae). D: Female. E: Male. Both: gland opening at anterior end of ovoid area of textured cuticle, area much larger in male. F: Male, close-up of textured cuticle. G, H: Hydropsychinae. G: *Cheumatopsyche speciosa* male, female very similar, gland opening on small bald bulge. H: *Hydropsyche placoda* male, female very similar, gland opening on bulge. Go, gland opening. Scale bars: A-D, F, H = 10 μm; E = 100 μm; G = 1 μm.


A
Go
B
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C
Go
D
E
Go
F
G
H
Go

FIGURE 2-20. SEMs of external structures. A-F: Hydropsychidae. A-C: *Diplectrona* sp. (Diplectroninae) female, male very similar. A: Protuberance on sternum V, note the groove running from the gland opening to the apex. B: Close-up of gland opening and start of groove. C: Close-up of apex with microtrichia. D-F: *Asmicridea edwardsii* (Smicrideinae), male and female very similar. D: Male, protuberance on sternum V, note the groove running from the gland opening to the apex. E: Female, close-up of gland opening and start of groove. F: Male, close-up of apex, note the dense brush-like cover of microtrichia. Go, gland opening. Scale bars: A, D = 100 μm; B = 1 μm; C, E, F = 10 μm.

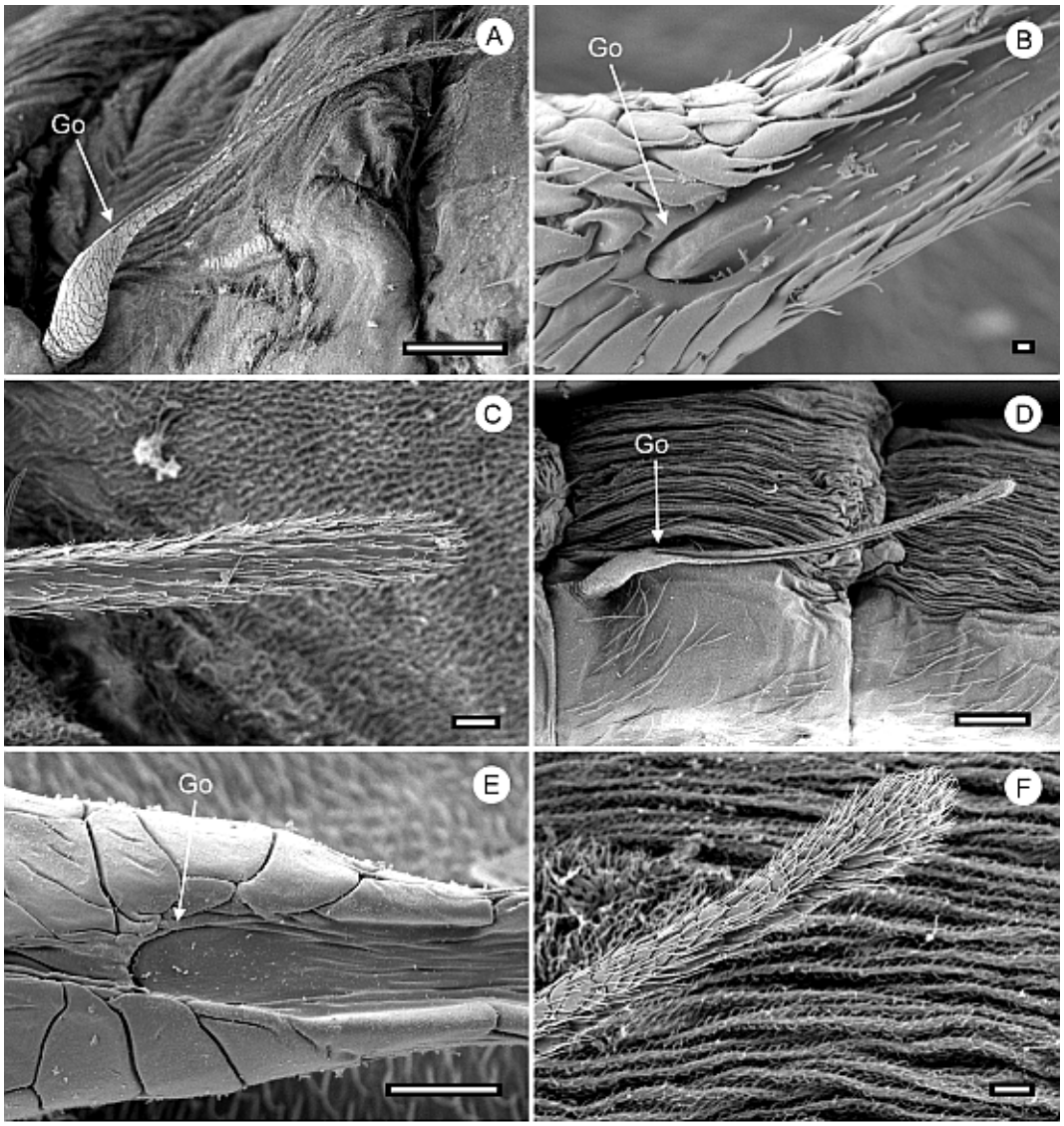

A
Go
B
Go
C
D
Go
E
Go
F

FIGURE 2-21. SEMs of external structures. A-D: Hydrobiosidae. A-C: *Apsilochorema segitiga* female. A: Overview of gland opening, connecting groove and ventral protuberance with stout setae. B: Close-up of gland opening, note how the direction of the microtrichia would guide gland products towards the groove. C: Close-up of setae of ventral protuberance, note the wear on the setae, indicating that the protuberance is dragged along the substrate. D: *Atopsyche callosa* male, protuberance with gland opening. E, F: *Rhyacophila arnaudi* (Rhyacophilidae). E: Female, protuberance with gland opening and setae. F: Male, part of protuberance with gland opening and setae. Go, gland opening. Scale bars: A, E = 100 μm; B-D, F = 10 μm.

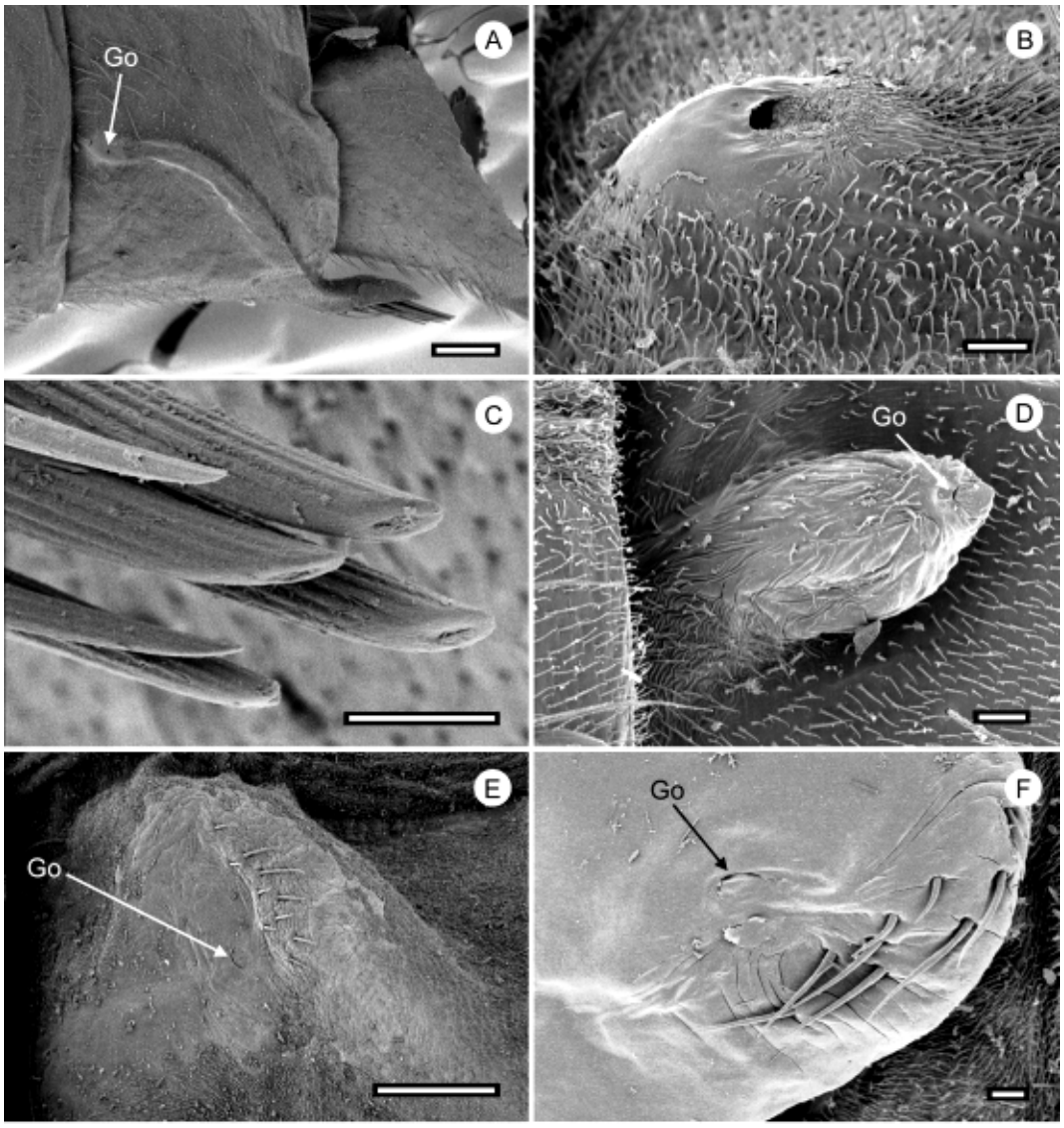

A
Go
B
C
D
Go
E
Go
F
Go

FIGURE 2-22. SEMs of external structures. A-C: Glossosomatidae. A, B: *Anagapetus debilis* female, male very similar. A: gland opening on protuberance. B: Close-up of reticulated cuticle on protuberance. C: *Protoptila cana* female, gland opening in bald area adjacent to transverse groove. D-F: Hydroptilidae. D: *Palaeagapetus guppyi* (Ptilocolepinae) female, male very similar, gland opening on protuberance. E, F: Hydroptilinae sp. female, male very similar. E: Overview of protuberance with gland opening and long three-flanged seta. F: Close-up of three-flanged setae. Go, gland opening. Scale bars: A, C-E = 10 μm; B, F = 1 μm.

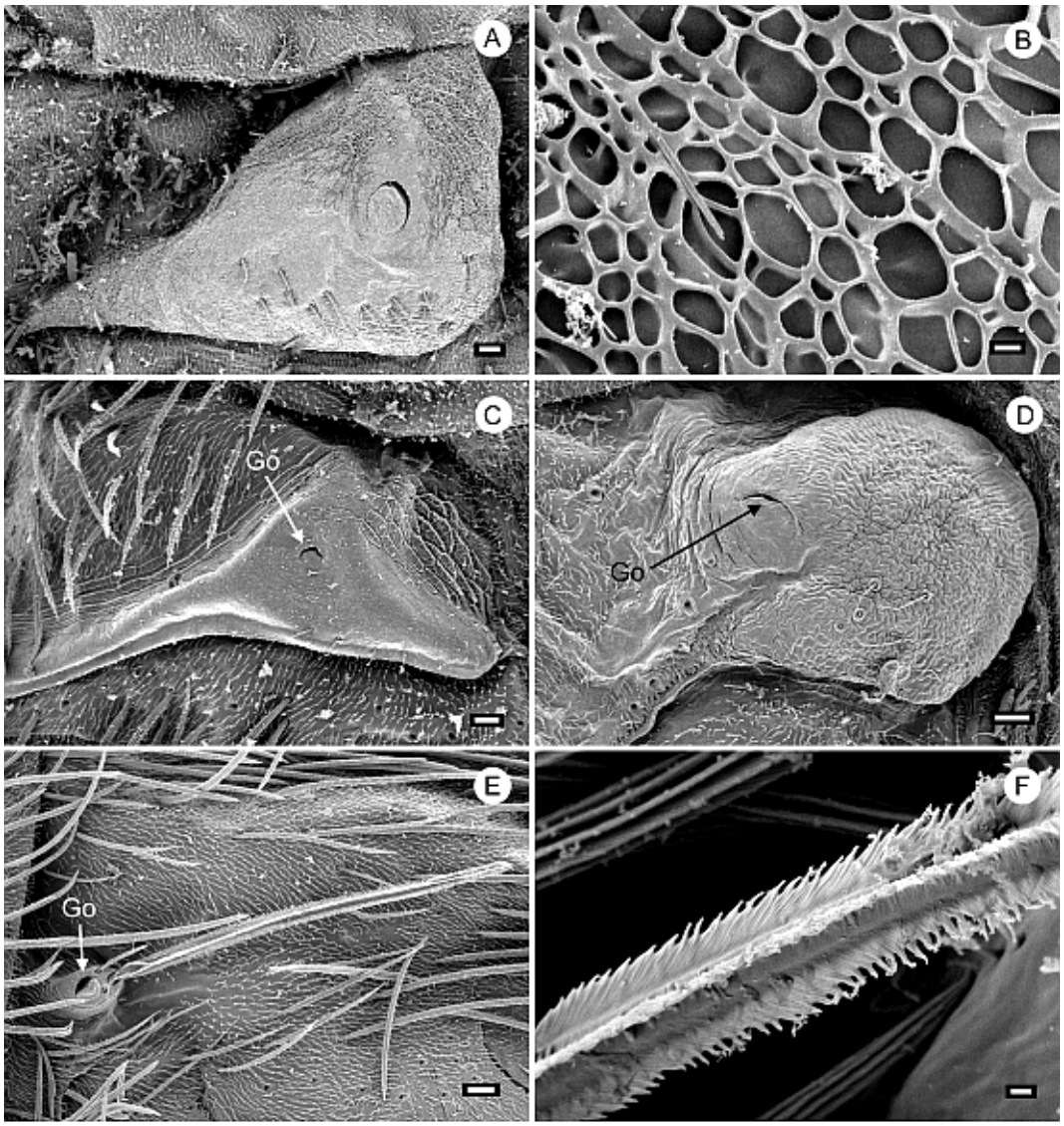

A
B
C
Go
D
Go
E
Go
F

FIGURE 2-23. SEMs of external structures. A, B: *Brachycentrus occidentalis* (Brachycentridae), male and female very similar. A: Female, hexagons with grooves in between covering sternum V. B: Male, gland opening connecting with grooves between hexagons. C, D: Phryganeidae. C: *Yphria californica* male, gland opening in bald, slightly raised area. Female similar, but bald area not raised. D: *Agrypnia straminea* male, gland opening in small bald area. E: *Oeconesus maori* (Oeconesidae) male, gland opening on bald thumb-shaped protuberance. F: *Neophylax concinnus* (Uenoidae) male, gland opening in small bald area, bald area larger in female. Go, gland opening. Scale bars: A, B, D, F = 10 μm; C, E = 100 μm.

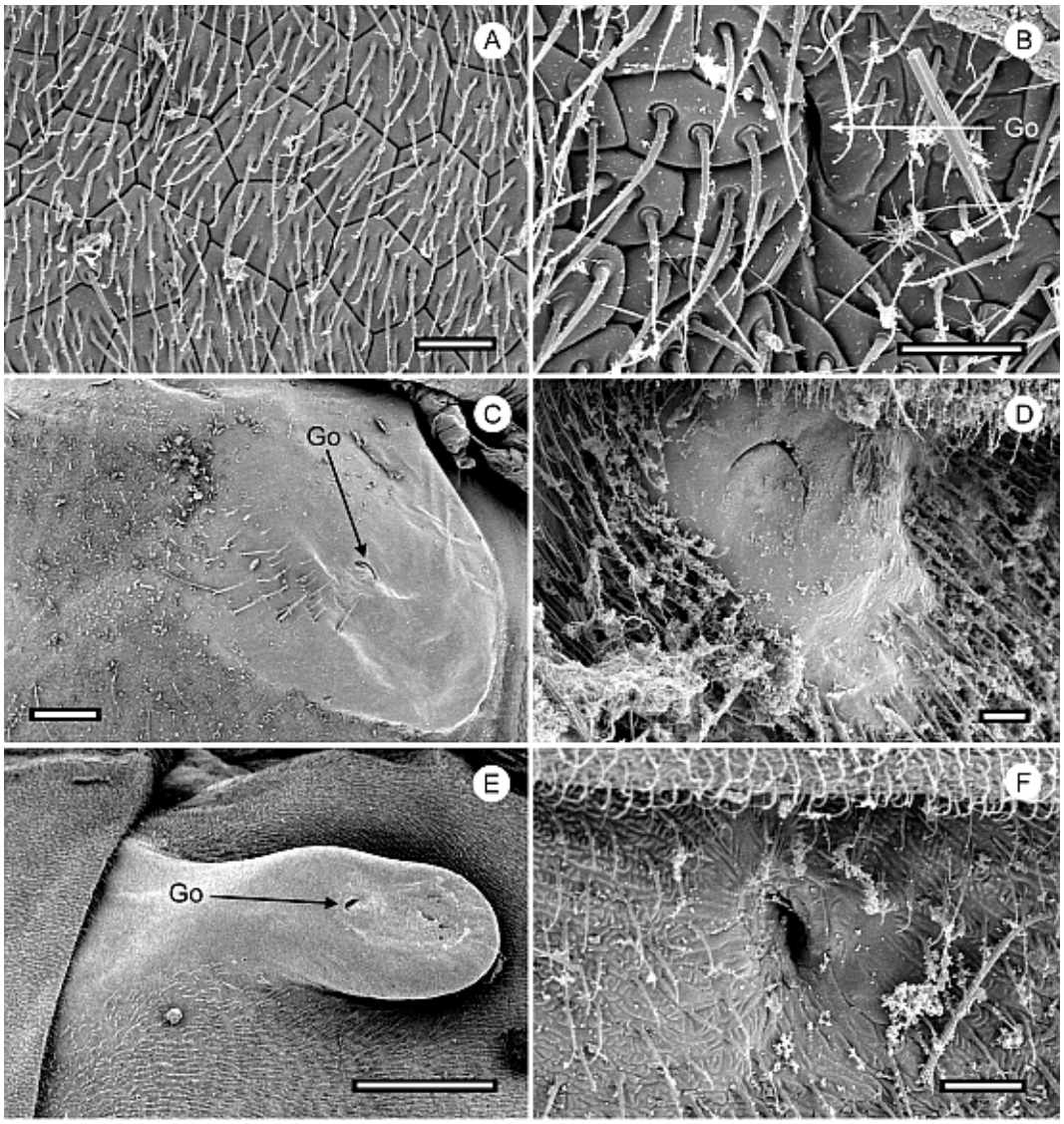

A
B
Go
C
Go
D
E
Go
F

FIGURE 2-24. SEMs of external structures. A: *Apatania zonella* (Apataniidae) female, male very similar, gland opening connecting with grooves between hexagons. B-E: Limnephilidae. B: *Onocosmoecus unicolor* (Dicosmoecinae) male, female very similar, gland opening adjacent to small sculpted patch. C: *Pseudostenophylax sparsus* (Pseudostenophylacinae) male, female very similar, gland opening and edge of area covered with hexagons. D, E: Limnephilinae. D: *Limnephilus secludens* male, gland opening, other examined *Limnephilus* specimens similar. E: *Pycnopsyche lepida* male, gland opening on protuberance, protuberance smaller in female. F-G: Brevitentoria, other examined brevitentorians very similar. F: *Limnocentropus grandis* (Limnocentropodidae) female, gland opening in bald area extending from front edge. G: *Gumaga griseola* (Sericostomatidae) male, female very similar, gland opening in bald area extending from front edge. H: *Austrocentrus griseus* (Helicophidae) female, gland opening in bald area extending from front edge. Go, gland opening. Scale bars: A-D, F-H = 10 μm: E = 100 μm.

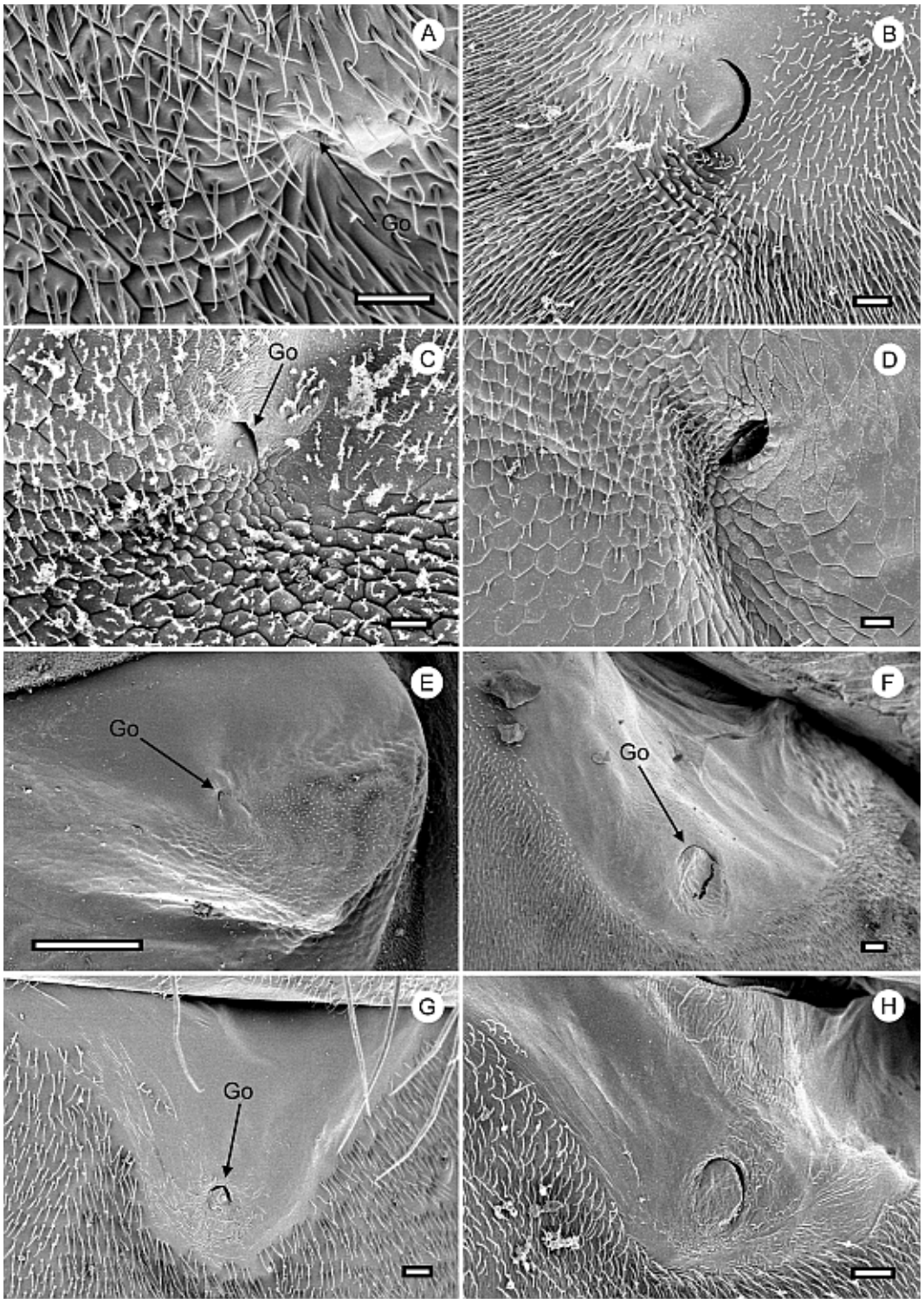

A
Go
B
C
Go
D
E
Go
F
Go
G
Go
H

FIGURE 2-25. SEMs of internal structures, all specimens have been treated with KOH. A-D: *Arctopsyche grandis* (Hydropsychidae) female. A: Overview showing gland reservoir. B: Close-up of gland duct. Note the U-shape (in cross section) of the duct and how the muscle fibres insert on inside curve of the U-shaped duct. C: Close-up of surface of reservoir, note the numerous ductules connecting to the reservoir. The ductules each connect a secretory cell to the reservoir (secretory cells dissolved by KOH treatment). D: Close-up of end apparatus of a single ductule. The end apparatus is part of the ductule cell, but is surrounded by the secretory cell. E: *Psychomyia flavida* (Psychomyiidae) female. Note how the gland duct connects to the membranous cuticle between sternite IV and V. In the live animal, the gland reservoir is pressed against the inside of the perforated patch. F: *Anagapetus debilis* (Glossosomatidae) female. Overview showing reservoir and gland duct, in the live animal the reservoir is situated along the body wall, anteriad from the gland opening. Dt, gland duct; Go, gland opening; Ip, insertion point; Mf, muscle fibres; Ppi, perforated patch, internal; Rs, gland reservoir; S IV, sternum IV; S V, sternum V. Scale bars: A-C, E = 10 μm, D = 100nm; F = 100 μm.

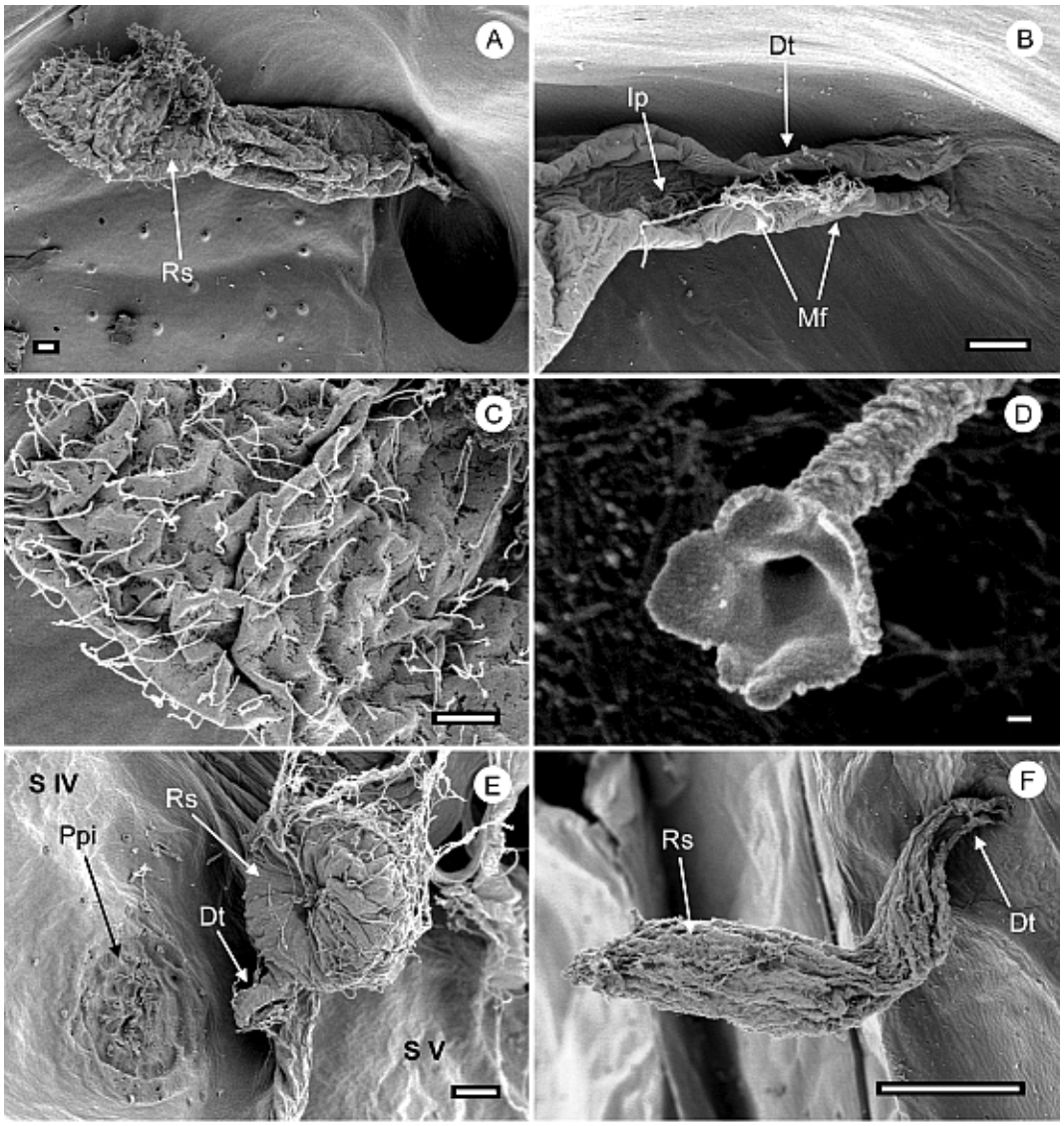

A
Rs
B
Dt
lp
Mf
C
D
S IV
Rs
Ppi
E
Dt
S V
F
Rs
Dt

FIGURE 2-26. SEMs of internal structures, A, B, E were freshly killed specimens dissected in saline, C, D, F-H have been treated with KOH. A, B: *Phryganea cinerea* (Phryganeidae) male. A: Overview showing sternum V gland in situ. B: Close-up of criss-crossing muscle fibres surrounding gland reservoir. C-E: Limnephilidae. C, D: *Onocosmoecus unicolor* female. C: Overview showing the reniform gland reservoir, note the short gland duct. D: Close-up of gland duct, note the U-shape (in cross section). E: *Limnephilus harrimani* female, gland reservoir in situ. F: *Limnocentropus grandis* (Limnocentropodidae) female, gland reservoir. G, H: *Gumaga griseola* (Sericostomatidae) female. G: Overview showing gland reservoir and internal cuticular ridge extending from antecosta on both sides of gland opening and connecting in a smooth curve posterior to gland opening. In the live animal the reservoir is situated along the body wall, anteriad from the gland opening. H: Close-up of end apparatus of a single ductule. The end apparatus is part of the ductule cell, but is surrounded by the secretory cell (secretory cells dissolved by KOH treatment). Cr, cuticular ridge; Rs, gland reservoir; S V g, sternum V gland. Scale bars: A, C, E = 100 μm; B, D, F, G = 10 μm; H = 1 μm.

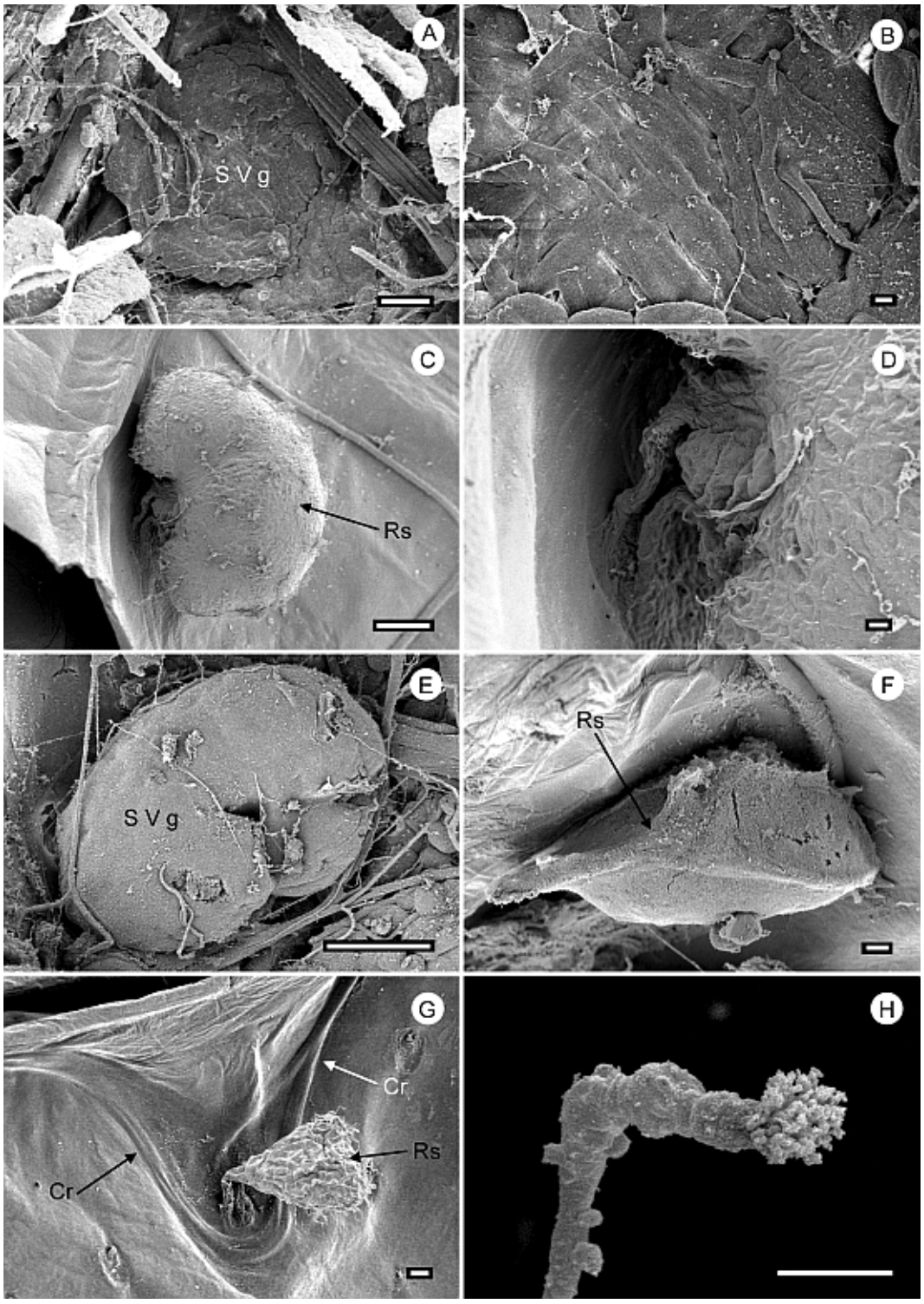
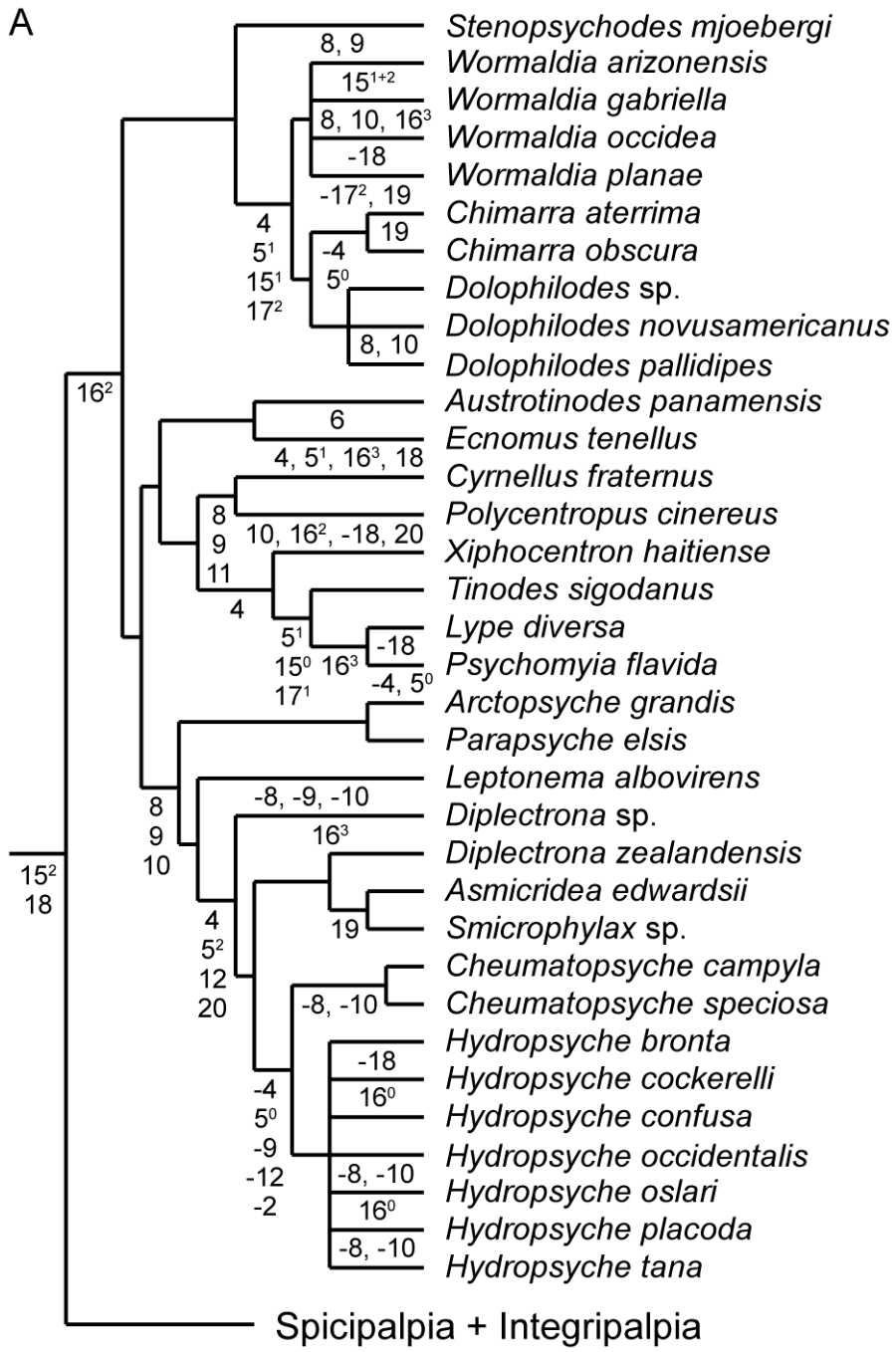
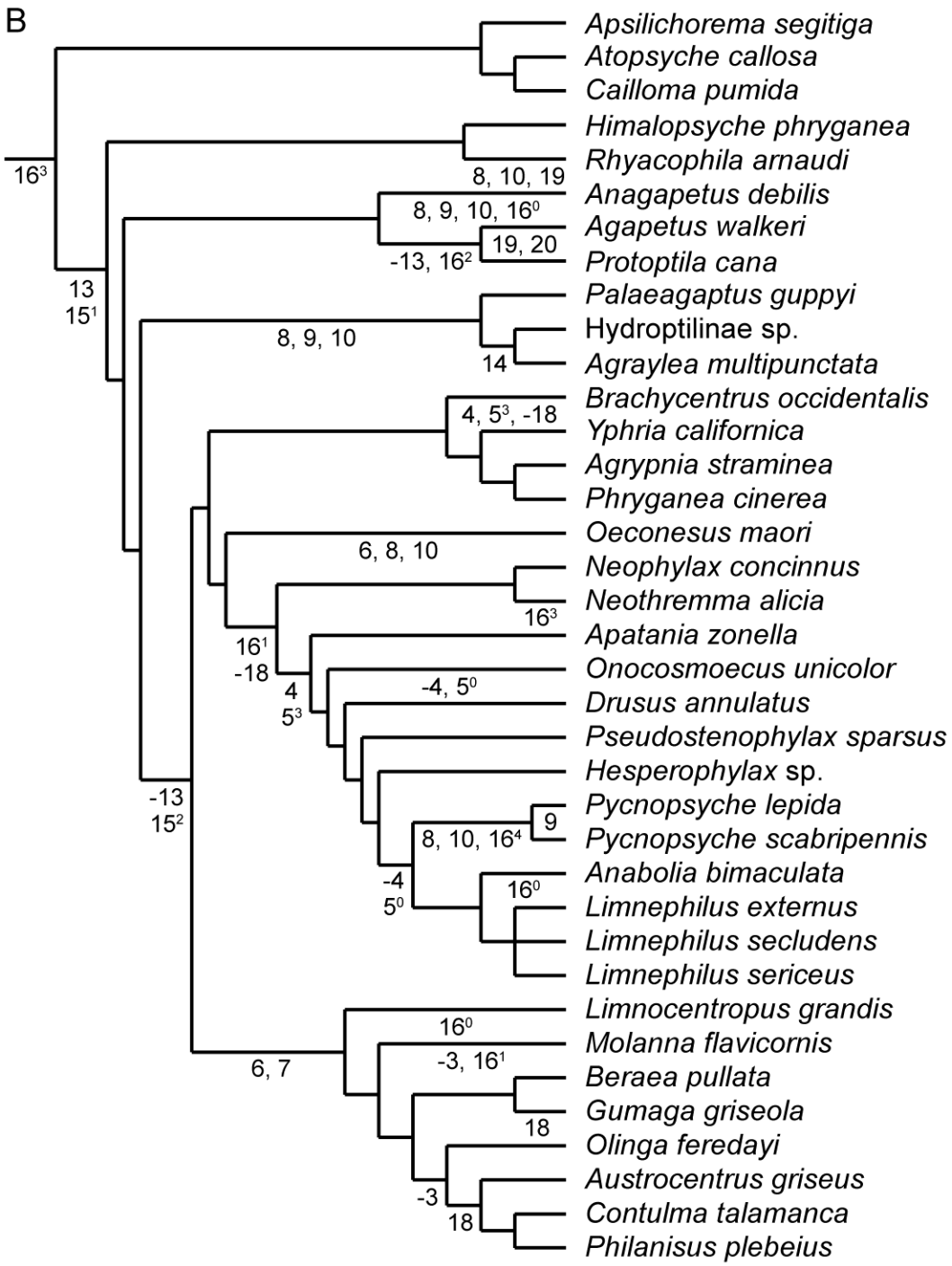

A
SVg
B
C
Rs
D
E
SVg
F
Rs
G
Cr
Rs
Cr
H

FIGURE 2-27. Tree illustrating mapping of gland characters with parsimony reconstruction of ancestral states. Only taxa in which the gland is present are included as presence/absence is illustrated in Figure 2-1. Apart from taxa in which the gland is absent, I excluded *Limnephilus harrimani* (Limnephilidae) as most characters for this species were scored as unknown, and several other *Limnephilus* species are included. 2-27 A shows Annulipalpia and 2-27 B shows Spicipalpia + Integripalpia. The characters are described in Materials and Methods, with references to appropriate figures. Characters 1 (gland presence/absence) and 2 (gland presence/absence in females) are not included here; character 1 because it is present in all included taxa, character 2 because it is present or unknown in all included taxa. If two or more equally parsimonious reconstructions were presented for a single trait, I chose to minimize independent origins of the trait. The exception to this is character 10 (presence/absence of protuberance in males). This character is a subset of character 8 (presence (in either sex)/absence of protuberance), and was treated as such; character 10 could not be present if character 8 was not. Ancestral reconstruction of character 10 by Mesquite differed from character 8 due to a larger number of taxa scored as unknown for character 10. For binary characters a minus in front of the character number indicate a loss. For multistate characters the character state is indicated with superscript numbers appended to the character numbers, and all state changes are indicated.


A
Stenopsychodes mjoebergi
Wormaldia arizonensis
Wormaldia gabriella
Wormaldia occidea
Wormaldia planae
Chimarra aterrima
Chimarra obscura
Dolophilodes sp.
Dolophilodes novusamericanus
Dolophilodes pallidipes
Austrotinodes panamensis
Ecnomus tenellus
Cyrnellus fraternus
Polycentropus cinereus
Xiphocentron haitiense
Tinodes sigodanus
Lype diversa
Psychomyia flavida
Arctopsyche grandis
Parapsyche elsis
Leptonema albovirens
Diplectrona sp.
Diplectrona zealandensis
Asmicridea edwardsii
Smicrophylax sp.
Cheumatopsyche campyla
Cheumatopsyche speciosa
Hydropsyche bronta
Hydropsyche cockerelli
Hydropsyche confusa
Hydropsyche occidentalis
Hydropsyche oslari
Hydropsyche placoda
Hydropsyche tana
Spicipalpia + Integripalpia


B
Apsilichorema segitiga
Atopsyche callosa
Cailloma pumida
Himalopsyche phryganea
Rhyacophila arnaudi
Anagapetus debilis
Agapetus walkeri
Protoptila cana
Palaeagaptus guppyi
Hydroptilinae sp.
Agraylea multipunctata
Brachycentrus occidentalis
Yphria californica
Agrypnia straminea
Phryganea cinerea
Oeconesus maori
Neophylax concinnus
Neothremma alicia
Apatania zonella
Onocosmoecus unicolor
Drusus annulatus
Pseudostenophylax sparsus
Hesperophylax sp.
Pycnopsyche lepida
Pycnopsyche scabripennis
Anabolia bimaculata
Limnephilus externus
Limnephilus secludens
Limnephilus sericeus
Limnocentropus grandis
Molanna flavicornis
Beraea pullata
Gumaga griseola
Olinga feredayi
Austrocentrus griseus
Contulma talamanca
Philanisus plebeius
16^3
8, 10, 19
8, 9, 10, 16^0
19, 20
-13, 16^2
13
15^1
8, 9, 10
14
4, 5^3, -18
6, 8, 10
16^3
16^1
-18
4
5^3
-4, 5^0
-13
15^2
9
8, 10, 16^4
-4
5^0
16^0
16^0
-3, 16^1
6, 7
18
-3
18

Chapter 3

The sternum V glands in Lepidoptera

INTRODUCTION

The sternum V gland is found in many of the most basal lepidopterans as well as in Trichoptera. Since it is not found in any other insects it is considered one of the autapomorphies of Amphiesmenoptera (Lepidoptera and Trichoptera) (Kristensen, 1981). Lepidoptera are one of the 'big four', the four most speciose insect orders, and lepidopterans are a vital part of terrestrial ecosystems and important indicators of environmental status. Understanding the basic 'bauplan' of the Lepidoptera is important for understanding the evolutionary trajectories at the base of this diverse order. The sternum V gland has been included in several morphological studies of basal lepidopterans (mainly Davis, 1975, 1978; Kristensen & Nielsen, 1979; Kristensen, 1984; Nielsen & Kristensen, 1996), but the most recent study focusing entirely on the sternum V gland in Lepidoptera is a short paper by Razowski (1975).

The sternum V glands are a pair of invaginations from the anterolateral part of sternum V, and the basic structure of each gland consists of a duct leading to an anteriad reservoir with associated secretory cells (Kristensen, 1984). An opening muscle that originates on the anterior edge of sternum VI inserts on the gland duct just inside the gland orifice, and is likely a neoformation (Kristensen, 1984). The sternum V glands are present in representatives of five lepidopteran families (Micropterigidae, Agathiphagidae, Heterobathmiidae, Eriocraniidae and Lophocoronidae) with features associated with the gland present in representatives of two additional families (Neopseustidae and Nepticulidae) (Davis, 1975; Kristensen & Nielsen, 1979).

In micropterigids the gland is absent in members of two genera, *Micropterix* and *Hypomartyria*, but when present it occurs in both sexes (Philpott,

1925; Razowski, 1975; Kristensen, 1984). The gland opening might be situated on a setose protuberance (representatives of *Sabatinca*, *Agrionympha* and *Squamicornia*) or in a hyaline area surrounded by serrate scale-like structures (representatives of *Epimartyria*, *Paramartyria*, *Palaeomicroides* and *Neomicropteryx*) (Philpott, 1925; Kristensen, 1984). The opening muscle runs internally to the ventrolongitudinal musculature (Kristensen, 1984). The internal gland structures are quite uniform throughout the family, with a narrow duct and a terminal saccular reservoir covered with type 3 secretory cells; the entire reservoir is surrounded by a network of muscle fibres contraction of which will cause expulsion of the gland contents (Kristensen, 1984).

In agathiphagids, only males possess the sternum V gland; the gland itself is tube-like and coiled, with a thin-walled reservoir, which is considered an autapomorphy of the family (Kristensen, 1984, 1998).

In heterobathmiids, the gland is present in both sexes. The opening is situated on a tongue-shaped protuberance with domed epidermal cells that cover the distal surface and ridges between the epidermal cells around the base; the gland reservoir is nearly spherical (Kristensen & Nielsen, 1979).

In eriocraniids, the gland is generally present in both sexes, although absent in males in some species (Le Cerf, 1926; Davis, 1978). The gland orifice is situated on a protuberance or in a raised oval area with a reticulate network which may enhance the dispersal of gland products (Le Cerf, 1926; Davis, 1978). The rounded gland reservoirs are closely associated with the cuticle, and in females the cuticle in this area is transparent; the transparent cuticle is denoted fenestra (Davis, 1978). The function of the fenestra has been the subject of some speculation. Razowski (1975) proposed a separate glandular function for the fenestra. Davis (1975), like Philpott (1925), noted that the fenestrae have no external openings or internal ducts and suggested that the fenestrae are not necessarily glands, but might have a chemosensory function. Davis later (1978) concluded that the fenestrae simply mark where the gland reservoirs are pressed against the cuticle and do not have a glandular function. Ivanov and Melnitsky

(2002) suggested that fenestrae not associated with a reservoir or an excurrent duct were the most primitive form of the sternum V glands.

In lophocoronids, the gland is present in both sexes, with the gland orifice situated in an ellipsoid patch of sculptured cuticle with polygonal domes; the reservoir is covered with type 3 secretory cells and surrounded by a loose network of muscle fibres (Nielsen & Kristensen, 1996).

In female neopseustids and nepticulids, Davis (1975) found no glands, but did find fenestrae, a pair of transparent patches on sternum IV, resembling those found in female eriocraniids.

The sternum V gland's general function is pheromone production, more specifically production of sex pheromones in females (Wood & Resh, 1984; Resh & Wood, 1985; Solem, 1985; Löfstedt et al., 1994; Zhu et al., 1995; Kozlov et al., 1996; Bjostad et al., 1996; Jewett et al., 1996; Bergman et al., 2001, 2002). In Lepidoptera, the function of the sternum V gland is only directly known in some eriocraniids in which females use it to attract males (Zhu et al., 1995; Kozlov et al., 1996). However, Kozlov and Zvereva (1999) did not find any evidence for attraction pheromones in two species of *Sabatinca* (Micropterigidae). Tóth et al. (1995) found that female nepticulids attracted males with substances similar to those isolated from the sternum V glands in Eriocraniidae and various trichopterans (e.g. Löfstedt et al., 1994; Zhu et al., 1995; Kozlov et al., 1996; Bergman et al., 2001, 2002). However, Tóth et al. (1995) did not link the pheromone production to a specific body part. Nevertheless, this could indicate that structures associated with the fenestrae are involved in pheromone production and are derived from the sternum V glands. Löfstedt and Kozlov (1997) stated that identification of the pheromone-producing glands in nepticulids is critical to understanding the evolution of pheromone communication in Lepidoptera.

I examine representatives of all the major groups of basal Lepidoptera that possess either the gland or features associated with the gland. As the gland function is generally unknown in males, I specifically investigate morphological differences in the sternum V gland between the sexes. I investigate whether the fenestrae 1) are simply areas of hyaline cuticle (Davis, 1978); 2) have a separate

glandular function (Razowski, 1975); 3) are associated with normal, but overlooked sternum V glands in neopseustids and nepticulids; or 4) are reduced, but still functional sternum V glands. Based on the well known phylogeny of the most primitive Lepidoptera (Kristensen & Skalski, 1998), I discuss the evolutionary history of the sternum V gland.

MATERIALS AND METHODS

Taxon sampling

I examined representatives from all lepidopteran families known to possess the sternum V gland. I also examined representatives from the two families known to have fenestrae, a pair of patches of transparent cuticle on sternum IV, in the females without known associated secretory or glandular structures. The families in which the gland is known to be present in at least some representatives are: Micropterigidae, Agathiphagidae, Heterobathmiidae, Eriocraniidae and Lophocoronidae. The two families whose representatives are only known to possess fenestra are Neopseustidae and Nepticulidae. All the investigated species are listed in Table 3-1.

Specimen preparation

Some of the material supplied by the Natural History Museum of Denmark was in the form of prepared slides (wholemounts and some series of histological sections), prepared using standard methods. Material prepared by the author was fixed in either Bouin's fluid or 70% ethanol, and stored in 70% ethanol. Generally, I used one specimen of each sex for each preparation. In cases in which a preparation had been of insufficient quality, a replacement preparation was made if there were a sufficient number of specimens available. I checked results from different preparations as well as possible replacement preparations against each other for individual differences. In general, I did not find individual differences; however, it must be noted that in several cases all the studied specimens of a species were from a single population as possible differences between populations were not part of this study.

For wholemounts, the ventral mid-abdomen was used. Specimens were lightly stained in a very dilute solution of clorazol black (diluted with 70% ethanol), cleared in cedar oil and mounted in Canada balsam. I studied wholemounts with brightfield and polarized light microscopy.

For histology, either the entire abdomen (*Eriocrania cicatricella*) or metathorax and abdomen minus the genital region was used. Abdomens were embedded in paraffin wax and cut in 8 μm sections and stained with either Masson's trichrome stain (Harris' hematoxylin, Ponceau-acid fuchsin and acetic aniline blue) or Harris' hematoxylin and acidified eosin and mounted with DPX resin. One female of *Synempora andesae* and one male of *Agathiphaga vitiensis* were embedded in resin, cut in 1 μm sections and stained with Richardson's stain (methylene blue and azure II). I studied histological sections with brightfield microscopy.

For scanning electron microscopy (SEM), the ventral mid-abdomen was critical point dried, mounted on SEM stubs with carbon tape, and coated with gold using a sputter coater.

Transmission electron microscopy (TEM) micrographs were provided by N. P. Kristensen (Natural History Museum of Denmark).

The methods employed for each species are indicated in Table 3-1. Drawings of wholemounts and histological sections were made with a Camera Lucida.

3D reconstruction

3D reconstructions of the sternum V gland in *Eriocrania cicatricella* were prepared using Avizo®. The reconstruction was based on transverse sections through the abdomen. The different gland structures were traced on digital photos of the sections in Photoshop. The photos and tracings were manually aligned in Avizo, after which Avizo assembled 3D reconstructions based on the tracings.

OBSERVATIONS

Micropterigidae

In *Epimartyria auricrinella* (Figure 3-1 A, B), the gland opening is situated in fairly undifferentiated flat cuticle, with a few folds anterior to the gland opening; the folds are more pronounced in the male. A gland-opening muscle that originates on the anterior edge of sternum VI inserts on the gland duct, just inside the aperture. This muscle runs internally to the longitudinal body wall musculature. In the male, the gland duct expands smoothly into a round reservoir, in the female the reservoir is more ovoid and the border between duct and reservoir is sharply defined. The gland duct in both sexes is about as long as the greatest width of the reservoir excluding the secretory cells. Both sexes have criss-crossing muscle fibres surrounding the reservoir; the musculature is better developed in the male. Secretory cells surround the gland reservoir and muscle fibres. The gland reservoir is slightly larger in males, but the surrounding layer of secretory cells is much better developed (2-3x thicker) in females.

In *Zealandopterix zonodoxa* (Figure 3-1 C-D), the gland opening is situated on a small protuberance with 8-9 setae (Figure 3-2 A). Apart from being grouped together and somewhat longer, the setae are indistinguishable from ordinary body setae. A gland-opening muscle, originating on the anterior edge of sternum VI, inserts just inside the gland aperture (Figure 3-1 C, D). This muscle runs internally to the longitudinal body wall musculature. The gland duct gradually expands into an ovoid gland reservoir surrounded by a network of muscle fibres. The secretory cells are situated around the anterior end of the gland sac, forming several rounded clusters. In females, the gland duct is very short, less than a third of the length of the reservoir, while in males it is slightly longer than the reservoir.

Agathiphagidae

Agathiphaga vitiensis has a highly derived configuration of the sternum V gland. The gland opening is situated on a smooth protuberance, and an opening muscle that originates on the anterior edge of sternum VI inserts on the gland duct just

inside the aperture. The secretory cells are arranged around a coiled duct at the farthest point of the invagination, and a section of the gland duct has been expanded into a reservoir. For further details of the gland configuration in *A. vitiensis*, please refer to Chapter 4.

Heterobathmiidae

In *Heterobathmia pseuderiocrania*, the gland opening is situated on a large thumb-shaped protuberance with elaborate surface sculpturing (Figure 3-2 B, C). The distal part of the protuberance is covered with domes, and around the base, sharp ridges demarcate the borders between individual, and often domed, epidermal cells. A gland-opening muscle that originates on the anterior edge of sternum VI inserts just inside the gland aperture (Figure 3-3 A). This muscle runs externally to the longitudinal body wall musculature. The gland duct leads to a round to ovoid reservoir which is pressed against the body wall. Layers of secretory cells surround the internal side (towards the body cavity) of the reservoir (Figure 3-3 B). The layers of secretory cells are somewhat thicker in the female. The gland duct in the female is a little longer than the greatest width of the reservoir including the secretory cells, the duct in the male is 1.5 times the greatest width of the reservoir including secretory cells. Muscle fibres attach on the cuticle around the reservoir, surrounding both the reservoir and the secretory tissue. Most of the fibres run parallel to the axis of the abdomen, with a few running at a right angle.

Eriocraniidae

In *Dyseriocrania subpurpurella* the gland opening is situated in a slightly raised oval area or very flattened protuberance of cuticle covered with domes/scale-like structures. The gland is present in both sexes, but had been removed during the dissection of the female. The following description is based on the male. A gland-opening muscle that originates on the anterior edge of sternum VI inserts just inside the gland aperture. The reservoir is ovoid and the gland duct is about twice as long as the length of the reservoir. The secretory tissue is possibly clustered in

small nodules, or is a loose layer of individual cells. Muscle fibres attach on the cuticle around the reservoir, run across the reservoir, between the reservoir and the secretory cells, and attach on the cuticle on the opposite side of the reservoir. The reservoir itself is pressed against the body wall.

In *Erocrania cicatricella* the gland is only present in the female. The gland opening is situated on a small flattened thumb-shaped protuberance, the distal part of which is covered with domes (Figure 3-2 D). A gland-opening muscle that originates on the anterior edge of sternum VI inserts just inside the gland aperture (Figure 3-3 C). This muscle runs internally to the longitudinal body wall musculature. The gland duct leads to an ovoid reservoir and is 1.5-2 times as long as the diameter of the reservoir. A highly unusual feature in this species is that the secretory cells not only surround the reservoir, but are also associated with the gland duct (Figures 3-3 C; 3-4 A, C). The secretory cells are organised in ca. 15 rounded to tear-shaped clusters with their tips oriented towards the reservoir or the duct. Muscle fibres insert on the cuticle around the reservoir, run across the reservoir, between the reservoir and the secretory cells, and insert on the cuticle on the opposite side of the reservoir (Figures 3-3 D; 3-4 B, C). The reservoir itself is pressed against the body wall cuticle (Figures 3-3 D; 3-5 A): this spot is marked by a patch of transparent cuticle, the fenestra. There is a peculiar arrangement of muscle fibres that originate on the cuticle around the fenestra and converge on the reservoir where they insert on the side pressed against the cuticle (Figures 3-3 C, D; 3-5 B). Together these muscle fibres resemble a sunburst. SEM investigation has shown that, in addition to being unsclerotised, the fenestral cuticle is also perforated by tiny pores (20-220 nm in diameter) (Figure 3-2 E, F). Light microscopy of semi thin sections (8 μm) indicates that these pores continue all the way through the cuticle.

In *E. semipurpurella* the gland opening is situated on a thumb-shaped protuberance covered in domes/scale-like structures. The protuberance is wider and flatter in the male. A gland-opening muscle that originates on the anterior edge of sternum VI inserts just inside the gland aperture. This muscle runs internally to the longitudinal body wall musculature. The gland duct leads to an

ovoid reservoir and is about the length of the greatest diameter of the reservoir. Muscle fibres insert on the cuticle around the reservoir, run across the reservoir and the secretory tissue, and insert on the cuticle on the opposite side of the reservoir. Parts of the secretory tissue bulge out between the muscle fibres (Figure 3-6 A, B). The reservoir is closely associated with the cuticle; however, there is a layer of large secretory cells between the centre of the gland reservoir and the cuticle, at least in females. The same 'sunburst' arrangement of muscle fibres as in *E. cicatricella* is present between the cuticle and reservoir. The position of the reservoir is marked by a patch of transparent cuticle, the fenestra, in the female.

Lophocoronidae

Only males of *Lophocorona pediasia* were available. The gland opening is situated on an extremely flattened protuberance, where the epidermal cells resemble flattened domes (Figure 3-2 G, H). A gland-opening muscle that originates on the anterior edge of sternum VI inserts just inside the gland aperture (Figure 3-7 A). This muscle does not cross any ventrolongitudinal muscles. The gland ducts lead to an elongated reservoir that is situated close to the cuticle, with secretory cells surrounding the internal side of the reservoir (Figure 3-7 A, B). There is no sharp demarcation between duct and reservoir, but the secretory cells start to surround the reservoir about halfway between the gland opening and the distal tip of the reservoir. A few possible neck/accessory cells (sensu Noirot & Quennedy, 1974) can be discerned in the histological sections. Some muscle fibres originate on the cuticle laterad of the reservoir and insert on the median side of the reservoir, surrounding both the reservoir and the secretory tissue (Figure 3-7 A, B).

Neopseustidae

Synempora andesae does not have gland openings, and thus no gland-opening muscles, but females have well-developed glandular structures associated with the transparent fenestrae on sternum IV (Figures 3-5 C; 3-7 D). The cuticular gland reservoirs are surrounded by secretory cells with additional secretory cells

arranged in nodules around the edges (Figure 3-7 C). The secretory cells are clearly separate from the cuticular reservoir. Muscle fibres run across the reservoir including the secretory cells. As in *Eriocrana cicatricella* and *E. semipurpurella* short muscle fibres originate on the cuticle around the fenestra and converge on the reservoir where they insert on the side towards the cuticle (Figures 3-5 D; 3-7 C). However, they are more irregularly placed than in *E. cicatricella* (Figure 3-7 D). A few muscle fibres run between the reservoir and the secretory cells on the cuticle side of the reservoir. In SEM, the fenestra looks like a collection of domes, each with tiny pores in it (20-50 nm in diameter) (Figure 3-8 A, B). Each dome covers a large secretory cell in the layer of secretory cells between the cuticle and the reservoir (Figure 3-7 C).

Nepticulidae

Like *Synempora andesae*, nepticulids do not have gland openings and thus no gland-opening muscles. However, in females there are well-developed perforated fenestrae (Figures 3-5 E, F; 3-8 C, D). Histological sections show that there is a cuticular reservoir and secretory tissue associated with the fenestra (Figure 3-7 E). The reservoir is situated at an angle to the cuticle, and the secretory tissue is wedged between the reservoir and the cuticle, extending slightly farther along the cuticle. No muscle fibres were observed, but this may be due to the minute size of the animals, and thus the diminutive size of the glandular structure and any potential muscle fibres.

General observations

The secretory cells are generally separated from the reservoir, sometimes with muscle fibres running between the reservoir and the secretory cells. Furthermore, the end apparatus of the ductules connecting the secretory cells to the reservoir is visible with TEM (Figure 3-6 B). Thus, the cells are most likely type 3 and certainly not type 1 as defined by Noirot and Quennedy (1974).

DISCUSSION

Comparisons to earlier studies

Kristensen (1984) stated that the internal structures of the gland in Micropterigidae were uniform throughout the family and between the sexes. I found differences between the species as well as subtler differences between the sexes. In *Epimartyria auricrinella*, a layer of secretory cells of approximately uniform thickness covers the entire reservoir. In *Zealandopterix zonodoxa*, the reservoir is more elongate than in *E. auricrinella*, and the secretory tissue is organised into distinct lobes, concentrated around the anterior part of the reservoir (Figure 3-1 A-D). In *E. auricrinella*, the reservoir in the male is smaller, and is surrounded by more muscle fibres than in the female, while the layer of secretory tissue is 2-3 times thicker in the female than in the male (Figure 3-1 A, B). In *Z. zonodoxa*, the reservoir in the female is longer, and the duct shorter than in the male (Figure 3-1 C, D). The secretory tissue is also restricted to a more anterior position than in the male.

Agathiphaga vitiensis had additional unique traits compared to those observed by Kristensen (1984, 1998), and a so highly derived gland structure that it is treated separately in Chapter 4.

I found that the gland reservoir in *Heterobathmia pseuderiocrania* is ovoid and somewhat flattened against the cuticle (Figure 3-3 A, B), contrary to Kristensen and Nielsen's (1979) observation that the reservoir in Heterobathmiidae is spheroidal.

SEM examinations revealed that the fenestrae, a pair of patches of tranparent cuticle on sternum IV, are perforated, and thus are not simply demarcating the position of the reservoir (Figures 3-2 F; 3-8 B, D). In addition to being perforated, a peculiar muscle arrangement is associated with the fenestra in eriocraniids and neopseustids (Figures 3-3 C, D; 3-5 B; 3-7 C, D). The muscle fibres are situated between the cuticle and the reservoir, originate on the former and converge to insert on the latter.

Nielsen and Kristensen (1996) stated that the gland configuration in Lophocoronidae was similar to that in Micropterigidae. I found that the reservoir

is very elongate and slender (Figure 3-7 A); the muscle fibres associated with the reservoir surround both reservoir and secretory tissue (Figure 3-7 B) while in the investigated micropterigids the muscle fibres surround only the reservoir (Figure 3-1).

Davis (1975) investigated the occurrence of fenestrae in Lepidoptera, and observed that they are associated with the sternum V gland reservoir in female eriocraniids. He did not find an external opening or a duct associated with the fenestrae in female neopseustids and nepticulids (Davis, 1975). I did not find one large external opening or a duct associated with the fenestrae in neopseustids and nepticulids, but I found that the cuticle is perforated (as is also the case in eriocraniids) (Figures 3-2 F; 3-8 B, D). Additionally, I found a reservoir and secretory tissue associated with the reservoir (Figure 3-7 D, E). In neopseustids, muscle fibres insert on the reservoir, confirming the cuticular nature of the reservoir (Figure 3-7 C). As the reservoir is cuticular, it must have originated as an invagination. Please see Chapter 5 for a detailed discussion of the probable origin of the fenestral glands. I did not observe any muscle fibres associated with the fenestra in nepticulids. This may be an artefact caused by the minute size of nepticulids. However, I did observe secretory tissue and a reservoir that is probably of cuticular origin.

Gland-opening muscles

Gland-opening muscles are present in all lepidopteran species with a sternum V gland opening, and as they have the same points of origin and insertions, these muscles are homologous. However, the position of these muscles with respect to the intersegmental muscles differs between taxa. In micropterigids, the gland-opening muscles run internally to the intersegmental muscles. In *Agathiphaga vitiensis*, the gland-opening muscles run externally to or on both sides of the intersegmental muscles. In Heterobathmia pseuderiocrania, the gland-opening muscles run externally to the intersegmental muscles while in eriocranids, the gland-opening muscles run internally to the intersegmental muscles. The gland-

opening muscles in *Lophocorona pediasia* do not cross any intersegmental muscles.

The sternum V glands are only present in adults, and Kristensen (1984) suggested that the gland-opening muscles were a neoformation. Thus the gland-opening muscles presumably develop de novo during metamorphosis from myoblasts (Heming, 2003). My observations indicate that these muscles can form on either side of the intersegmental muscles, in *A. vitiensis* there is even individual variation in the placement of the gland-opening muscles with respect to the intersegmental muscles. There is probably no functional difference between gland-opening muscles running internally or externally to the intersegmental muscles, and the distribution of the two states does not suggest a phylogenetic significance.

Expulsion and dispersion of gland products

I found musculature surrounding the reservoir or reservoir and associated secretory tissue in all investigated species, except *Agathiphaga vitiensis* and Nepticulidae spp. Previously, musculature associated with the reservoir has only been documented in representatives of Micropterigidae and Lophocoronidae. Contraction of the muscle fibres surrounding the reservoir would force gland products out, and enable a rapid and controlled release. Contraction of the gland-opening muscle will open the gland duct, thereby allowing release of gland products, but does not actively expel the gland products

Razowski (1975) proposed that the external cuticular structures associated with the gland opening serve to increase the evaporative surface, enhancing the dispersal of gland products. Davis (1978) supported the same function, referring explicitly to the interconnected grooves surrounding the opening in eriocraniids, while Kristensen (1984) noted that the setose protuberance in representatives of *Sabatinca*, *Agrionympha* and *Squamicornia* (all Micropterigidae) resembled a pheromone sender.

I found structures that provide an enlarged available evaporative surface in *Zealandopterix zonodoxa* in the form of a setose protuberance (Figure 3-2 A).

Heterobathmia pseuderiocrania (Heterobathmiidae) has a protuberance with well-defined domes (Figure 3-2 B), resulting in a network of minute canals as well as an increased surface area. Like Davis (1978) did, I found a similar arrangement in the various species of Eriocraniidae (e.g. Figure 3-2 D). The flattened domes in *Lophocorona pediasia* result in an increased evaporative surface, but the grooves between the domes seem too shallow to support capillary action (Figure 3-2 G). While *Agathiphaga vitiensis* have a well developed protuberance, the smooth surface does not provide an enlarged evaporative surface.

My observations thus support the suggestion that most of the various structures associated with the gland opening increase the evaporative surface, and in some cases provide a network of tiny grooves that would disperse the gland products through capillary action. However, as there is evidence that *Sabatinca* does not employ pheromones (Kozlov & Zvereva, 1999), the evaporative structure is not necessarily a pheromone sender as suggested by Kristensen (1984).

Evolutionary history

The phylogeny of the basal Lepidoptera (Figure 3-9) is well known, and constitutes the basis for my discussion of the evolution of the sternum V gland in Lepidoptera.

The setose protuberance associated with the gland opening in *Zealandopterix* is unparalleled in Lepidoptera outside Micropterigidae, although a similar setose protuberance occurs in Hydroptilinae (Trichoptera, Hydroptilidae) (Chapter 2). The gland opening in *Agathiphaga vitiensis* is situated on a smooth protuberance of even thickness, also unparalleled in Lepidoptera, and although several different types of protuberances are associated with the gland opening in various Trichoptera, none have this configuration (Chapter 2). However, the domed/scaly protuberances in heterobathmids (Kristensen & Nielsen, 1979; present study) and some eriocraniids (representatives of *Eriocrania* and *Neocrania*) (Davis, 1978; present study) are similar and likely homologous. Other eriocraniids (representatives of *Dyseriocrania*) have the gland opening in a raised

oval area or on a flat protuberance covered with domes/scale-like structures, and this resembles a flattened version of the protuberance in members of *Eriocrania* and is probably derived from the *Heterobathmia*/*Eriocrania* type protuberance. The gland opening in lophocoronids is situated on a flat protuberance covered with flattened domes, this cuticular modification might be an extremely flattened version of the protuberance in representatives of *Heterobathmia* and *Eriocrania* (independently derived from the one found in representatives of *Dyseriocrania*), or it might be completely independently derived.

In *Zealandopterix zonodoxa*, the reservoir is close to the cuticle, but not closely associated with it. While *Agathiphaga vitiensis* does not have the typical configuration of a reservoir with associated secretory tissue, the secretory and product storage areas of the gland are not closely associated with the cuticle. However, in Heterobathmiidae + Glossata, the gland reservoir is closely associated with the cuticle in all investigated species. *Heterobathmia pseuderiocrania* has muscle fibres running across the reservoir, attaching on the cuticle on either side of the reservoir, holding the reservoir firmly against the cuticle, with no layer of secretory cells between the reservoir and the cuticle. In eriocraniids, the reservoir is pressed against the cuticle (Figure 3-3 D), held in place with muscle fibres as in *H. pseuderiocrania*, and with the addition of 'sunburst' musculature in the females. In *Lophocorona pediasia* (Lophocoronidae), muscle fibres that originate on the cuticle run across the reservoir including secretory tissue and insert on the reservoir on the opposite side (Figure 3-7 B). In *Synempora andesae* (Neopseustidae), the reservoir is pressed against the fenestra by muscle fibres that originate on the cuticle and run across the reservoir, and a less regular version of the 'sunburst' musculature is also present (Figure 3-7 C, D). In the nepticulids, the reservoir and secretory tissue is closely associated with the fenestra (Figure 3-7 E). Thus there seems to be an evolutionary trend towards the reservoir being closely associated with the cuticle. However, the reservoir is also closely associated with the cuticle in some Trichoptera, and this condition may be the ancestral state of the sternum V gland (Chapter 5). Hence the disassociation of the distal part of the gland from the

cuticle might be an independently derived condition in micropterigids and agathiphagids.

In representatives of Lepidoptera, the muscle fibres associated with the reservoir in some cases surround only the reservoir (micropterigids and some eriocraniids), and in some cases both the reservoir and the secretory tissue (heterobathmiids, some eriocraniids, lophocoronids and neopseustids). In representatives of Trichoptera, including representatives of the basal lineages, muscle fibres generally surround only the reservoir (Chapter 2), and as this is also the case in micropterigids, this is probably the ancestral state in Amphiesmenoptera. Thus the arrangement of muscle fibres in heterobathmiids, *Eriocrania semipurpurella* (Eriocraniidae), lophocoronids and neopseustids with respect to the secretory tissue are secondary developments. However, this seems to be a rather labile trait. In representatives of Trichoptera muscle fibres surrounding only the reservoir or both reservoir and secretory tissue might be found in conspecific males and females (e.g. *Wormaldia arizonensis* (Ling)) and in several species some muscle fibres surround only the reservoir while other fibres surround both reservoir and secretory tissue (Chapter 2).

The secretory tissue is arranged in lobes or clusters in *Zealandopterix zonodoxa* (Micropterigidae) (Figure 3-1 C-F) and *Eriocrania cicatricella* (Eriocraniidae) (Figure 3-3 C, D), but in a layer in *Epimartyria auricrinella* (Micropterigidae) (Figure 3-1 A, B), *Heterobathmia pseuderiocrania* (Heterobathmiidae) (Figure 3-3 B) and *Lophocorona pediasia* (Lophocoronidae) (Figure 3-7 B). In female *E. semipurpurella*, a layer of secretory cells is situated between the cuticle and the centre of the reservoir, and on the side facing away from the body wall a layer of secretory cells bulges out between the muscle fibres, forming lobes (Figure 3-6). In *Synempora andesae* (Neopseustidae), a single layer of large secretory cells surrounds the entire reservoir, but around the edges of the reservoir are small lobes of secretory cells (Figure 3-7 C). In representatives of Trichoptera, the secretory tissue is generally organised as a layer around the reservoir, but the secretory tissue also forms lobes in some groups, including some species in the basal lineages (Chapter 2). The ancestral state in this case is

uncertain, but there does seem to be a large degree of plasticity in the organisation of the secretory cells. The occurrence of secretory cells along the otherwise well-defined gland duct in *E. cicatricella* (Figure 3-3 C) was not found in any other species, nor in representatives of Trichoptera (Chapter 2).

Functional considerations

The gland is present in both sexes in micropterigids, only in males in agathiphagids, in both sexes in heterobathmiids, generally in both sexes in eriocraniids (absent in males in six species (Karsholt et al., in prep.), in both sexes in lophocoronids (Nielsen & Kristensen, 1996), and is only present in females (without gland openings) in neopseustids and nepticulids. The sternum V gland or sternum IV fenestrae are not known from representatives of any other lepidopteran families. Thus there has been a general tendency to lose the sternum V gland, especially in males although the situation is reversed in agathiphagids. In lepidopterans, the tendency to retain the gland in females while losing it in males is only found in the groups in which the females possess fenestra. While gland products can presumably be secreted through the fenestra (Chapter 5), this is unlikely to be as rapid as the release through the gland opening.

Sternum V gland type pheromones[1] are known from females of both Eriocraniidae (Zhu et al, 1995; Kozlov et al., 1996) and Nepticulidae (Tóth et al., 1995). The presence of 'sternum V gland' type pheromones in nepticulids suggests that these tiny moths still possess a functional version of the sternum V gland. I propose that the secretory tissue associated with the reservoir is the production site for these pheromones and is a derived form of the sternum V gland. The most likely method of secretion is through the fenestra as no other means of secretion has been identified. Thus it seems that fenestra can be used for the release of pheromones, and that pheromone production is the retained function when the gland is only present in females.

[1] The pheromones produced by the sternum V gland are short chain, contrasting with the long chains of pheromones produced by higher Lepidoptera (Tóth et al., 1995; Zhu et al, 1995; Kozlov et al., 1996; Löfstedt & Kozlov, 1997).

As mentioned above, the gland is present in males of Micropterigidae, Agathiphagidae (not present in females), Heterobathmiidae, most Eriocraniidae and Lophocoronidae, and it is as well developed, albeit sometimes differently, as in the females. This strongly argues against the sternum V gland being non-functional in males. The general function of the sternum V in male Lepidoptera seems to require the ability to rapidly release gland products, as no males are known to possess the sternum V gland in combination with the presumably slow-releasing perforated fenestra.

The best known function of the sternum V gland is production of female sex pheromones (Wood & Resh, 1984; Resh & Wood, 1985; Solem, 1985; Löfstedt et al., 1994; Zhu et al., 1995; Kozlov et al., 1996; Bjostad et al., 1996; Jewett et al., 1996; Bergman et al., 2001, 2002), but this does not explain the function of the sternum V gland in the males. Based on the presence of the sternum V gland in both sexes, several authors have suggested a defensive function for the gland (Eltringham, 1934; Kristensen, 1972, 1984; Davis, 1975, 1978; Kozlov & Zvereva, 1999). There are some data on the function of the sternum V gland in male trichopterans: aggregation pheromones produced by some male *Hydropsyche* (Hydropsychidae) (Löfstedt et al., 1994), and defensive substances produced by both sexes in *Pycnopsyche scabripennis* (Rambur) (Limnephilidae) (Duffield et al., 1977). The only study to investigate the function of the sternum V gland in male Lepidoptera was Kozlov and Zvereva (1999) in representatives of Micropterigidae. However, Kozlov and Zvereva (1999) focused on pheromones and concluded that neither sex produced pheromones. My experiments using either male or female *Epimartyria auricrinella* as bait in livetraps supports this conclusion; see Appendix 3 for details.

The glands in the micropterigids included in the present study are well developed, and the protuberances in *Zealandopterix zonodoxa* have long setae that would increase the evaporative surface for any gland secretions. However, members of some micropterigid genera (*Micropteryx* and *Hypomartyria*) do not possess the sternum V gland (Kristensen, 1984). Kozlov (1986) concluded that species of *Micropteryx* use visual cues to detect a partner once brought in visual

range by habitat heterogenity, e.g. adults often congregate on flowers. Kozlov and Zvereva (1999) observed that *Sabatinca* moths have a highly uneven distribution, and suggested that 'swarming' males search for females using visual cues. Thus visual recognition of potential mates might be the general rule in micropterigids.

While this explains how micropterigids may locate potential mates without the use of pheromones, it leaves the unanswered question of the function of the sternum V gland in this group. If not used to produce pheromones, then a defensive function is a possibility as this has been demonstrated in some Trichoptera (Duffield et al., 1977); this function was also suggested by Kozlov and Zvereva (1999). Futhermore, several species of Trichoptera produce substances (e.g. acetophenone and organic acids) (Ansteeg & Dettner, 1991; Löfstedt et al., 1994; Bergmann, 2002; Bergmann et al., 2002) that are known to be irritants or toxic (Ansteeg & Dettner, 1991; Mohsen et al., 1995; Lewis, 2000; Pohanish, 2002). A defensive function could explain the presence of sternum V glands in the absence of pheromone production, and is a possible explanation for the widespread presence of well developed sternum V glands in males. A defensive function of the gland, with substances released in response to an attack or threat of attack from a predator, is consistent with a rapid release of gland products being required.

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TABLE 3-1. List of all taxa studied showing the treatments employed for each species and description of gland morphology (presence/absence of the sternum V gland, if the gland-opening muscles runs internally or externally to the intersegmental muscles, the presence or absence of fenestra (transparent and perforated cuticle) on sternum IV and whether the reservoir musculature surrounds only the reservoir (exclusive) or both reservoir and secretory cells (inclusive)). '?' denotes that a structure could either not be detected with the treatment(s) used, or that the structure was absent, but the absence was presumed to be due to artefacts. F, female; M, male.

Taxa	Treatments	Sternum V gland	Opening muscles int. or ext.	Fenestra (Perforated cuticle)	Reservoir musculature incl./excl.
Micropterigidae					
Epimartyria auricrinella Walsingham F + M	wholemount	present	internally	absent	exclusive
Zealandopterix zonodoxa (Meyrick) F + M	wholemount, external SEM, histology	present	internally	absent	exclusive
Agathiphagidae					
Agathiphaga vitiensis Dumbleton M	wholemount, external SEM, histology	present	externally or internally & externally	absent	absent
Heterobathmidae					
Heterobathmia pseuderiocrania Kristensen & Nielsen F + M	wholemount, external SEM, histology	present	externally	absent	inclusive
Eriocraniidae					
Dyseriocrania subpurpurella (Haworth) F + M	wholemount	present	?	present in F only	exclusive
Eriocrania cicatricella (Zetterstedt) F + M	wholemount, external SEM, + internal SEM, histology of F	present in F absent in M	internally in F, n/a in M	present in F only	exclusive
E. semipurpurella (Stephens) F + M	wholemount, + histology, TEM of F	present	internally	present in F only	inclusive
Lophocoronidae					
Lophocorona pediasia Common M	wholemount, external SEM, histology	present	n/a	absent	inclusive
Neopseustidae					
Synempora andesae Davis & Nielsen F	Wholemount, ext. & int. SEM, histology	present w/o duct	n/a	present	inclusive
Nepticulidae					
Nepticulidae spp. F	wholemount, external SEM, histology	present w/o duct	n/a	present	absent[a]
Ectoedemia heringiella Doets F	internal SEM	?	?	present	?

[a]At least not observed, could be an artefact due to the small size of the specimens

FIGURE 3-1. Drawings of Micropterigidae based on wholemounts (A-D) or histological sections (E and F). The drawings based on wholemounts are viewed through the cuticle and anterior is towards the left with down being mesad; thus the drawings show the left gland. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is not indicated. A and B: *Epimartyria auricrinella*, female and male respectively. Note the relatively larger reservoir in the male and the relatively thicker layer of secretory cells in the female. Also note the sharply defined border between the duct and the reservoir in the female. C-F: *Zealandopterix zonodoxa*. C: Male. D: Female, note the very short gland duct. In both C and D note the setae on the protuberance. E: Male. F: Female. In both E and F, note the organisation of the secretory cells into a rounded cluster. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; St, setae; L-t, Lepidoptera-type opening muscle. Scale bars: A-D = 20 μm; E, F = 10 μm.

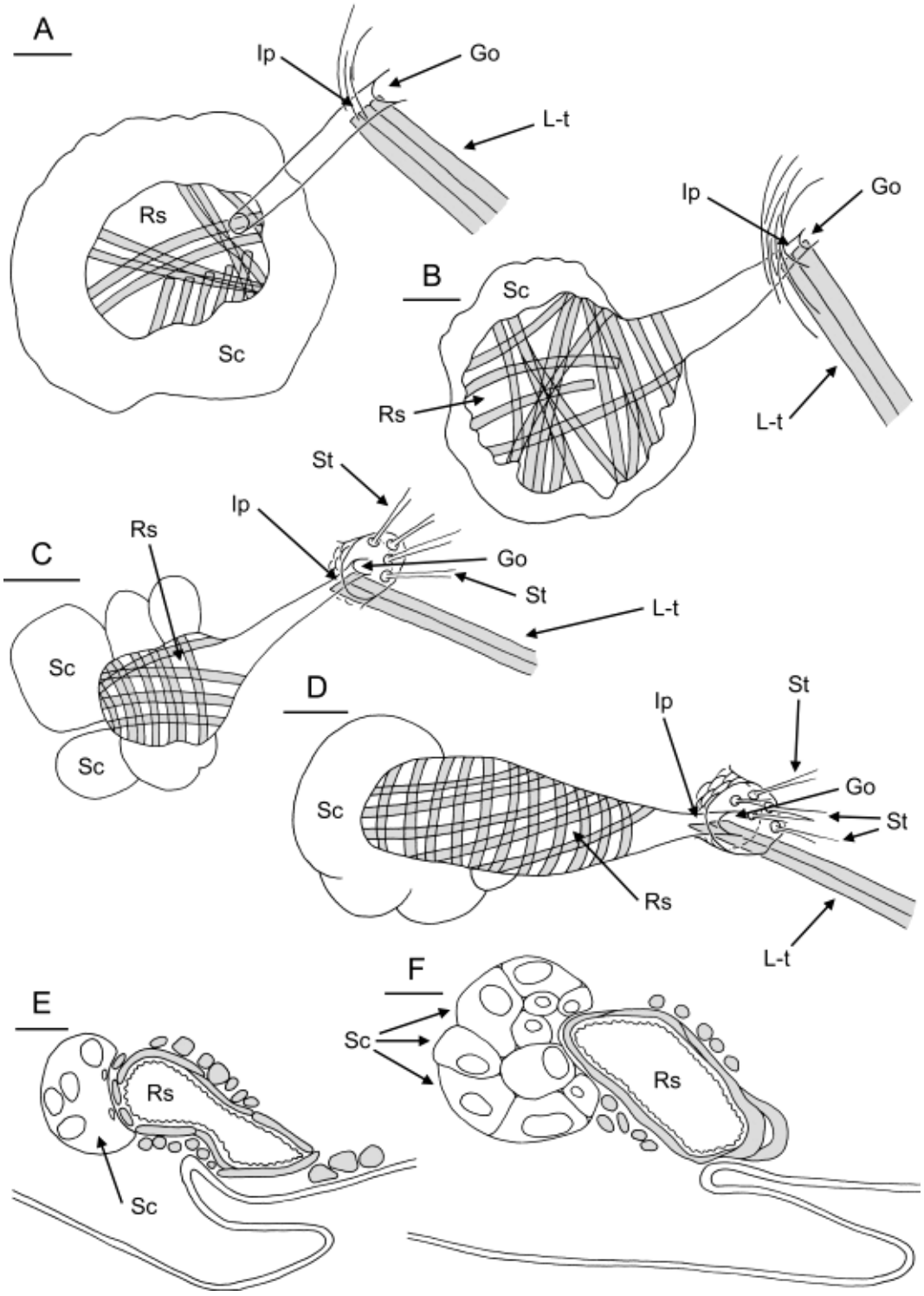

A
Ip
Go
L-t
Rs
Sc
B
Ip
Go
Sc
Rs
L-t
St
Ip
Rs
C
Go
St
L-t
Sc
Sc
D
Ip
St
Go
St
Sc
Rs
L-t
E
Rs
Sc
F
Sc
Rs

FIGURE 3-2. SEMs of external structures. A: *Zealandopterix zonodoxa* (Micropterigidae) female, male similar. Protuberance with gland opening, the bases of the long setae are visible. B and C: *Heterobathmia pseuderiocrania* (Heterobathmiidae) male, female similar. B: Protuberance with gland opening, note the domes that cover the distal part of the protuberance and the ridges demarcating the borders between individual epidermal cells. C: Close-up of ridges. D-F: *Eriocrania cicatricella* (Eriocraniidae) female. D: Protuberance with gland opening; note the domes that cover the distal part of the protuberance. E: Overview of fenestra; the fenestra is the area of wrinkled cuticle devoid of microtrichia. F: Close-up of fenestra; note the perforations. G and H: *Lophocorona pediasia* (Lophocoronidae) male. G: Gland opening on very flat protuberance with flattened domes. H: Close-up of gland opening. Go, gland opening. Scale bars: A, C, H = 1 μm; B, D, E, G = 10 μm; F = 100 nm.

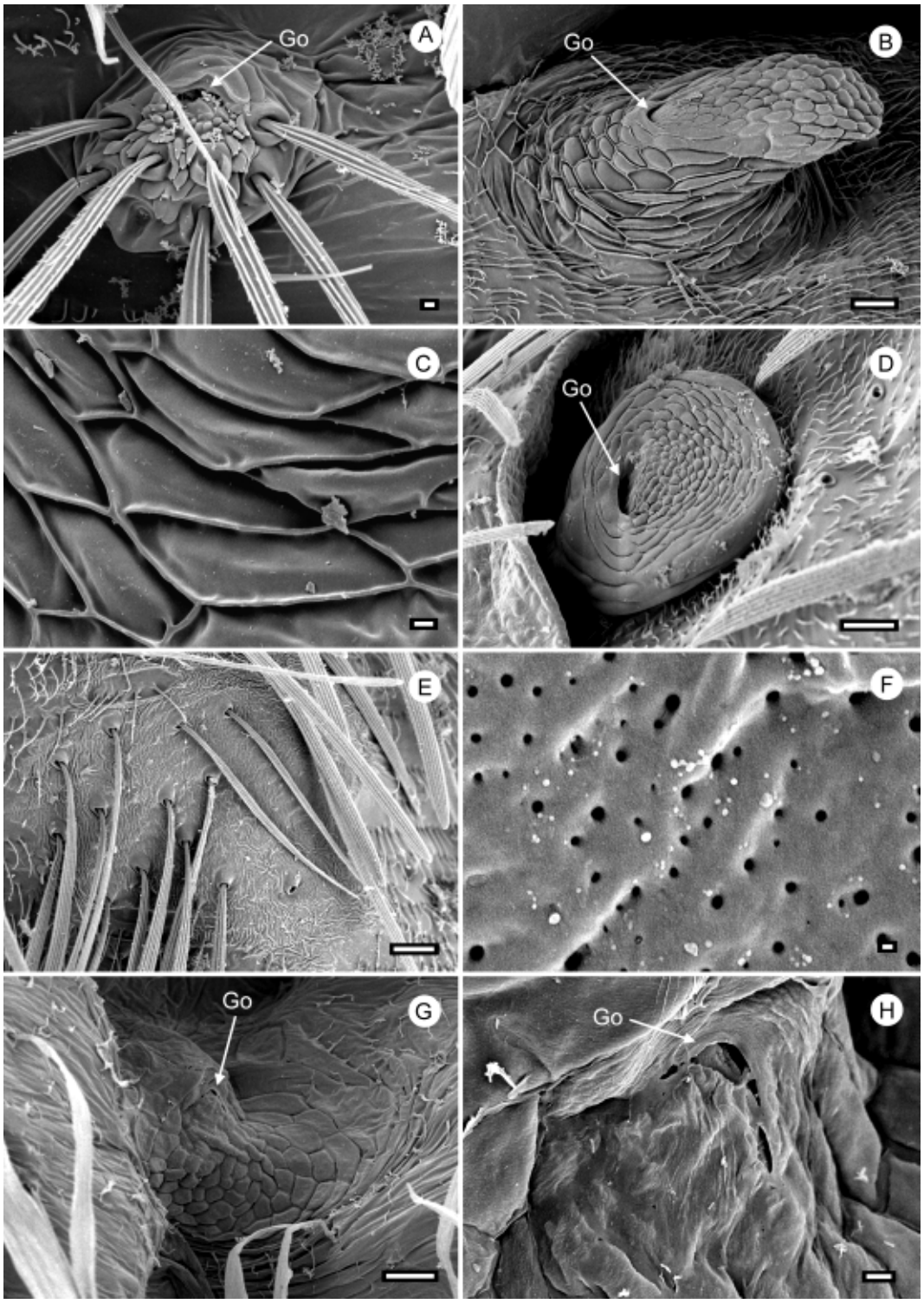

Go
A
Go
B
C
Go
D
E
F
Go
G
Go
H

FIGURE 3-3. Drawings based on wholemounts (A and C) or histological sections (B and D). The drawings based on wholemounts are viewed through the cuticle and anterior is towards the left with down being mesad; thus the drawings show the left gland. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is not indicated. A and B: *Heterobathmia pseuderiocrania* (Heterobathmiidae), male and female respectively. Note the muscle fibres attaching on the cuticle on both sides of the reservoir and how the reservoir is pressed against the body wall. C and D: *Eriocrania cicatricella* (Eriocraniidae) female. Note the secretory cells along the gland duct and the sunburst-like arrangement of muscle fibres originating on the cuticle and inserting on the reservoir. Also note how the reservoir is pressed against the cuticle by the muscle fibres attaching on the cuticle on either side of the reservoir. At, attachment point for muscle; Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; L-t, Lepidoptera-type opening muscle. Scale bars: A-D = 20 μm;

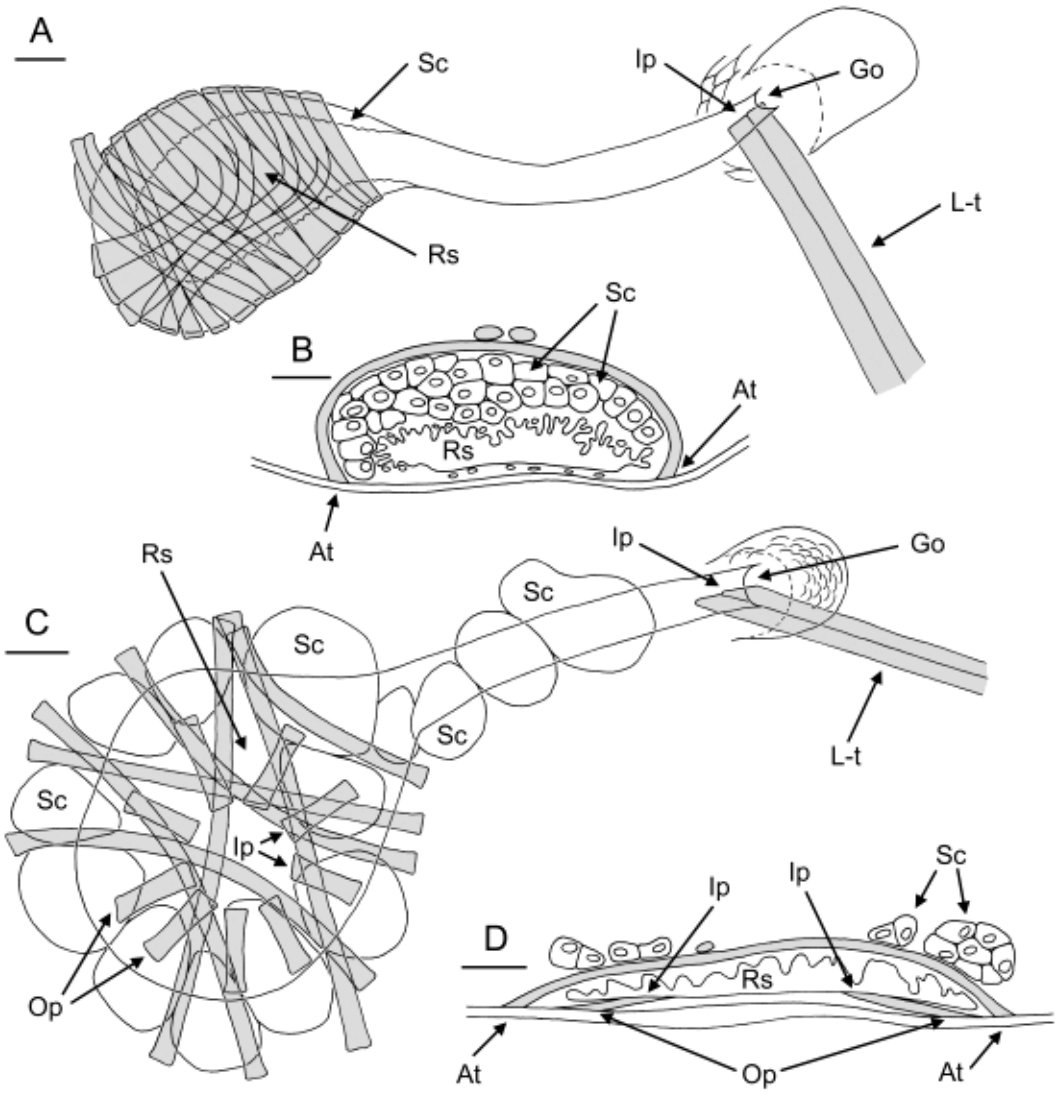

A
Sc
Ip
Go
L-t
Rs
B
Sc
At
Rs
At
C
Rs
Sc
Ip
Go
Sc
Sc
Sc
L-t
Sc
Ip
Op
D
Ip
Ip
Sc
Rs
At
Op
At

FIGURE 3-4. Digital 3D reconstructions of *Eriocrania cicatricella* (Eriocraniidae) female. The viewpoint is out towards the cuticle and anterior is towards the left with down being mesad; thus the right side gland is shown. A: Gland reservoir and duct. B: Musculature associated with gland: gland-opening muscle and transverse and longitudinal muscle fibres across the gland reservoir. C: Secretory tissue and musculature, note the secretory tissue along the gland duct. Dt, gland duct; Lm, longitudinal muscle fibres; Tm, transverse muscle fibres; Sc, secretory tissue; L-t, Lepidoptera-type opening muscle.

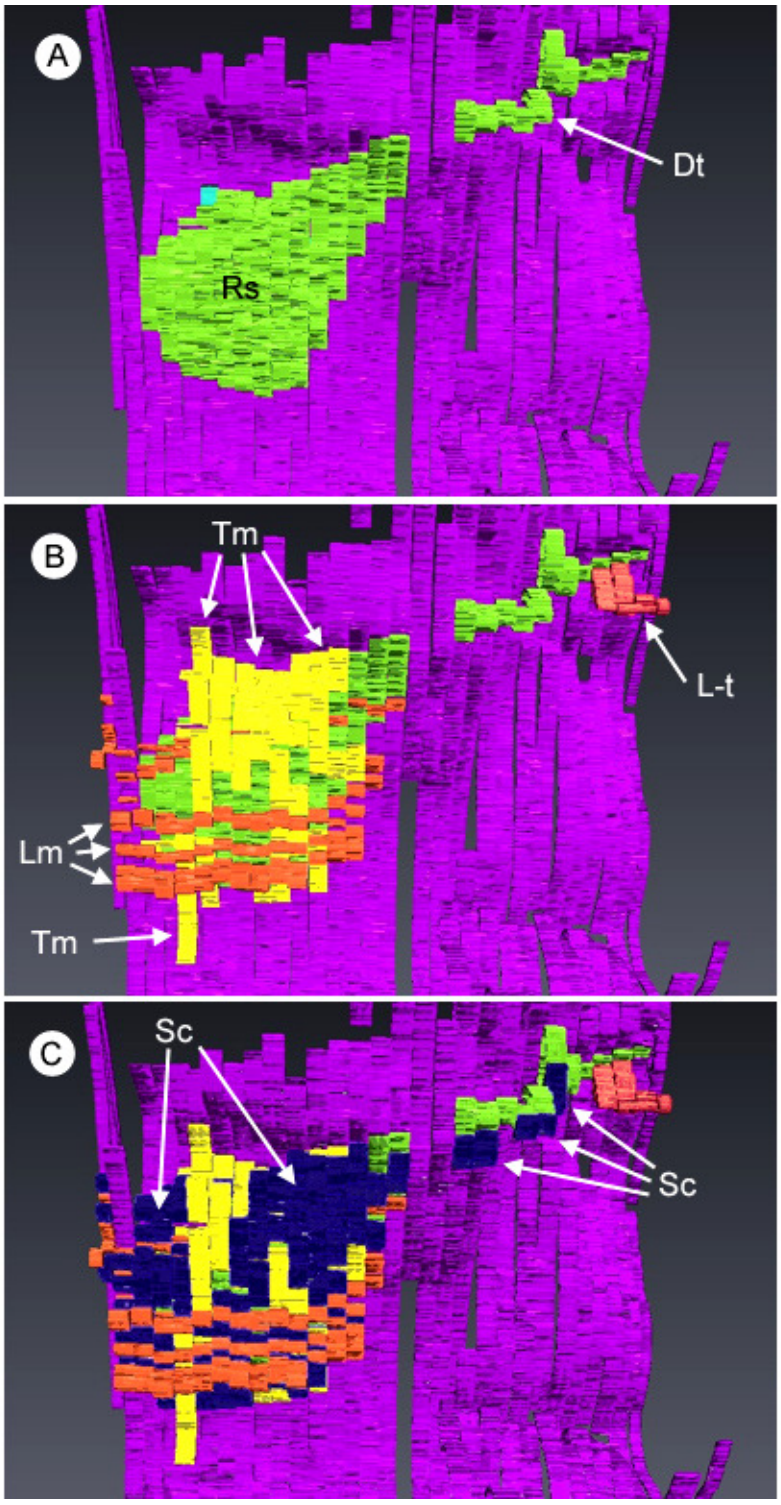

A
Dt
Rs
B
Tm
L-t
Lm
Tm
C
Sc
Sc

FIGURE 3-5. SEMs of internal structures. A and B: *Eriocrania cicatricella* (Eriocraniidae) female. A: Gland reservoir in situ. B: Reservoir has been removed, revealing the 'sunburst' musculature between the reservoir and the cuticle. C and D: *Synempora andesae* (Neopseustidae) female. C: Gland structure, note the absence of excurrent duct. D: Close-up of edge of structure showing muscle fibres and secretory cells. E and F: *Ectoedemia heringiella* (Nepticulidae) female. E: Appearance of fenestra on inside of cuticle. F: Close-up of same. Mf, muscle fibres; Rs, gland reservoir. Scale bars: A = 100 μm; B, D, E = 10 μm; C = 20 μm; F = 1 μm.

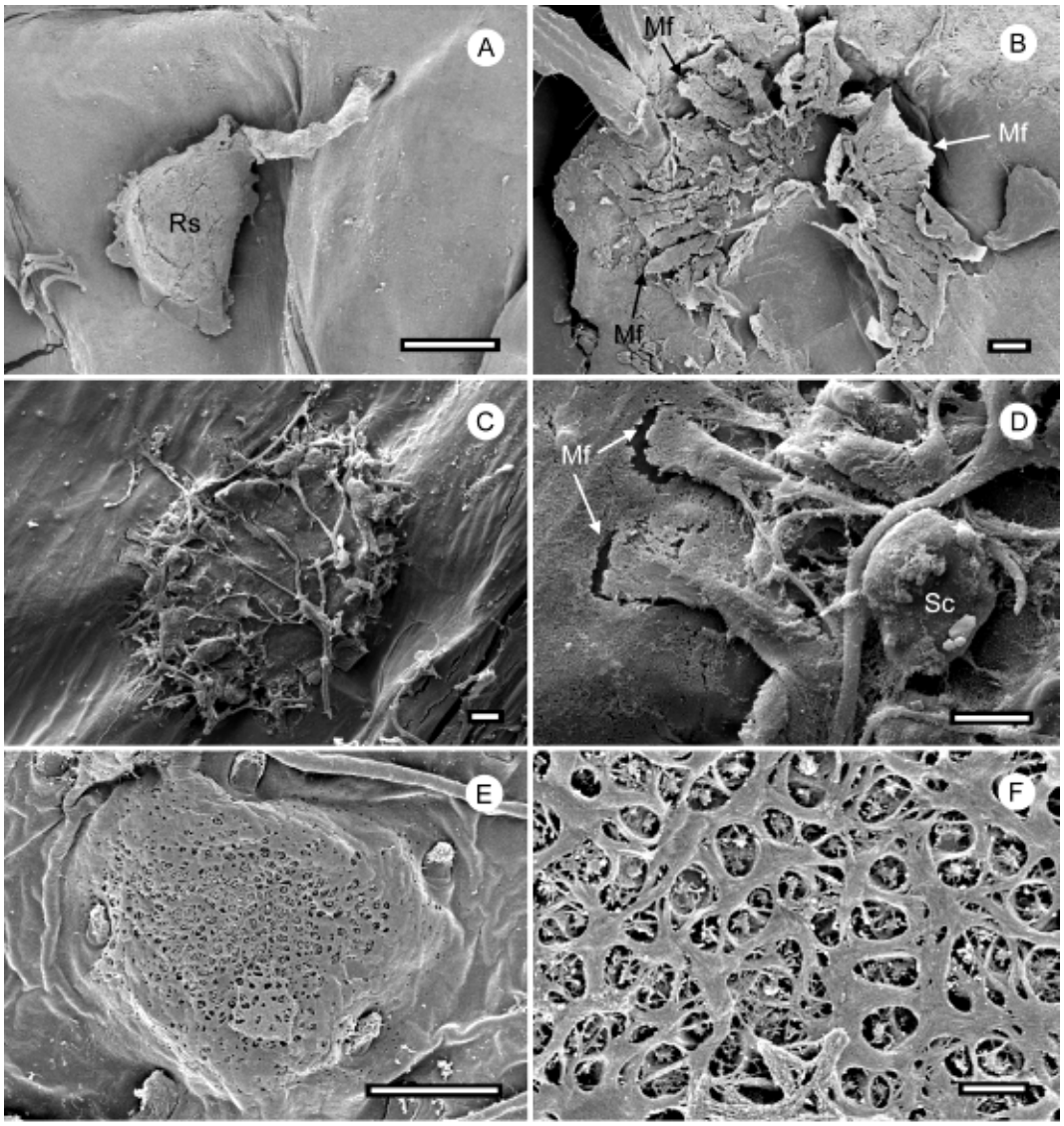

A
Rs
B
Mf
Mf
Mf
C
D
Mf
Sc
E
F

FIGURE 3-6. TEMs of sternum V gland in *Eriocrania semipurpurella* (Eriocraniidae) female. A: Overview of gland structures. Note the bulges of secretory cells, the layer of secretory cells between the reservoir and the cuticle and the muscle fibres between the reservoir and the cuticle (parts of the 'sunburst' musculature). B: Close-up of gland structures showing end apparatus with tiny tubuli. In the very bottom of the picture, part of the gland reservoir is visible. Ct, cuticle; Ea, end apparatus; Mf, muscle fibres; Rs, gland reservoir; Sc, secretory cells. Scale bars: A = 10 μm; B = 1 μm.

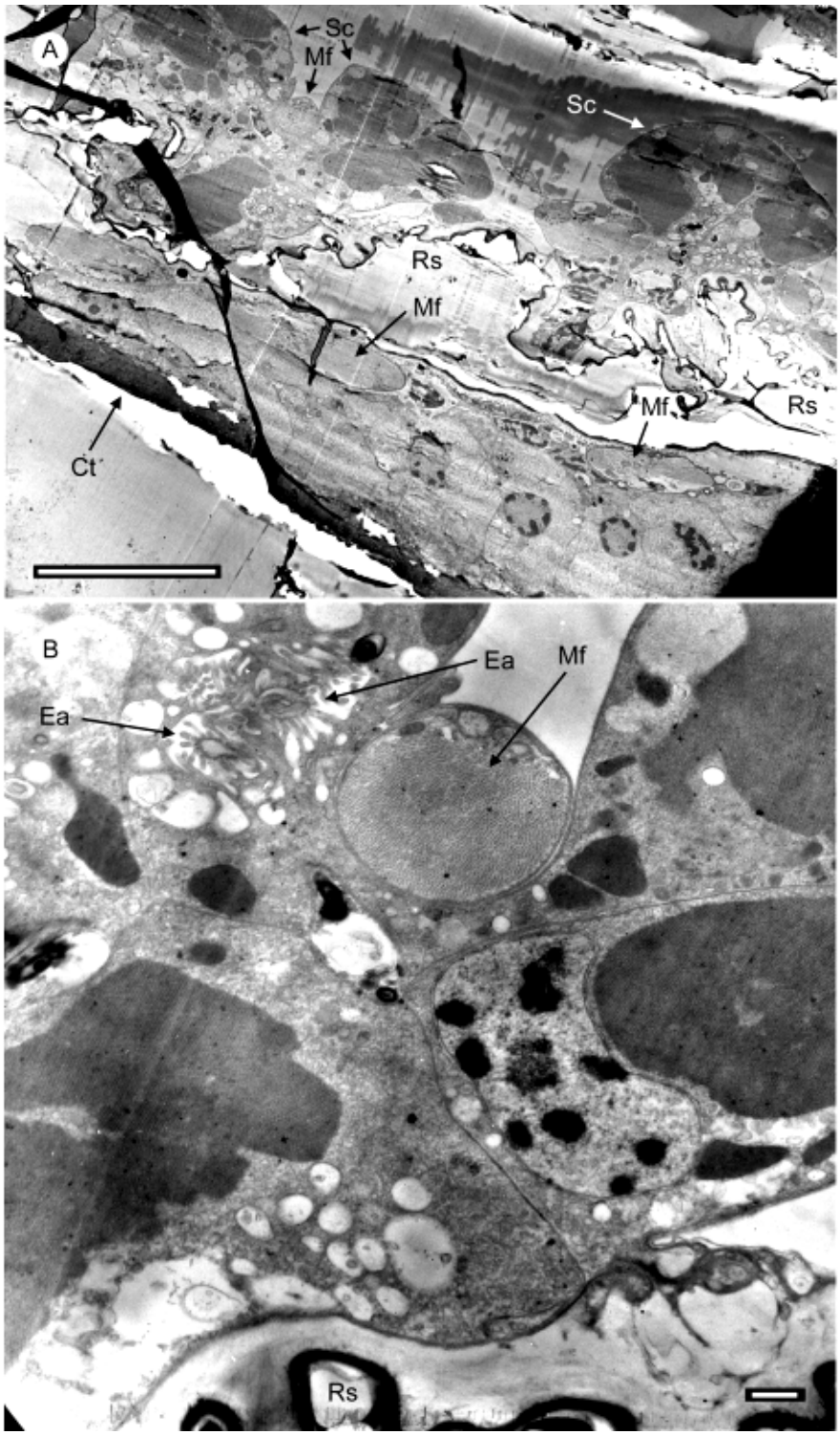
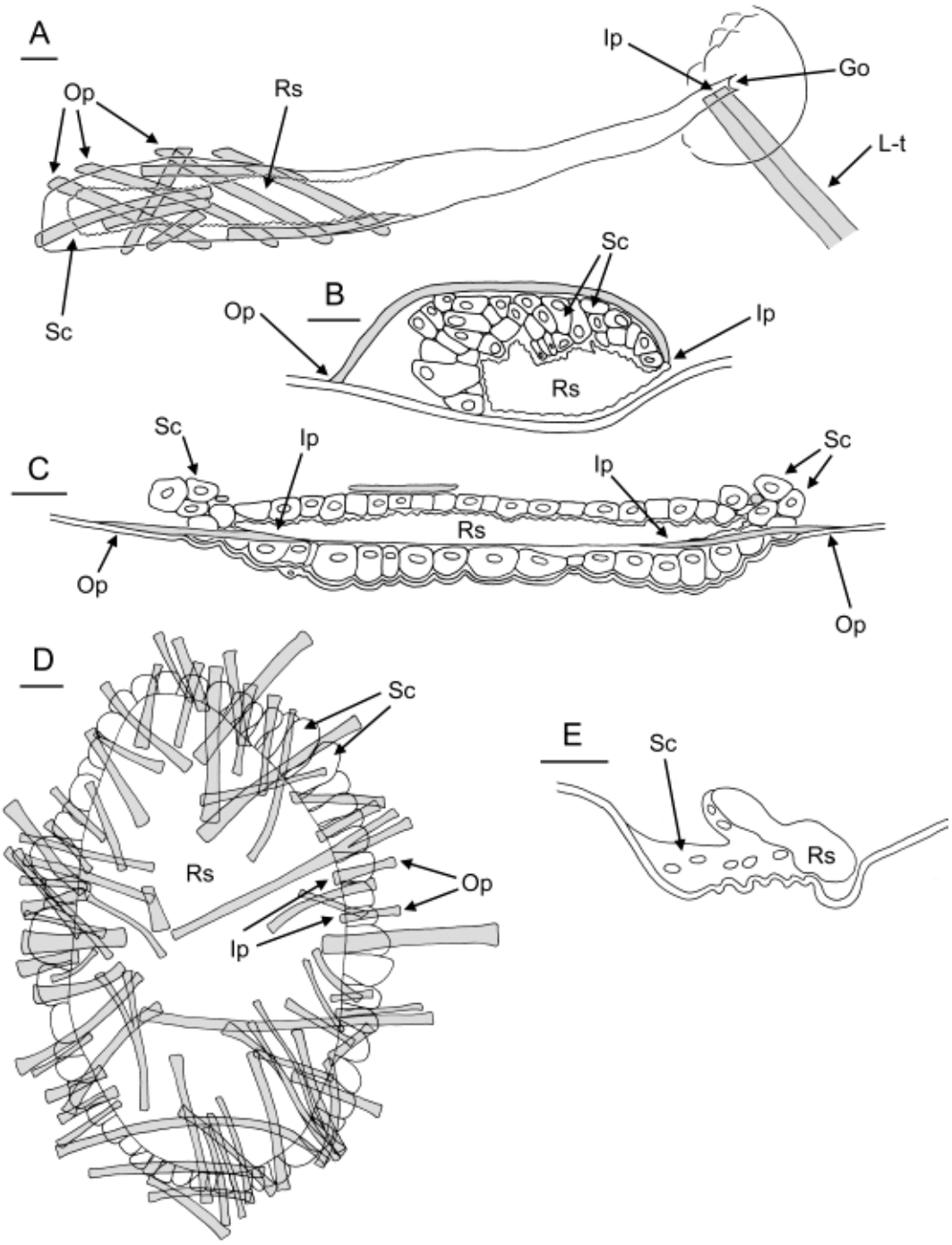

A
Sc
Mf
Sc
Rs
Mf
Mf
Rs
Ct
B
Ea
Mf
Ea
Rs

FIGURE 3-7. Drawings based on wholemounts (A and D) or histological sections (B, C and E). The drawings based on wholemounts are viewed through the cuticle and anterior is towards the left with down being mesad; thus the drawings show the left gland. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is not indicated. A and B: *Lophocorona pediasia* (Lophocoronidae) male. Note the very elongate shape of the reservoir and how muscle fibres originating on the cuticle cross the secretory tissue and the reservoir before inserting on the reservoir. C and D: *Synempora andesae* (Neopseustidae) female. Note the irregular 'sunburst' of muscle fibres originating on the cuticle and inserting on the reservoir. C is based on camera lucida drawings of three sections, two 1 μm sections, and one 8 μm section. E: Nepticulidae sp. female. Go, gland opening; Ip, insertion point for muscle; Op, origin point for muscle; Rs, gland reservoir; Sc, secretory cells; L-t, Lepidoptera-type opening muscle. Scale bars: A-D = 20 μm; E = 10 μm.


A
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Go
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C
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Op
D
Sc
Rs
Op
Ip
E
Sc
Rs

FIGURE 3-8. SEMs of external structures. A and B: *Synempora andesae* (Neopseustidae) female. A: Fenestra, note the distinctive domed appearance of the cuticle. B: Close-up of domes, note the perforations (black and white arrows). C and D: Nepticulidae sp. female. C: Fenestra. D: Close-up of same showing perforations and unusual finger-like microtrichia. Mt, microtrichia. Scale bars: A = 10 μm; B-D = 1 μm.

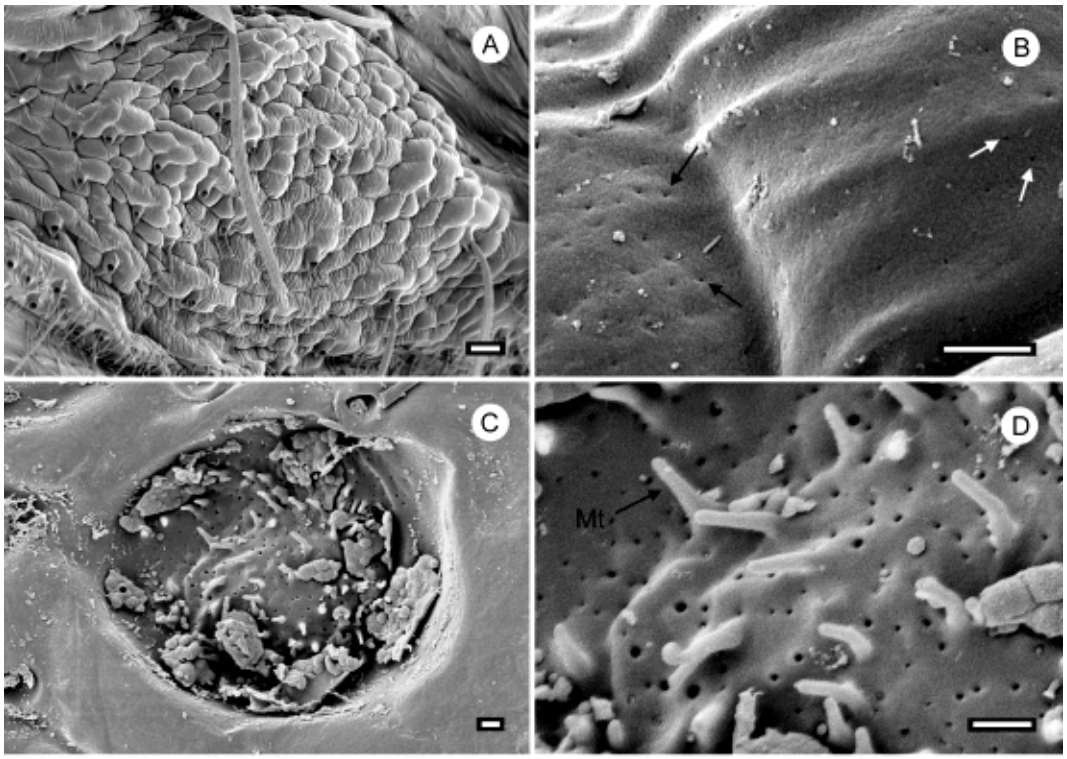
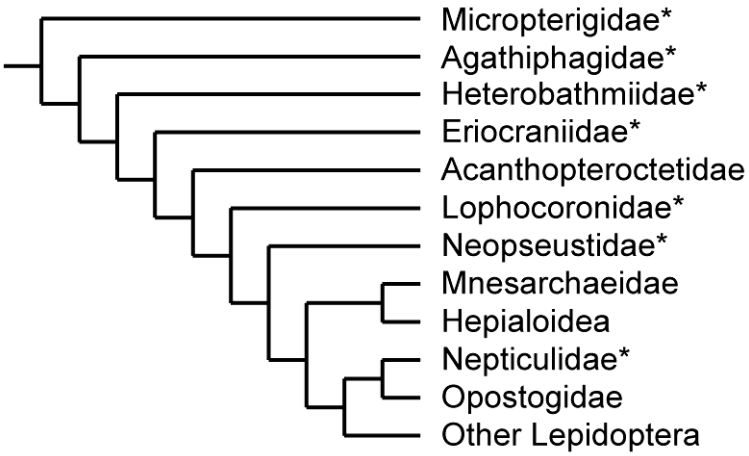

A
B
C
D
Mt

Figure 3-9. Tree showing family level relationships of basal Lepidoptera based on Kristensen and Skalski (1998). Families in which the sternum V gland or associated structures are present are marked with an asterisk.


Micropterigidae*
Agathiphagidae*
Heterobathmiidae*
Eriocraniidae*
Acanthopteroctetidae
Lophocoronidae*
Neopseustidae*
Mnesarchaeidae
Hepialoidea
Nepticulidae*
Opostogidae
Other Lepidoptera

Chapter 4

Derived morphology in a basal moth: the uniquely specialized sternum V glands of *Agathiphaga* (Lepidoptera: Agathiphagidae)[1]

INTRODUCTION

The presence of paired glands opening on abdominal sternum V is among the prominent groundplan autapomorphies of the superorder Amphiesmenoptera (Lepidoptera + Trichoptera), one of the (arguably *the*) best supported supraordinal groupings within the insects. In Trichoptera, these glands are retained in at least some members of all major subordinate lineages, while in the Lepidoptera they may have been lost in the stem-lineage of the Neolepidoptera (Kristensen 1998a), which comprises the vast majority of the members of the order. Remarkably, the functional significance of these glands is diverse within the superorder, their secretion being sex or aggregation pheromones in some subordinate lineages, and defensive substances in others (e.g. Duffield et al., 1977; Löfstedt et al., 1994). Equally remarkably, according to current knowledge this functional diversity is not clearly reflected in structural diversity.

The region of sternum V that surrounds the gland orifice may be conspicuously produced and/or otherwise modified, and the presence of gland-associated processes has been mentioned in passing in classical descriptive works by Trichoptera taxonomists (see, e.g., Ulmer, 1907). However, targeted study was only initiated with Philpott's (1925) descriptions of the cuticular structure of the gland-orifice area in Lepidoptera (representatives of Micropterigidae and Eriocraniidae) as well as in a philopotamid caddisfly, followed by subsequent work on representatives of Eriocraniidae by Le Cerf (1926), Razowski (1975) and

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Davis (1978). 'Soft morphology' of the glands in various representatives of Trichoptera was investigated by Eltringham (1931, 1934), Nielsen (1980), Ivanov and Melnitsky (1999, 2002) and most recently by Hashimoto and Kobayashi (2009), while that of the glands in some lepidopterans has been described by Kristensen and Nielsen (1979), Kristensen (1984) and Nielsen and Kristensen (1996). In almost all taxa, the cuticle-lined gland complex consists of a duct leading from the orifice to a saccular reservoir located in segment IV or V; an opener muscle that originates either on the anterior edge of sternum VI or on sternum V inserts on the duct just inside the orifice (Chapter 2, 3). 'Type 3' glandular cells (term of Noirot & Quennedy (1974)) discharge into the saccular reservoir, and there is often a network of muscle fibres surrounding either both or the latter.

The small SW Pacific moth family Agathiphagidae (with the single genus *Agathiphaga* and but two described, overall similar species) has attracted particular attention because of its basal position in the Lepidoptera; it is either the sister group of all other non-micropterigid Lepidoptera or even (but arguably less likely) the sister group of *all* other Lepidoptera (see, e.g., Kristensen et al. (2007) and references therein). In the original description of this family (Dumbleton, 1952), the processes bearing the gland openings were mentioned and sketched; the processes are longer than in any other Lepidoptera. Subsequently it has been reported (Kristensen (1984, 1998b) that the gland configuration differs markedly from that of other amphiesmenopterans in that the secretory component is a long twined tube which is eventually followed by a large thin-walled reservoir; the gland-orifice region has remained unstudied. It is the principal purpose of the present work to provide the first detailed description and pictorial documentation of the highly autapomorphic gland design in this taxon, with considerations of function and evolutionary implications.

MATERIALS AND METHODS

This study is based on material of *Agathiphaga vitiensis* from the Aneityum (New Hebrides) population that was successfully reared in the Natural History Museum

in the 1970s (Robinson & Tuck 1976). The moths were fixed in Bouin's shortly after emergence and subsequently stored in 70% ethanol. Male specimens were studied using wholemount slide preparations (2.5 specimens), histological sections and scanning electron microscopy (SEM).

Wholemounts (2.5 specimens) were prepared either from alcohol preserved material (70% ethanol) or from dried material rehydrated following the protocol of Birket-Smith (1965). The abdomen was divided horizontally and internal genitalia and the digestive tract were removed from the ventral part. Specimens were lightly stained with chlorazol black, cleared with clove or cedar oil and mounted with CAEDEX or Canada balsam. Specimens were studied with bright field, interference contrast and polarization microscopy.

Histological sections were prepared from material fixed in Bouin's fluid and stored in 70% ethanol. Metathorax and abdomen was dehydrated in an ethanol-butanol series, embedded in paraplast and cut sagittally in 8 µm sections (one specimen) or 12 µm sections (one specimen). Sections were stained with a trichrome stain; Weigert's haemetoxylin, bluish erythrosine and fast green. Standard 3D reconstructions were made from photographic prints of the 8 µm sections. The gland itself was also studied in semithin (1µm) sections made from a specimen embedded in epoxy resin and stained with Richardson's stain (methylene blue and azure II).

SEM observations were made on an alcohol preserved specimen that was freeze-dried following dehydration in an ethanol-benzol series and coated with gold.

OBSERVATIONS

The sternum V gland in *Agathiphaga vitiensis* is a complex structure with several parts distributed over sternum III to V. Figure 4-1 A shows an overview of the entire gland structure.

External cuticular specializations

The sternum V glands in *A. vitiensis* open near the bases of a pair of elongate, smooth tubular protuberances (Figure 4-2 A) which arise from the anterior sternal region and are directed posteromediad. From each gland orifice a shallow depression extends towards the apex of the protuberance; it is lined by a pair of narrow furrows which are not, however, continuous along their entire length. Near the gland orifice are a couple of socketed sensilla (one damaged and one biforked in the examined specimen) and an apparently unsocketed hair (Figure 4-2 B). At the base of the protuberance, the cuticle is transversely wrinkled as on the adjacent sternal area, but unlike the latter it is devoid of microtrichia.

Internal structures

Secretory section

The secretory gland section of each gland is formed by the apical portion of the long invagination from sternum V; it is an extremely elongate tubular sac coiled like a ball of yarn (Figures 4-1 A; 4-3 A), of which the bulk is located laterally in segment III. The secretory apparatus consists of 'type 3' gland cells (Noirot & Quennedey, 1974; Quennedy, 1998) which are so densely clustered that they appear as a continuous epithelium rather than as discrete units (Figure 4-2 C). The conducting ductule cells form an apparently single layer around the cuticular intima of the common duct (Figure 4-3 B).

Efferent system

The duct leading from the secretory, apical part of the gland is a long simple tube the distal part of which is entwined with the secretory part; together these parts form the above-mentioned 'yarn ball' (Figure 4-3 A). Eventually the duct is expanded into an elongate fusiform reservoir (Figure 4-1 A). The longitudinally plicate surface of the reservoir indicates a considerable capacity for expansion. The duct wall consists of a thin epithelium and a strongly folded cuticular intima (Figure 4-2 D). The epithelium and intima of the spindle-shaped reservoir are extremely thin, much thinner than that of the duct. Prior to entering the base of the

cuticular protuberance the gland duct goes through a series of bends (Figure 4-1 A).

Accessory sac

Near the gland opening a small accessory sac connects with the gland duct (Figure 4-1 A). The accessory sac is situated close to the body wall, and is dorsoventrally flattened (Figure 4-4 B, 4-5). The sac extends in a lateroanteriad direction from the point where it connects to the gland duct. The adjacent sternal region accommodates the origins of numerous muscle fibres (Figures 4-4; 4-5; 4-6 and 4-7), some of which connect the sac with the sternal cuticle, while others insert elsewhere on the sternum itself. These muscle fibres can be roughly classified as external fibres that run along the accessory sac adjacent to the body wall (Figure 4-7 B), and internal fibres that run along the accessory sac on the side facing away from the body wall (Figure 4-7 C).

External muscle fibres (Figure 4-7 B): Most of the external fibres inserting on the sac duct (ext. 1) originate on the anterior edge of sternum V. One or two fibres (ext. 2) that originate on sternum V beyond the lateral edge of the sac cross these duct fibres and insert near the centre of the posterior half of the sac. Some muscle fibres (ext. 3) have their posterior insertion on the cuticle of sternum V mesoanteriad of the point where the accessory sac connects to the gland duct, these fibres proceed anteriad and insert on the cuticle anteriad to the accessory sac. Some muscle fibres (ext. 4) that originate on sternum V anteriad to the accessory sac insert on the mesoanterior sac region; these fibres might extend farther than shown. A few muscle fibres near the anterior edge of the accessory sac run transversely near the point of origin and are most likely part of ext. 1.

Internal muscle fibres (Figure 4-7 C): Most (int. 1, 3, 5-7) of these insert on the short duct that connects the accessory sac to the gland duct. At least half of these (int. 3) originate on sternum V, some near the anterior edge, some slightly more laterally and less close to the anterior edge. One fibre (int. 7) originates near the anterior edge of sternum V and extends backwards along the median edge of the sac. Other fibres have their anterior insertions on the accessory sac itself: one

large fibre (int. 1) inserts anteriorly at the lateromost point, several fibres (int. 5) insert on the median half of the anterior edge, and a few (int. 6) insert between the centre and the median edge

A few muscle fibres (int. 4) that originate near the anterior edge of sternum V have their posterior insertion on the accessory sac itself; one near-longitudinal inserts near the lateral edge, a more oblique fibre inserts near the centre. A few muscle fibres (int. 2) attach to the sternum at both ends; their anterior insertion is in the same small region as int. 4 and some int. 3. They run alongside the int. 3 fibres till they reach the accessory sac, at which point int. 2 bends sharply lateroanteriad and run alongside the laterad int. 3 fibres and insert on the sternum in the same region as the latter.

Other musculature ((Figure 4-7 D): A large muscle fibre (oth. 1) that originates on the front edge of sternum V runs along the anterior edge of the accessory sac; it inserts at the mesoanteriad edge of the sac. Two muscle fibres (loop m.) insert on the internal side of the sac at the anterior edge, forms a loop around oth. 1 and insert on the external side of the sac at the anterior edge. One muscle fibre (oth. 2) inserting on the accessory sac duct runs along the gland duct, and eventually across the latter to join the muscle fibres surrounding the accessory sac where the latter reaches its farthest median point (Figures 4-3; 4-7 D). Due to compression of and subsequent displacement in wholemount specimens this does not appear to be the most median point of the accessory sac in Figure 4-1 A.

Gland opening mechanism

The duct intima just inside the gland orifice is thick, likely elastic and U-shaped in cross section. An opener muscle that inserts on this concave duct wall originates near the anterior edge of the contralateral part of sternum VI; hence the two gland opener muscles cross in the body midline (Figure 4-1 B). The fibre bundle that constitutes each gland opener muscle either splits (specimen used for reconstructions) when it crosses the longitudinal intersegmental muscles, thus approximately half of its fibres cross on either side of the longitudinal fibres (Figure 4-6 A), or run externally to the longitudinal muscles (other specimens).

Two of the muscle fibres (d.op.m.) from the gland-opening muscle follow the same path as the other fibres from the anterior edge of sternum VI until the bundle comes into close proximity with the gland duct near the base of the gland protuberance. At this point the two d.op.m. fibres arch below the gland duct and join the muscle fibres that surround the accessory sac instead of continuing to the main attachment area on the duct inside the gland opening (Figures 4-6 B; 4-7 B, D). In addition to the gland opener muscle, a single fibre (duct m.) that originates on sternum V anteriad of the accessory sac proceeds mesad and inserts on the gland duct at the first major bend (Figures 4-6 B; 4-7 D).

DISCUSSION

External body wall modifications

Elaborate cuticular specializations associated with the amphiesmenopteran sternum V gland opening have been described from several taxa. These modifications come in many forms: sculptured protuberance with setae and/or piliform scales in some micropterigids (Philpott, 1925; Kristensen, 1984; Chapter 3), sculptured but naked protuberances in representatives of Heterobathmiidae, Eriocraniidae and Lophocoronidae (Davis, 1978; Kristensen & Nielsen, 1979; Nielsen & Kristensen, 1996; Chapter 3) (all Lepidoptera), setose protuberances in representatives of Hydroptilidae (Marshall, 1979; Ivanov & Melnitsky, 1999, Chapter 2), grooved protuberances in representatives of Polycentropodidae (Ansteeg, 1989; Ansteeg & Dettner, 1991; Ivanov & Melnitsky 2002; Chapter 2) as well as well-developed protuberances in many other trichopterans (Eltringham, 1934; Mosely & Kimmins, 1953; Duffield et al., 1977; Ivanov & Melnitsky, 1999, 2002; Chapter 2). These structures are supposed to act as pheromone senders by increasing the evaporative surface (Razowsky, 1975; Davis, 1978; Kristensen, 1984; Ansteeg & Dettner, 1991; Chapter 2, 3). *Pycnopsyche scabripennis* (Rambur) (Trichoptera-Limnephilidae) uses its hind legs to smear gland secretions over the abdominal surface (Duffield et al., 1977).

While the cuticular protuberance in *A. vitiensis* is considerably longer than in any other lepidopteran, its smooth wall is without features that would increase

the surface area or transport gland secretions to a larger area. This lack of structures to enhance evaporation of gland product indicates that either such evaporation of secretion is not of major importance in this taxon, or that the secretion is transferred to another surface more suited for evaporation.

Secretory epithelium

The glandular epithelium in *A. vitiensis* is type 3 according to current terminology (Noirot and Quennedy 1974, Quennedy 1998), as in other representatives of Lepidoptera (Kristensen, 1984; Nielsen & Kristensen, 1996; Chapter 3). Eltringham (1931, 1934) described glandular cells with ducteoles in representatives of *Agapetus* (Trichoptera: Glossomatidae), *Diplectrona* (Trichoptera: Hydropsychidae) and *Polycentropus* (Trichoptera: Polycentropodidae), and thus these trichopterans must have type 3 gland cells as well. Furthermore, Hashimoto and Kobayashi (2009) described type 3 secretory cells in the glandular epithelium in representatives of *Stenopsyche* (Trichoptera: Stenopsychidae), *Eubasilissa* (Trichoptera: Phryganeidae) and *Nemotaulius* (Trichoptera: Limnephilidae). Assuming that type 3 secretory cells are part of the amphiesmenopteran ground plan is clearly most parsimonious.

In other lepidopterans, the secretory cell units discharge into the glandular reservoir (Kristensen, 1984; Nielsen & Kristensen, 1996; Chapter 3). Generally, this is also the case in representatives of Trichoptera (Ivanov & Melnitsky, 2002; Hashimoto & Kobayashi, 2009; Chapter 2), although some representatives of Hydropsychidae and Polycentropodidae have the secretory cells separate from the reservoir (Eltringham, 1934; Chapter 2). *Agathiphaga vitiensis* is unique in having the secretory cells discharge into a part of the invagination distant from the part which has been enlarged to function as a reservoir.

Expulsion of gland products

Gland opening

Opener muscles have been found in all lepidopteran families known to possess the sternum V gland (Kristensen, 1984; Nielsen & Kristensen, 1996; Chapter 3) as

well as in 24 trichopteran families (Nielsen, 1980; Ansteeg, 1989; Hashimoto & Kobayashi, 2009; Chapter 2). In other Lepidoptera, the gland-opening muscle originates on the front edge of sternum VI and attaches on the gland duct just inside the gland opening; if it crosses any ventrolongitudinal muscles, it runs either internally or externally (Kristensen, 1984; Nielsen & Kristensen, 1996; Chapter 3). The gland-opening muscles in *A. vitiensis* show two and sometimes three unique conditions: 1) they originate on the side of the midline opposite their target gland and hence cross each other (Figure 4-1 B); 2) a couple of fibres from the gland-opening muscle are not directed towards the main insertion area, but instead run in an arch below the gland duct and join the muscle fibres surrounding the accessory sac (Figures 4-6 B; 4-7 B, D); 3) in some specimens they split and run both internally and externally to the ventrolongitudinal muscles (Figure 4-6 A).

Contraction of the gland opener muscles opens the gland orifice, thereby allowing discharge of gland contents, but they do not actively assist in the process. The active expulsion would in many cases be effected by muscle fibres surrounding the gland reservoir as such fibres are found in representatives of four families of Lepidoptera and in representatives of several trichopteran families (Kristensen, 1984; Nielsen & Kristensen, 1996; Ivanov & Melnitsky, 2002; Hashimoto & Kobayashi, 2009; Chapter 2, 3). In *A. vitiensis*, contraction of the gland-opening muscle would open the gland orifice as in other amphiesmenopterans, but contraction of the two d.op.m. fibres (Figures 4-6 B; 4-7 B, D) would press the gland duct against the oth. 2 muscle fibre (Figures 4-5; 4-7 D) from the accessory sac musculature. If the d.op.m. fibres and the regular gland-opening muscle fibres contract simultaneously, it would apparently result in the concurrent opening of the gland orifice and closing of the gland duct. This effect would be amplified if the duct m. muscle fibre (Figures 4-6 B; 4-7 D) that inserts on the other side of the gland duct also contracts at the same time.

In *A. vitiensis*, gland products are stored in the fusiform reservoir which is completely separate from the secretory part of the gland. The plicate surface structure of the reservoir indicates an ability to expand to a larger size than shown

in Figure 4-1 A, but as there is no muscle tissue surrounding the reservoir, it cannot be emptied through direct muscular activity. However, a general increase in hemocoel pressure in the abdomen (mediated by contraction of body wall musculature) would compress the reservoir and force the gland product downstream. The main mechanism of active expulsion from the sternum V gland in *A. vitiensis* seems to be tied to the accessory sac. The accessory sac is surrounded by muscle fibres (e.g. Figures 4-4 A; 4-6 B). Some of these insert on the sac itself and originate on sternum V (Figures 4-7 B: ext. 4; 4-7 C: int. 4), thus being able to expand the sac. Some fibres (Figure 4-7 B: ext. 3) attach on the sternum at both ends and likely cause the cuticle to bulge out, expanding the posterior half of the accessory sac which is attached to the cuticle. Other fibres (Figures 4-7 B: ext. 1; 4-7 C: int. 1, 3, 5-7) mostly attach on the short duct that connects the accessory gland to the gland duct and on the cuticle anteriad of the accessory sac or on the accessory sac itself and presumably functions to contract the sac. This arrangement is suggestive of a pump-like function by which relatively small amounts of gland product can be released in short bursts.

A concurrent opening of the gland orifice and closing of the gland duct would leave only the gland product stored in the accessory sac to be released. This, combined with the possible pump-like function of the accessory sac, makes it likely that sustained release of gland products would require multiple rounds of muscular contraction. The fusiform reservoir is vastly larger than the accessory sac (Figure 4-1 A), thus repeated actions of the accessory sac pump would be required to release a significant proportion of the stored gland product. In addition to the muscles described above, the gland duct is also affected by the duct m. muscle fibre (Figures 4-6 B; 4-7 D). Contraction of this muscle fibre would pull the most mesal part of the gland duct towards a more lateral position. Pulling the duct toward the oth. 2 muscle fibre (Figures 4-5; 4-7 D) running along it could assist its closure.

Evidently, if the muscle fibres (Figure 4-6 B: d.op.m.) arching below the gland duct contract independently of the other gland opening fibres, secretions in the fusiform reservoir could be released directly through combined effects of

hemocoel pressure, contraction of the gland-opening muscle and relaxation of d.op.m.; also, contraction of d.op.m. may not completely occlude the duct lumen. Nonetheless, the presence of the accessory sac and its elaborate musculature appears explicable only if the accessory sac content is the single, or at least the principal component of the finally expelled product. Since no gland cells have been observed to be associated with the sac, no additional components are likely added to the secretions received from the reservoir. However, it cannot be categorically rejected that chemical reactions in the stored secretion could make it change over time, and that therefore a mixture of stored and newly formed secretion would have properties different from both.

Gland closure

The closure of the gland orifice is probably effected by the elasticity of the thickened cuticular intima combined with the U-shaped profile of the gland duct, not unlike the closure of the similarly structured efferent duct of the silk glands in caterpillars (Snodgrass, 1935, fig. 167 F).

Evolutionary origin of agathiphagid gland configuration

The design of the agathiphagid sternum V gland complex, which is so different from the counterparts in other amphiesmenopterans, begs a question about its evolutionary origin. While the size and shape of the accessory sac correspond to conditions of the gland reservoir in 'typical' amphiesmenopteran glands, the fact that muscle fibres anchor the sac to sternum V rather than to IV, as in all other Lepidoptera, and especially, the lack of associated secretory tissue argues in favour of it being a neoformation. Hence, the agathiphagid gland configuration apparently evolved through a transformation of the anterior part of the ancestral reservoir into a long tube on which the glandular units became contiguous, but whether the non-secretory duct and fusiform reservoir are derived from parts of the ancestral reservoir from which the glandular units were lost, or from the much shorter ancestral gland duct remains wholly conjectural.

Functions of the sternum V glands in Amphiesmenoptera

Philpott (1925) suggested that the gland produced sex pheromones. Eltringham (1934) suggested that the glands could be defensive in nature, but considered it unlikely as he had found better developed glands in males than in females. Kristensen (1972) also suggested that the glands might have a defensive function. Davis (1975) agreed, finding it more likely that the glands produced defensive secretions rather that sex pheromones. Razowski (1975) proposed that the external cuticular structures could serve as a sender by increasing the evaporative area.

Demonstrated and likely pheromone function

The most widespread known function of the sternum V gland is production of sex pheromones by females as this has been demonstrated and/or implicated in representatives of one lepidopteran family (Eriocraniidae) and representatives of several trichopteran families (Apataniidae, Glossosomatidae, Limnephilidae, Molannidae, Philopotamidae, Phryganeidae, Polycentropodidae, Rhyacophilidae and Sericostomatidae) (Resh & Wood, 1985; Solem, 1985; Löfstedt et al., 1994, 2008; Zhu et al, 1995; Bjostad et al., 1996; Jewett et al., 1996; Kozlov et al, 1996; Bergmann et al., 2001, 2002; Bergmann, 2002). In one trichopteran, *Hydropsyche angustipennis* (Curtis) (Hydropsychidae), males employ aggregation/sex pheromones; the male gland secretions are attractive to both sexes, but more attractive to males (Löfstedt et al., 1994). In this genus, males aggregate in swarms (Gruhl, 1960; Schuhmacher, 1969; Benz, 1975), a behavior that might be pheromone mediated (Löfstedt et al., 1994). As the pheromones secreted by the males are also somewhat attractive to females (Löfstedt et al., 1994), females are likely to be drawn to the swarming males, and thus facilitate mating. In representatives of Eriocraniidae and the various trichopteran families, the pheromones are short chain (Löfstedt et al., 1994; Zhu et al, 1995; Bjostad et al., 1996; Kozlov et al, 1996; Bergman et al., 2001, 2002); this suggests a common origin for the pheromones in basal lepidopterans and representatives of Trichoptera, making sex pheromone production an ancestral trait (Löfstedt & Kozlov, 1997; Chapter 6).

However, long distance sex pheromones have not been identified in representatives of *Sabatinca* (Micropterigidae) (Kozlov & Zvereva, 1999), *Halesus, Potamophylax*, *Pycnopsyche* (Trichoptera: Limnephilidae) and *Polycentropus* (Trichoptera: Polycentropodidae) (Duffield et al., 1977; Solem & Petersson, 1987) although the sternum V gland is present and sex pheromone use have been investigated.

Demonstrated and likely defensive function

Duffield et al. (1977) demonstrated a defensive function of the gland in *Pycnopsyche scabripennis* (Rambur) (Trichoptera: Limnephilidae), showing that gland secretions deterred attacks by ants, and furthermore showed that gland extracts did not attract caddisflies, thus excluding a long distance sex or aggregation pheromone function. A defensive function has been implicated in other trichopterans as well: *Rhyacophila fasciata*, *R. nubila* (Rhyacophilidae), *Polycentropus flavomaculatus* (Polycentropodidae) and *Phryganea grandis* (Phryganeidae) (Ansteeg & Dettner, 1991).

In some trichopteran species (Glossomatidae: *Agapetus fuscipes* Curtis, *Mastigoptila longicornuta* (Schmid); Philopotamidae: *Wormaldia subnigra* McLachlan; Rhyacophilidae: *Rhyacophila fasciata* Hagen, *R. nubila* Zetterstedt, *R. obliterata* McLachlan), in which females produce sex pheromones or pheromonal compounds likely to function as sex pheromones, males produce less or none of the pheromonal compounds, but produce other substances such as acetophenone, acetic acid, 2-methyl propanoic acid, hexanoic acid and octanoic acid (Ansteeg & Dettner, 1991; Löfstedt et al., 1994; Bergmann, 2002; Bergman et al., 2002). In other species (Hydropsychidae: *Cheumatopsyche lepida* (Pictet), *Hydropsyche siltalai* Döhler, *Smicridea annulicornis* (Blanchard); Limnephilidae: *Monocosmoecus pulcherrimus* Schmid; Philopotamidae: *Philopotamus montanus* (Donovan); Polycentropodidae: *Neureclipsis bimaculata* (Linneaus); Psychomyidae: *Lype phaeopa* (Stephens)), these types of compounds have been found in females in addition to pheromone compounds (Bergmann, 2002; Bergman et al., 2002). In one species (Polycentropodidae: *Polycentropus*

flavomaculatus (Pictet)) in which females do not attract males with long distance pheromones, male glands contain primarily 2-methyl butanoic acid (Solem & Petersson, 1987; Ansteeg & Dettner, 1991). Acetophenone as well as the organic acid are toxic and/or irritants to mammals (Lewis, 2000; Pohanish, 2002) and several can be toxic to insects (Ansteeg & Dettner, 1991; Mohsen et al., 1995). Based on this, it is possible that a defensive function of the gland might be quite widespread. Furthermore, in most investigated species males have well developed glands (Trichoptera: Ivanov & Melnitsky, 1999; Hashimoto & Kobayashi, 2009; Chapter 2; Lepidoptera: Davis, 1978; Kristensen & Nielsen, 1979; Kristensen, 1984; Nielsen & Kristensen, 1996; Chapter 3), but the general function of the glands in this sex is unknown as targeted studies have generally failed to identify male sex pheromones (Resh & Wood, 1985; Solem, 1985; Löfstedt et al., 1994; Zhu et al., 1995; Bjostad et al., 1996; Kozlov & Zvereva, 1999; Bergman et al., 2002; Houghton, 2002). A defensive function could explain the presence of glands in the absence of pheromone production.

Functional implications of the *Agathiphaga* gland configuration

The sternum V gland is generally present in both sexes, or only in females. The latter is the case in representatives of several trichopteran families (Anomalopsychidae, Beraeidae, some Chathamiidae, Calocidae, Coenosucidae, Helicophidae, Molannidae, some Philopotamidae) and in some species of Eriocranidae (Ivanov & Melnitsky, 1999, 2002; Karsholt et al., in prep; Chapter 2, 3). The agathiphagid condition with the glands present only in the male is possibly unique. Ivanov and Melnitsky (1999) found that the gland is absent in female *Stenopsyche marmorata* Navas (Trichoptera: Stenopsychidae), but Hashimoto and Kobayashi (2009) reported it to be present and similar to the gland in conspecific males. When a glandular structure is only present in one sex, it is likely to be involved in courtship (sex pheromones) or interactions between members of the same sex.

The sternum V gland in *A. vitiensis* does not possess an enlarged evaporative surface, but the accessory sac musculature seemingly makes it

possible to eject gland products with some force. Hence, if the gland product is a pheromone, it is straightforward to suggest that the agathiphagid male, like the above-mentioned caddisfly *Pycnopsyche scabripennis*, would spray it on its legs and then perhaps smear it on other body parts as well. Alternatively, the pheromone could be sprayed or smeared on some other object (e.g. a plant surface) from where it would evaporate, attracting females, but not directly expose the male to predators that might also be tracking the pheromone. Predators tracking prey pheromones are known in several insects groups (Stowe et al., 1995 and references therein; Erbilgin & Raffa, 2001). Yet another possibility is that defensive properties of the gland have evolved for antagonistic male-male interactions, with males spraying gland contents at each other; in this case, however, one would arguably expect the gland orifice to be located apically on the sternal protuberance rather than on its base. All of this remains pure conjecture. As the structure of the sternum V gland in agathiphagids is extremely derived, the function might also be derived. Electroantennograms, observations of behaviour, and chemical analysis of gland contents will hopefully clarify the function of the agathiphagid sternum V gland eventually. However, such studies on these moths that are so notoriously difficult to breed in the laboratory, and of which the adults have been encountered so rarely in nature (see, e.g., Robinson & Tuck, 1976; Upton, 1997; Zobrowski & Edwards 2007), are presently a distant goal.

Agathiphaga: A living fossil with many derived traits

If the terms 'living fossils' or 'relict taxon' can be appropriately applied to any organisms, the two *Agathiphaga* species are certainly among them. In general appearance, the adult moths strongly resemble trichopterans, and the taxon retains a suite of plesiomorphic character states unknown from any other lepidopterans (Kristensen, 1998b).

But living fossils, like other organisms, are mixtures of character states which are plesiomorphic and apomorphic at the level in question. Notable autapomorphies of *Agathiphaga* (Kristensen, 1998b, 2003) include the lack of

ocelli, bifurcate antennal sensilla, simplified (likely non-functional) mandibles and the legless endophytic larvae with markedly reduced body setae. Previously known autapomorphic traits associated with the sternum V gland included the location of the gland opening on a long smooth process, secretory gland section a long coiled tube leading to separate thin-walled reservoir, absence of gland in female. To this can now be added the following autapomorphies: Gland-opening muscles crossing medially, some fibres of this gland-opening muscle diverted to different attachment point, presence of accessory sac with elaborate associated musculature.

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FIGURE 4-1. Drawings based on wholemounts. The drawings are viewed through the cuticle and anterior is up. Muscle fibres are grey and all structures are drawn as if they were transparent. A: Overview of the entire sternum V gland structure (left side gland shown). Note the protuberance, gland-opening muscle, accessory sac with associated musculature (only external musculature shown here, see Figure 4-7 for complete accessory sac musculature), fusiform reservoir and the coiled mass of the secretory part of the gland. B: Protuberances and crossed gland-opening muscles. AeSV, anterior edge of sternum V; AeSVI, anterior edge of sternum VI; PeSIV, posterior edge of sternum IV; As, accessory sac; Go, gland opening; Ip, insertion point for opening muscle; Op, origin point for opening muscle; Rs, gland reservoir; Sc, secretory cells; L-t, Lepidoptera-type opening muscle. Scale bars: A, B = 100 μm.

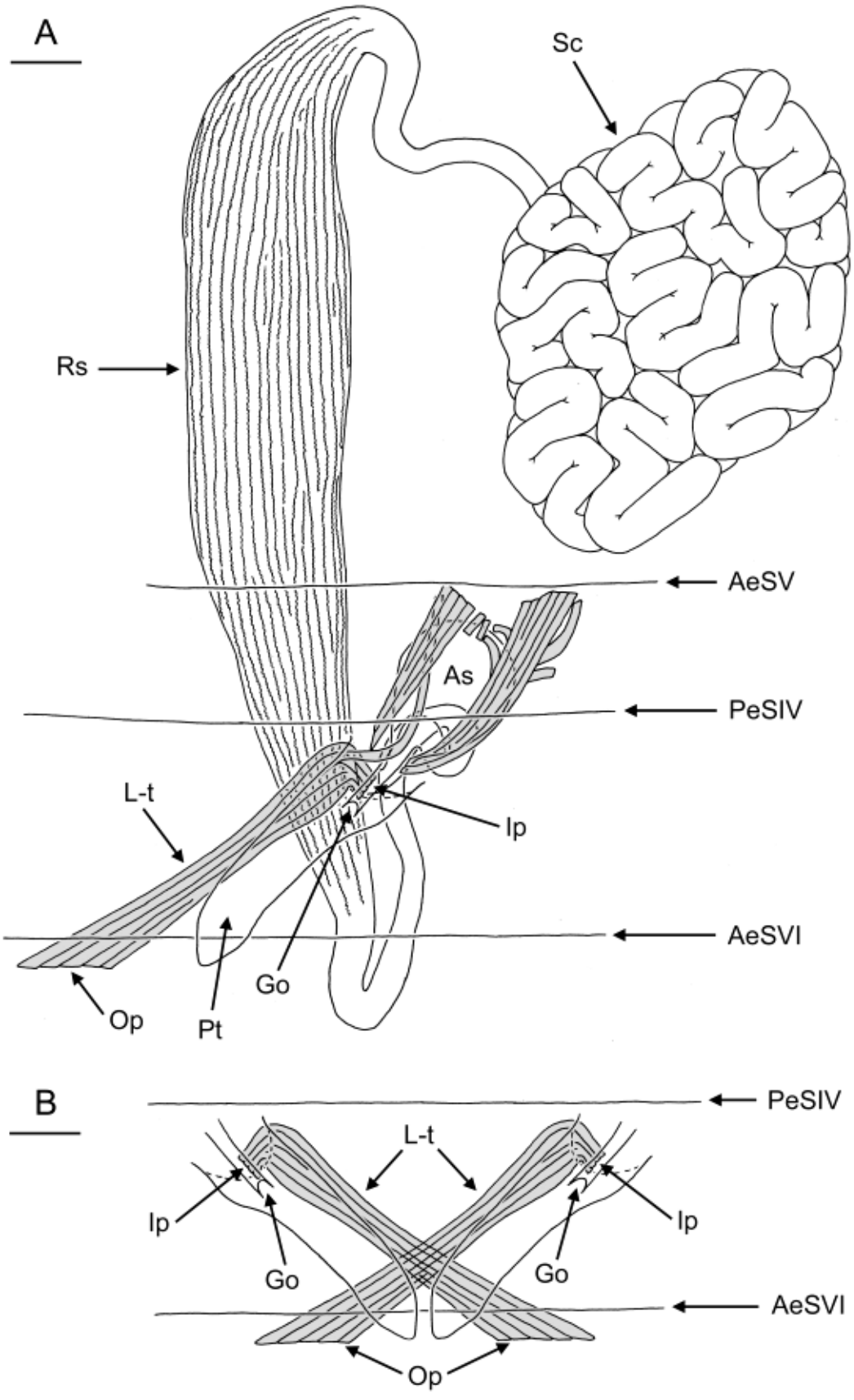

A
Sc
Rs
AeSV
As
PeSIV
L-t
Ip
AeSVI
Go
Op
Pt
B
PeSIV
L-t
Ip
Ip
Go
Go
AeSVI
Op

FIGURE 4-2. SEMs of external structures and light micrographs of 1 μm sections of secretory part of gland. A and B: SEMs. A: Overview showing protuberances. Note wrinkled cuticle around the bases, the position of the gland openings and the shallow depressions that run from the orifices towards the apices. B: Close-up of gland opening. Note the socketed sensilla (one broken, one biforked with one branch broken) and the narrow furrows lining the shallow depression. C and D: Light micrographs. C: Overview showing both gland duct (marked with black arrows) and secretory part of gland (around thin ducts marked with white arrows). Note how the secretory cells are so densely packed that they appear as a continuous epithelium. D: Close-up of gland duct within the 'yarn ball'. Note the thin epithelium and the strongly folded cuticular intima (black arrows). Fr, furrows; Go, gland opening; Sc, secretory cells; Sd, shallow depression; Sn, sensilla. Scale bars: A = 100 μm; B = 10 μm.

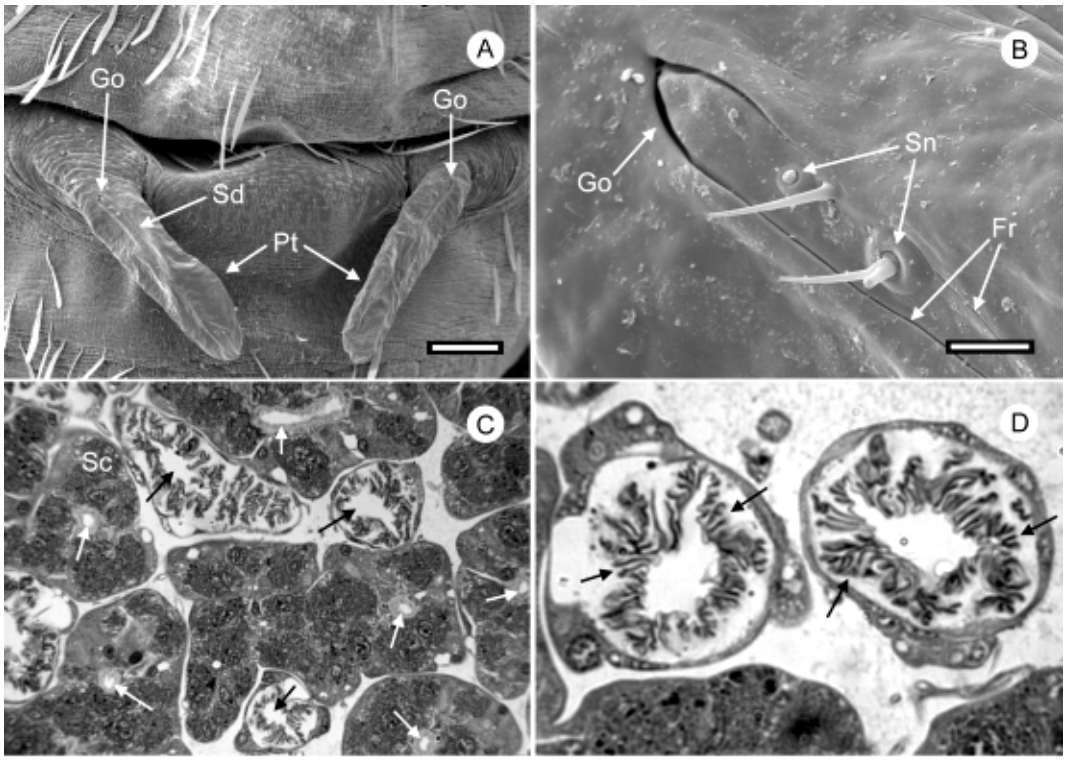

A
Go
Go
Sd
Pt
B
Go
Sn
Fr
C
Sc
D

FIGURE 4-3. Drawings based on 8 μm histological sections. A: Cross-section through the coiled mass, 'yarn ball', of the secretory part of the gland showing how the distal part of the gland duct is entwined with the secretory part of the gland. Thin-walled sections (Dt) are part of the gland duct while the thick-walled sections (Sc) are the secretory part of the gland. B: Cross-section through a single coil from the secretory part. Note the large secretory cells and the small cells surrounding the lumen (grey). The cross-hatched nuclei of some of the cells around the edge had a different appearance than the other nuclei. The slight amount of space between the cells is probably a shrinkage artefact of the preparation method (see Figure 4-2 A for a section without shrinkage), but this very artefact makes it possible to see how the cells are organised around the lumen of the gland. Dt, gland duct; Sc, secretory cells. A = 100 μm; B = 20 μm.

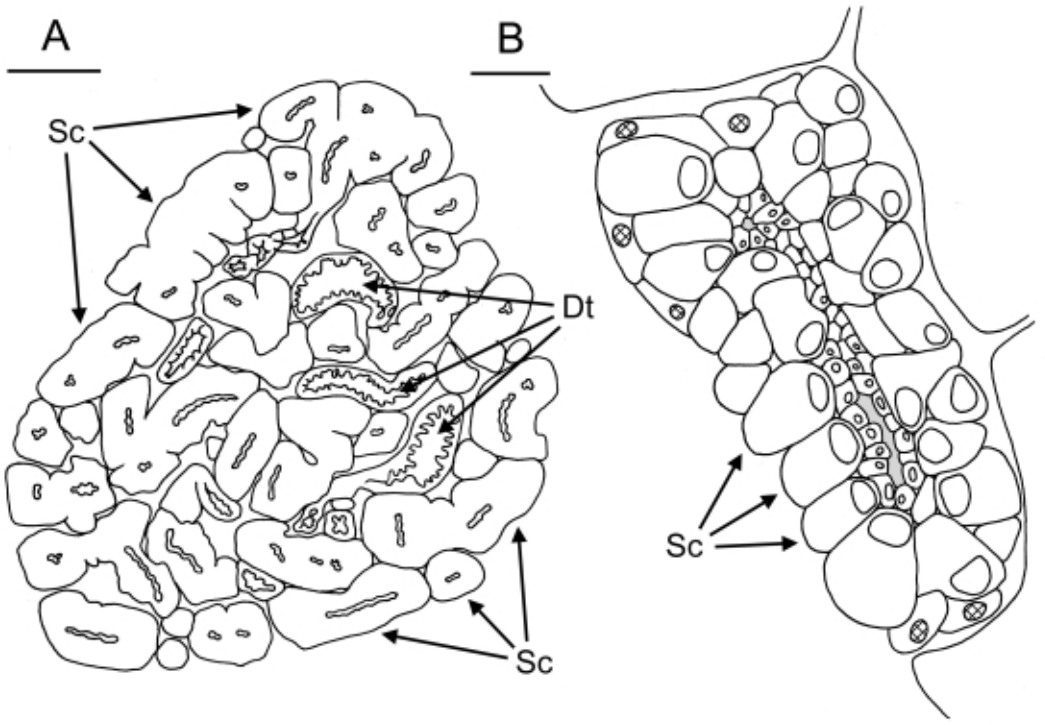

A
B
Sc
Dt
Sc
Sc

FIGURE 4-4. Three-dimensional reconstructions based on 8 μm histological sections. Anterior is towards the left and the view is towards the midline of the animal. Muscle fibres are coloured grey. A: Based on sections 1 through 39. Note the mass of muscle fibres that cover the accessory sac. B: Based on sections 16 through 34. Note the muscle fibres that surround the accessory sac. As, Accessory sac; Asm, accessory sac musculature; duct m.; muscle fibre originating on cuticle of sternum V and inserting on gland duct, see Figure 4-7 D; d.op.m., divergent fibres of gland-opening muscle, see Figure 4-7; Dt, gland duct; duct m., muscle fibre originating on sternum V and inserting on the gland duct, see Figure 4-7 D; Ism, intersegmental muscles; oth.2, muscle fibre crossing gland duct and inserting on accessory sac, see Figure 4-7 D; L-t, Lepidoptera-type opening muscle.

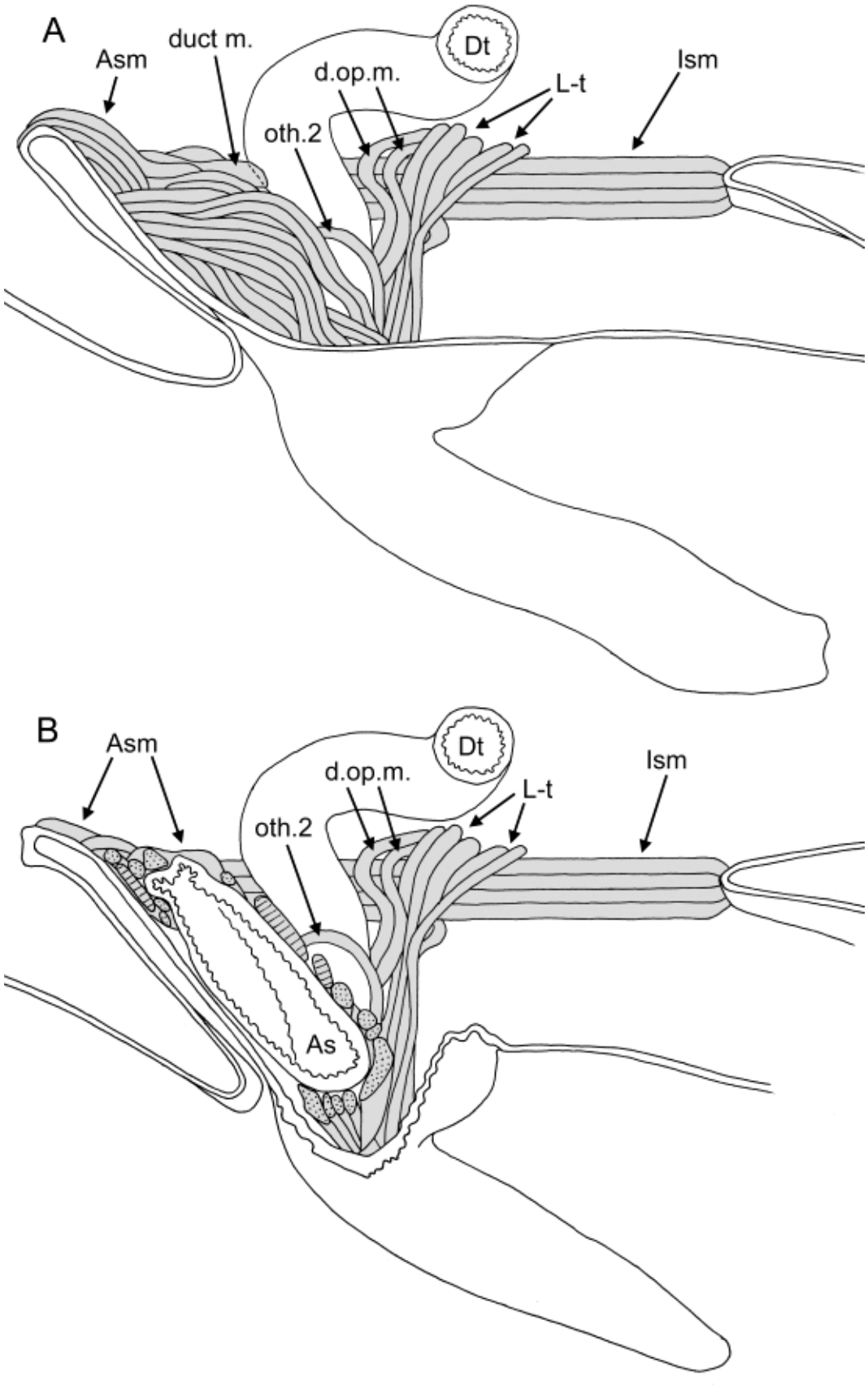

A
Asm
duct m.
Dt
d.op.m.
L-t
lsm
oth.2
B
Asm
Dt
d.op.m.
L-t
lsm
oth.2
As

FIGURE 4-5. Three-dimensional reconstruction based on 8 μm histological sections, 22 through 34. Anterior is towards the left and the view is towards the midline of the animal. Muscle fibres are coloured grey. Note how the accessory sac connects to the gland duct just inside the gland opening. Also note how the oth.2 muscle fibre crosses the gland duct then runs along the duct. As, Accessory sac; d.op.m., divergent fibres of gland-opening muscle, see Figure 4-7; Dt, gland duct; Go, gland opening; Ism, intersegmental muscles; oth.2, muscle fibre crossing gland duct and inserting on accessory sac, see Figure 4-7 D; L-t, Lepidoptera-type opening muscle.

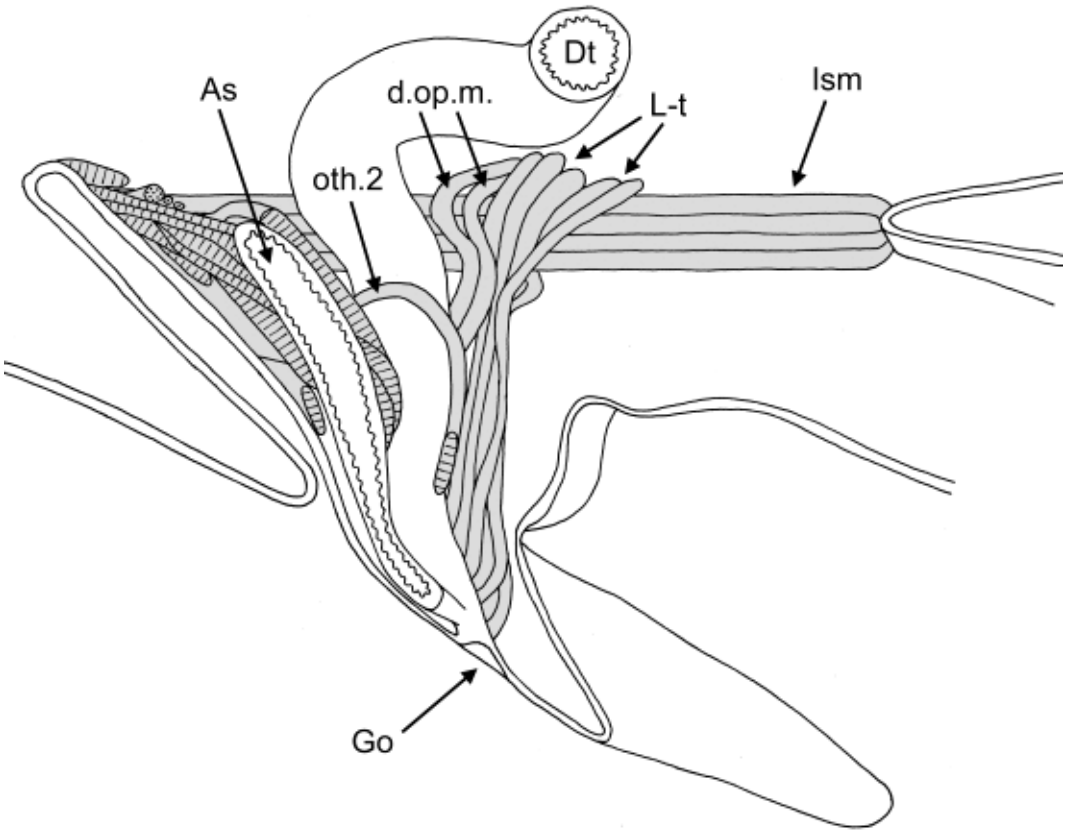

Dt
As
d.op.m.
L-t
lsm
oth.2
Go

FIGURE 4-6. Three-dimensional reconstructions based on 8 μm histological sections. Anterior is towards the left and the view is outwards from the midline of the animal. Muscle fibres are coloured grey. A: Based on sections 39 through 18. Note how the gland-opening muscle splits and runs both above and below the intersegmental muscle. B: Based on sections 33 through 16. The intersegmental muscle and most of the gland-opening muscle have been removed. Note the divergent opening muscle fibres crossing the gland duct and joining the accessory sac musculature. Also note the duct m. fibre inserting on the gland duct and the muscle fibres covering the accessory sac. duct m.; muscle fibre originating on cuticle of sternum V and inserting on gland duct, see Figure 4-7 D; d.op.m., divergent fibres of gland-opening muscle, see Figure 4-7; Dt, gland duct; duct m., muscle fibre originating on sternum V and inserting on the gland duct, see Figure 4-7 D; Go, gland opening; Ism, intersegmental muscles; L-t, Lepidoptera-type opening muscle.

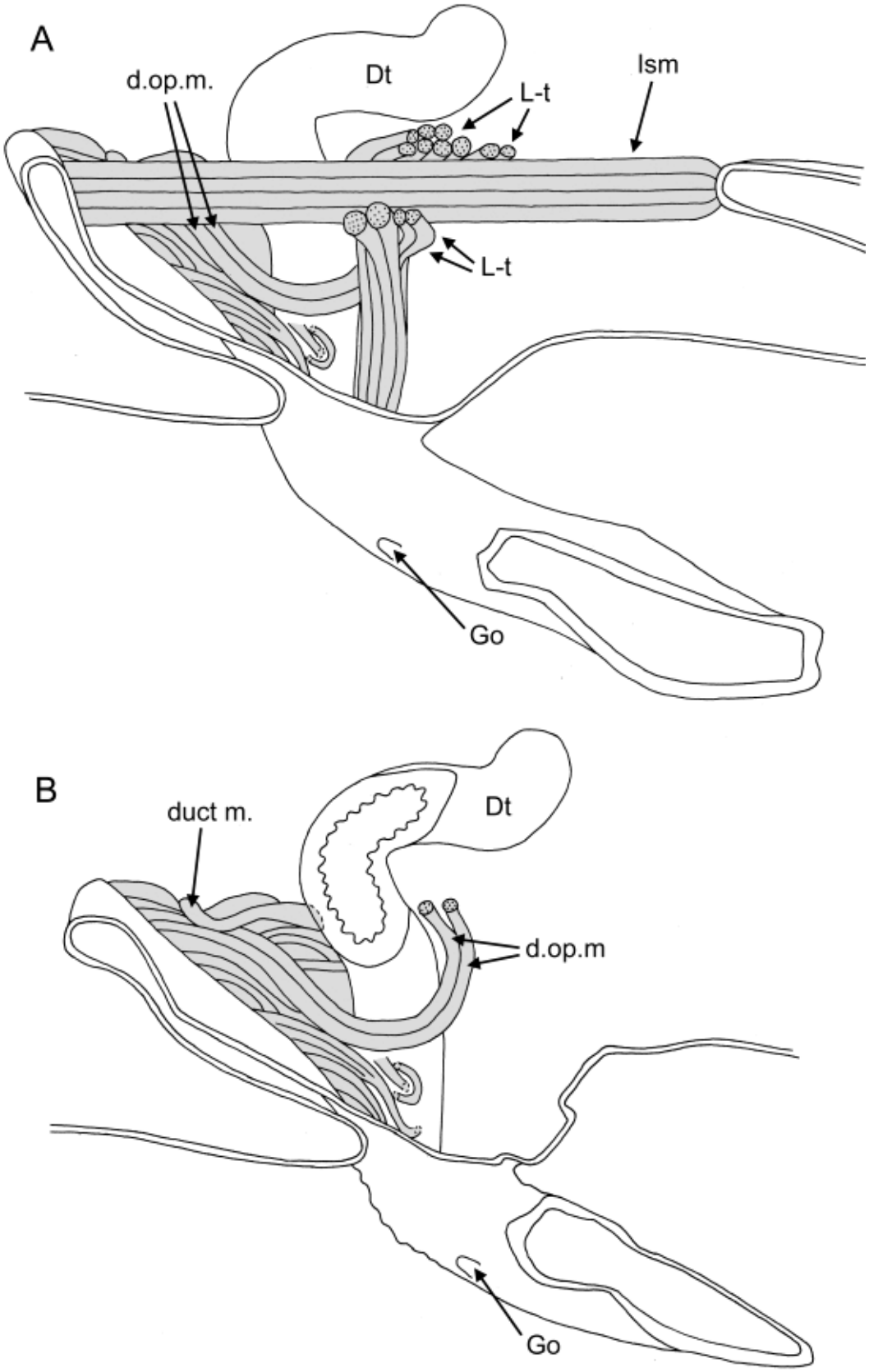

A
d.op.m.
Dt
lsm
L-t
L-t
Go
B
duct m.
Dt
d.op.m
Go

FIGURE 4-7. Transverse three-dimensional reconstructions of accessory sac musculature based on 8 μm histological sections. Anterior is up and mesad is towards the left side of the page. Muscle fibres are coloured grey, and attachment points of muscle fibres are marked with ellipses. Grey ellipses indicate attachment on the accessory sac, ellipses with black on white cross-hatch indicate attachment on the cuticle of sternum V, and ellipses with white on dark grey crosshatch indicate attachment on the gland duct.

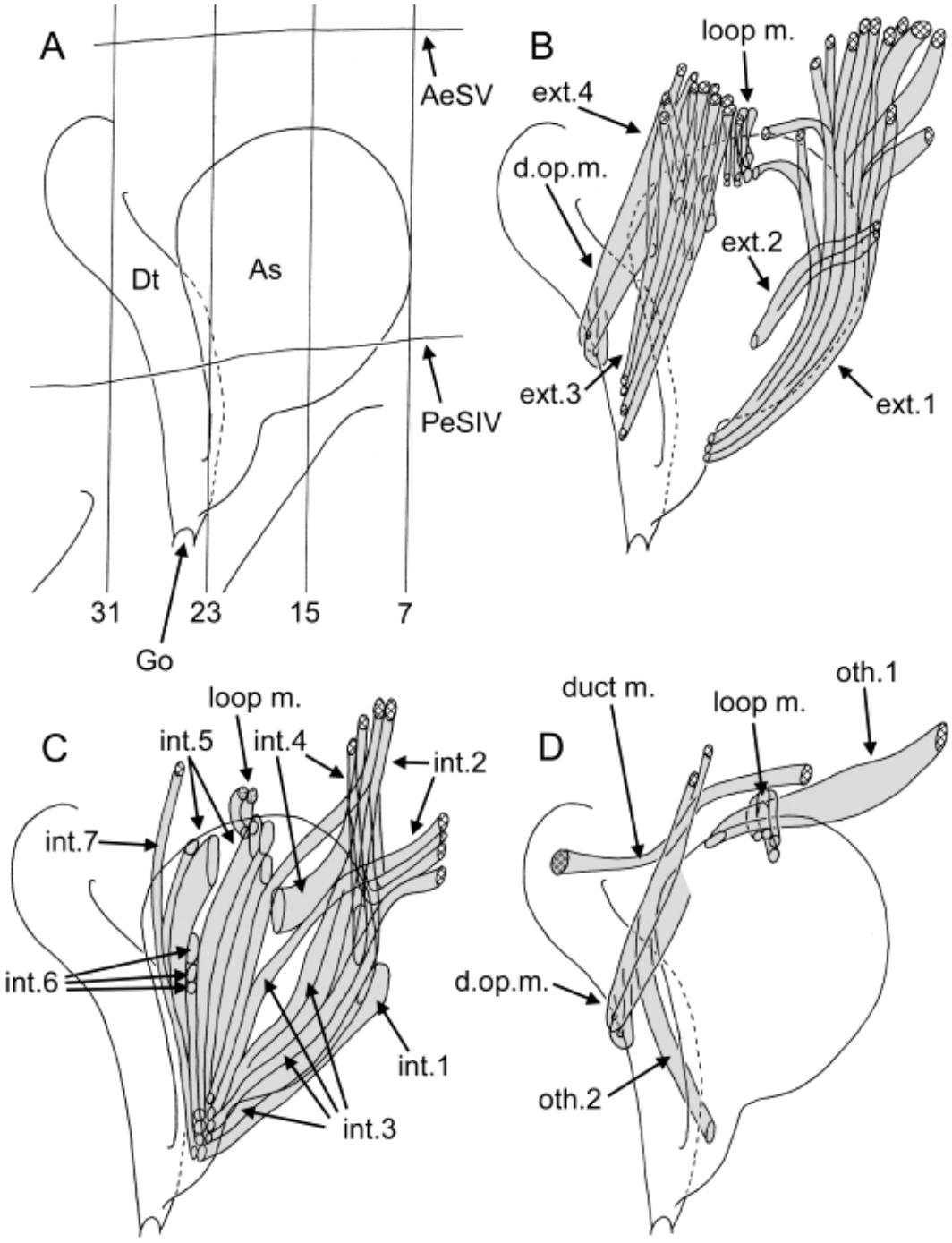
A: Overview showing cuticular structures: base of protuberance with gland opening, edges of sternites, the gland duct and the accessory sac. The numbered vertical lines refer to section numbers to facilitate cross-referencing between this figure and Figures 4-4 through 4-6. AeSV, anterior edge of sternum V; PeSIV, posterior edge of sternum IV; As, accessory sac; Dt, gland duct; Go, gland opening.

B: External accessory sac musculature. Ext. 1, fibres originating on the anterior edge of sternum V and inserting on the short accessory sac duct; ext. 2, one or two fibres originating on sternum V beyond the lateral edge of the sac cross the ext. 1 fibres and insert near the centre of the posterior half of the sac; ext. 3, fibres attaching on the cuticle at both ends: anterior attachment is anteriad of the accessory sac, posterior attachment is mesoanteriad of the point where the accessory sac connects to the gland duct; ext. 4, fibres originating on the cuticle anteriad to the accessory sac and inserting on the mesoanterior sac region (these fibres might extend farther than shown); d.op.m., divergent opening muscle fibres, see D; loop m., see D.

C: Internal accessory sac musculature. Int. 1, fibre attaching on the sac at both ends: anterior insertion is on the latero-most point of the sac, posterior insertion is on the short sac duct; int. 2, fibres attaching on the cuticle at both ends: anterior insertion is close the anterior edge of sternum V, after reaching the accessory sac the fibres bend sharply lateroanteriad and the posterior insertion point is on the cuticle laterad of the sac; int. 3, fibres originating on the cuticle and inserting on the sac duct: some originate close to the anterior insertion of int. 2, some close to the posterior insertion of int. 2, all int. 3 insert on the short duct of

the accessory sac; int. 4, fibres originating close to the anterior insertion of int. 2 and inserting on the anterior half of the sac; int. 5, fibres attaching on the sac at both ends: anterior insertion is on the median half close to the anterior edge of the sac, posterior insertion is on the short accessory sac duct; int. 6, fibres attaching on the sac at both ends: anterior insertion is between the centre of the sac and the median edge, posterior insertion is on the short accessory sac duct; int. 7, fibre originating near the anterior edge of sternum V, running along the median edge of the sac and inserting on the short accessory sac duct; loop m., see D.

D: Other accessory sac musculature and musculature near the accessory sac. Oth. 1, large muscle fibre originating on the front edge of sternum V, it runs along the anterior edge of the sac and inserts on the mesoanteriad edge of the sac; oth. 2, fibre inserting on the interior side of the short accessory sac duct, it runs along the gland duct, then across it before joining the accessory sac musculature at the farthest median point of the latter, the fibre most likely originate near the front edge of sternum V, see Figure 4-5 for a different view; loop m., two fibres inserting on the exterior side of the sac near the anterior edge, forming a loop around oth. 1 before inserting on the interior side of the sac near the anterior edge; d.op.m., divergent opening muscle fibres: after diverging from the other opening muscle fibres, the two divergent fibres arch below the gland duct and join the accessory sac musculature, the anterior attachment point is near the front edge of sternum V, see Figure 4-6 B for a different view; duct m., fibre originating on the cuticle anteriad of the accessory sac and inserting on the gland duct at the first major bend, see Figure 4-6 B for a different view.


A
AeSV
Dt
As
PeSIV
31
23
15
7
Go
B
loop m.
ext.4
d.op.m.
ext.2
ext.3
ext.1
C
loop m.
int.5
int.4
int.2
int.7
int.6
int.1
int.3
D
duct m.
loop m.
oth.1
d.op.m.
oth.2

Chapter 5

Evolutionary riddles and phylogenetic twiddles: the ground plan and early diversification of the sternum V glands in Amphiesmenoptera (Trichoptera + Lepidoptera)[1]

INTRODUCTION

Amphiesmenoptera (Trichoptera + Lepidoptera) is one of the best supported superordinal clades in the insects, and one 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 five 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, described the gland opening as 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 that the gland opening in three other

[1] A version of this chapter has been submitted for publication as: Djernæs, M. & Sperling, F. A. H. Evolutionary riddles and phylogenetic twiddles: the ground plan and early diversification of the sternum V gland in Amphiesmenoptera (Trichoptera + Lepidoptera). *Proceedings of the 13th International Symposium on Trichoptera.*

species of Trichoptera (in Limnephilidae, Phryganeidae and Stenopsychidae) is slit-like, although sometimes with associated 'notches' in the cuticle.

Gland-opening muscles

Gland-opening muscles[2] were observed by Nielsen (1980) in representatives of two 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 possess 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 a pair of transparent patches of cuticle, occur on sternum IV in females of Philopotamidae (Trichoptera), Eriocraniidae, Neopseustidae and Nepticulidae (Lepidoptera) (Davis 1975; Ivanov & Melnitsky 1999, 2002).

[2] Although Nielsen (1980) called them 'obturator', closing, muscles, he clearly describes them as opening the gland duct on p. 89.

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 reservoir of a normally developed sternum V gland; the reservoir is pressed against the fenestra (Davis 1975, 1978). However, in neopseustids and nepticulids the fenestrae apparently occur independently (Davis 1975, 1978). However, one nepticulid species (*Stigmella malella* (Stainton)) produces 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 that this type is 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 & 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 two 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 reduced form that is

nonetheless still functional and secretes 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.

MATERIAL AND METHODS

Taxon sampling

Representatives of thirty-eight 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 5-1.

Wholemounts, SEM, histology

For all species, both wholemount preparations and Scanning Electron Microscopy (SEM) of both sexes were used when possible (Table 5-1). In general, one specimen of each sex was used for each preparation.

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 with 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* (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 with a camera lucida.

Mapping

Gland opening muscles and perforated patches with associated 'sunburst' musculature were mapped on a phylogeny of Amphiesmenoptera. For Lepidoptera, we followed Kristensen and Skalski (1998) and for Trichoptera we followed Holzenthal et al. (2007). Mapping was done manually with both parsimony reconstruction of ancestral states and ancestral states reconstructed to reflect a single origin of each mapped character. For the parsimony reconstructions losses and gains were regarded as equally likely. For the single origin scenarios the relative probabilities of gains and losses were calculated based on the ancestral state reconstructions. Please refer to the Discussion for the actual calculations.

The reconstruction of ancestral states depend both upon the topology of the tree and the distribution of characters in recent taxa. Thus, if our reconstruction had been based on a different tree, or if future research reveals a different distribution of the relevant characters, the exact numbers of gains and losses would likely be different. This would in turn affect the relative probabilities of gains and losses in the single origin scenarios. However, the four spicipalpian families are consistently placed at the base of the trichopteran tree,

Philopotamidae is generally regarded as fairly basal within Annulipalpia, and Psychomyiidae is consistently grouped with Ecnomidae, Dipseudopsidae, Polycentropodidae and Xiphocentronidae (Frania & Wiggins 1997; Morse 1997; Ivanov, 2002; Holzenthal et al. 2007). Thus, even if the specific numbers change, our overall conclusions should remain stable.

OBSERVATIONS

Gland opening

SEM studies showed that the gland opening is a single slit in the sclerotised part of sternum V (Figure 5-1 A-C). The only exception was in *Psychomyia flavida* females (Trichoptera: Psychomyiidae) in which the slit-like gland opening was situated in the membranous cuticle between sternum V and IV (Figure 5-1 D). No trace of pores was found, even at magnifications at which 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 (Figure 5-2 H) with thickened cuticle, especially the inner wall. When the gland duct is compressed, the gland opening is closed.

Gland-opening muscles

Most trichopterans that possess the sternum V gland have gland-opening muscles that originate on the cuticle of sternum V mesad of the gland opening and insert on the walls of the gland duct just inside the gland opening. The opening muscle consists of one to several bundles of muscle fibres, which are typically fan-shaped with the tip of the fan inserting on the gland duct (Figure 5-3 A).

Some trichopterans (all species examined in Glossosomatidae, Hydroptilidae, Philopotamidae and Rhyacophilidae) have gland-opening muscles that originate on the anteromedial margin of sternum VI (Figure 5-3 B). A single species, *Wormaldia arizonensis* (Philopotamidae), possesses both types simultaneously (Figure 5-3 C). 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 (Figure 5-4 C). All species examined from the five

lepidopteran families known to possess the sternum V gland had gland-opening muscles that originate on the anterior margin of sternum VI.

Fenestrae/perforated patches

SEM investigations showed that the sternum IV fenestrae in females of Philopotamidae (Trichoptera), Eriocraniidae, Neopseustidae and Nepticulidae (Lepidoptera) consists of perforated cuticle (Figures 5-2 E, F; 5-5 A, 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 (Figures 5-2 A, B; 5-5 C, D), but to be otherwise absent in investigated species.

The fenestrae in female eriocraniids are known to be associated with the gland reservoirs; the reservoir subtends the fenestra (Davis, 1978) (Figures 5-2 C; 5-4 F). Our investigations showed the same relationship between the perforated patches and the gland reserviors in female psychomyiids (Figure 5-4 D). The fenestrae in female philopotamids were associated with glandular structures consisting of a cuticular gland sac surrounded by secretory cells (Figure 5-4 A, 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 (Figure 5-3 D). Male philopotamids only possess normal sternum V glands. The fenestrae in female neopseustids and nepticulids are also associated with glandular structures that consist of a cuticular gland sac surrounded by secretory cells (Figures 5-2 G; 5-4 G-I). Like the fenestral glands in female philopotamids, these lack excurrent ducts.

In philopotamids, psychomyiids and eriocraniids a distinctive ‘sunburst’ musculature is associated with the fenestrae/perforated patches. This musculature consists of muscle fibres that originate on the cuticle around the fenestrae/perforated patch and insert on the wall of the gland reservoir (Figures 5-2 D; 5-4 A-F). A more irregular version of this arrangement is associated with the fenestrae in *Synempora andesae* (Neopseustidae) (Figure 5-4 G, H) while no muscle fibres were found to be associated with the fenestrae in nepticulids.

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 167 F). 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 that gland-opening muscles are 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 only been reported by Eltringham (1934, figure 3), Nielsen (1980) and Hashimoto and Kobayashi (2009) in representatives of four families, although the two former 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 five families of Lepidoptera (Chapter 2, 3).

We found two completely different types of gland-opening muscles in Trichoptera, confirming Nielsen's (1980) observations. One type originates anteromedially on sternum VI (Figure 5-3 B) and is found in representatives of three spicipalpian families (Glossosomatidae, Hydroptilidae and Rhyacophilidae)

and of one annulipalpian family (Philopotamidae). The other type originates mesad on sternum V (Figure 5-3 A) 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 trichopteran types of opening muscles 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 spicipalpian) belong at the very base of Trichoptera with the trichopteran gland-opening muscle as a later development. Spicipalpia are probably not monophyletic, 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 gains of morphologically indistinguishable muscles (Figure 5-6 A), but we regard this as highly unlikely. Presuming only one 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 (Figure 5-6 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 1⅓ as likely as gains[3], then the single origin scenario becomes most parsimonious.

[3] Figure 5-6 A: seven losses and five gains, Figure 5-6 B: eleven losses and two gains:
7 losses + 5 gains = 11 losses + 2 gains <=> 1 gain = 1⅓ loss

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 in which 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 (Figures 5-2 D; 5-4 A-H). 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.

Nepticulids have glandular tissue and a reservoir associated with each fenestra, but no indications of a 'sunburst' musculature (Figure 5-4 I). 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 (Figure 5-5 I, J). 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 under Observations, the fenestral glands in female philopotamids co-occur with normally developed sternum V glands (Figure 5-3 D). However, the histological structure shows a cuticular reservoir (Figure 5-4 B) that 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 that the fenestral glands are derived from the sternum V gland, probably through increased compartmentalisation, eventually leading to complete physical separation of the part of the gland discharging through the gland duct and the part secreting through the fenestra (Figure 5-7).

Ansteeg (1989) observed the concurrence of both 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 semipurella* 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 glands.

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) (Figure 5-1 C). This transparent and scaled cuticle might superficially resemble the fenestrae on sternum IV. 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 (Figure 5-7).

Nevertheless, the occurrence of fenestrae/perforated patches, and in most cases also a distinctive and specialised muscular structure, the 'sunburst', in both Trichoptera and Lepidoptera (Figures 5-2 D; 5-4; 5-5) suggests that this arrangement originated in the common ancestor, with losses occurring in several members of both lineages (Figure 5-8 B). An alternative is to hypothesize five independent origins of perforated cuticle and four independent origins of the associated 'sunburst' musculature (Figure 5-8 A)[4]. 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 or 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

[4] 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:
Figure 5-8 A: nine gains, Figure 5-8 B: two gains and 29 losses
9 gains = 2 gains + 29 losses <=> 1 gain ≈ 4.14 loss

and associated structures might be an 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.

We have shown that the functional components (perforated cuticle and 'sunburst' musculature) of the fenestral complex can be present without the presence of a 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* showed a gland structure 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 (Chapter 3). Also of interest would be any species of Acanthopteroctetidae (Lepidoptera), including *Catapterix crimaea* in which 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 glands in Amphiesmenoptera. The glands are 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 inserts 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) (Chapter 2, 3). 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 facilitates secretion of gland products through the cuticle.

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TABLE 5-1. Taxa studied in families of particular interest with respect to sternum V gland-opening muscles and fenestra/perforated patches, including the treatments employed for each species and description of gland morphology (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 could either 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				
Annulipalpia				
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
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

FIGURE 5-1. SEMs of gland openings. Openings are clearly slit-like. A: *Agraylea multipunctata* female. B: *Limnephilus secludens* Banks female (Trichoptera: Limnephilidae). C: *Wormaldia planae* male. D: *Psychomyia flavida* female. Scale bars: A = 1 μm; B-D = 10 μm.

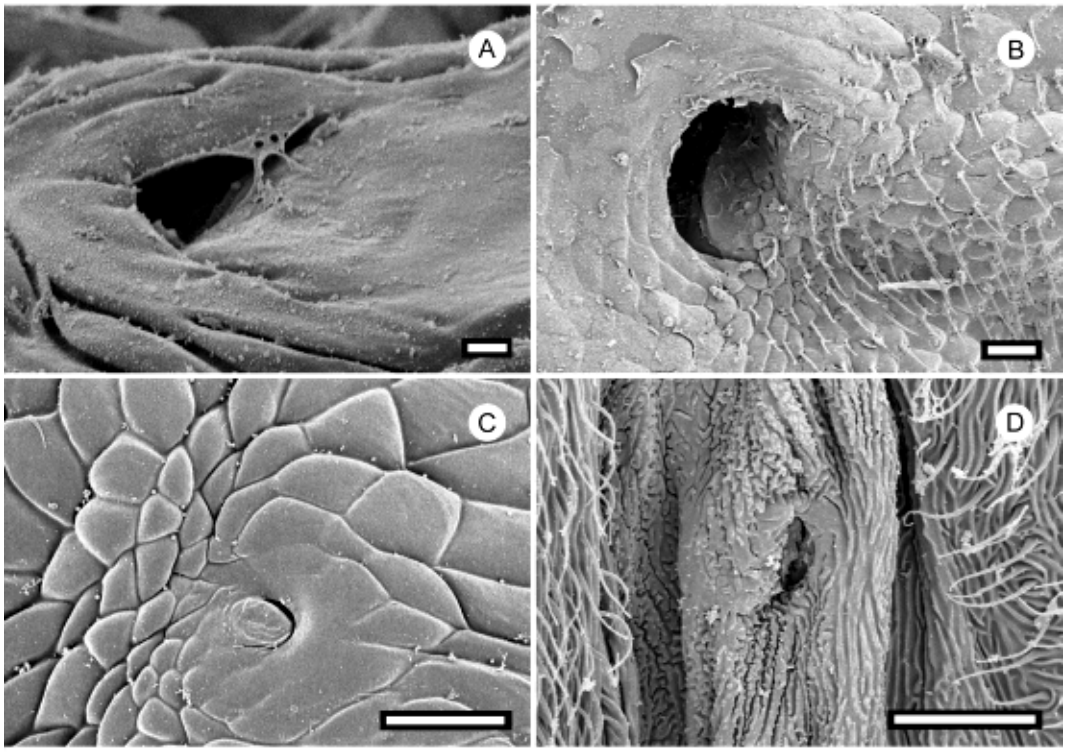

A
B
C
D

FIGURE 5-2. SEMs of internal structures. A-B: *Psychomyia flavida* female. A: Gland reservoir with excurrent duct which 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* female. 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: *Ectodemia heringiella* female. E: Internal surface of fenestra. F: Close-up of same. G: *Synempora andesae* female, 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.

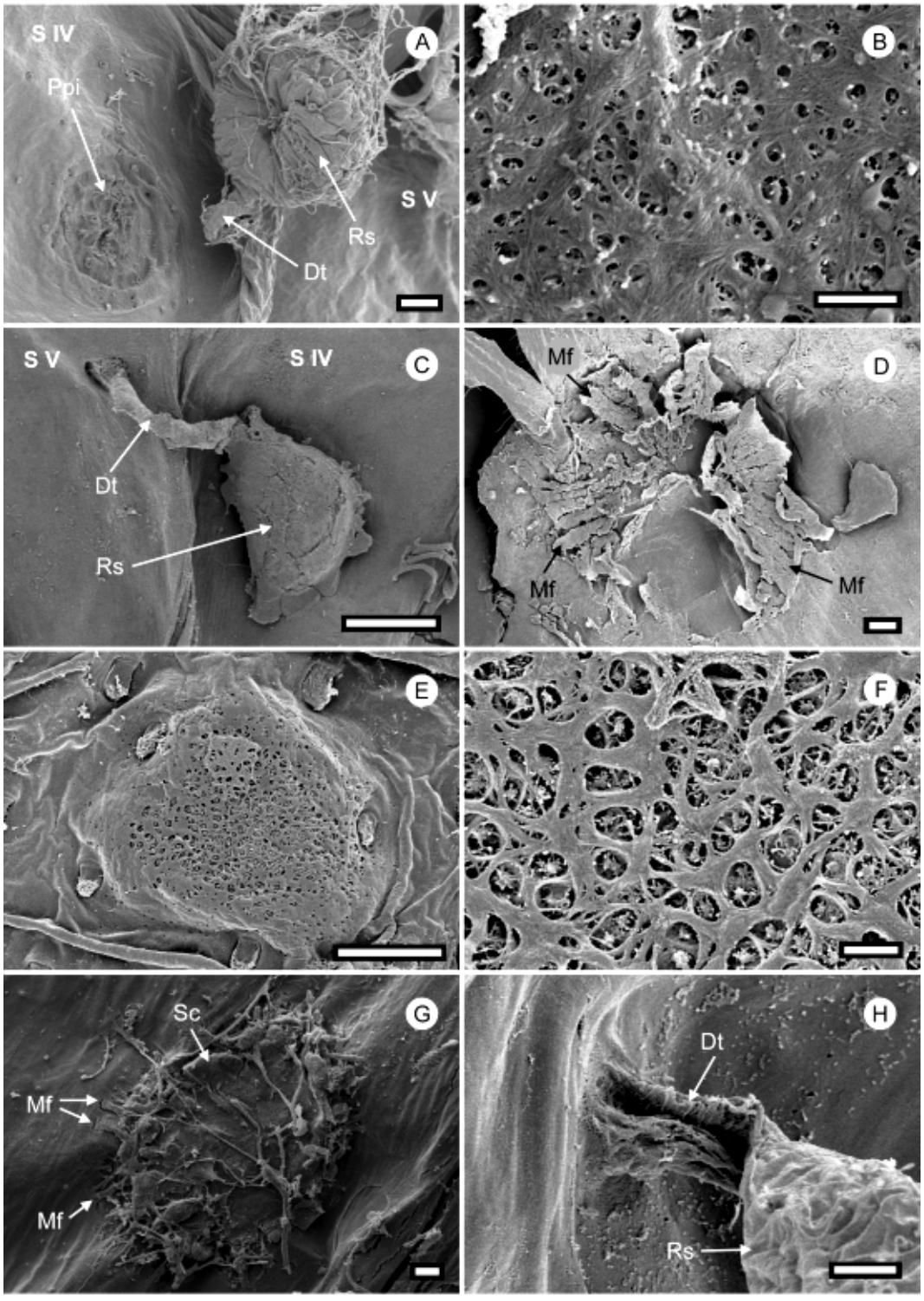

S IV
A
Ppi
S V
Rs
Dt
B
S V
S IV
C
Dt
Rs
Mf
D
Mf
Mf
E
F
Sc
G
Mf
Mf
Dt
H
Rs

FIGURE 5-3. Drawings based on wholemounts. The structures are viewed through the cuticle and the animals are facing right with down being mesad; thus all drawings show the right gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated. A: *Limnephilus secludens* male (Trichoptera: Limnephilidae), Trichoptera-type opening muscle that originate mesad on cuticle of sternum V and insert on the gland duct wall just inside the gland opening. B: *Rhyacophila arnaudi* female, Lepidoptera-type opening muscle that originate medioanteriad on sternum VI and insert on the gland duct wall just inside the gland opening. C: *Wormaldia arizonensis* female, co-occuring Lepidoptera-type and Trichoptera-type opening muscles. D: *Chimarra obscura* female, 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. Scale bars: A, B = 100 μm; C = 20 μm; D = 50 μm.

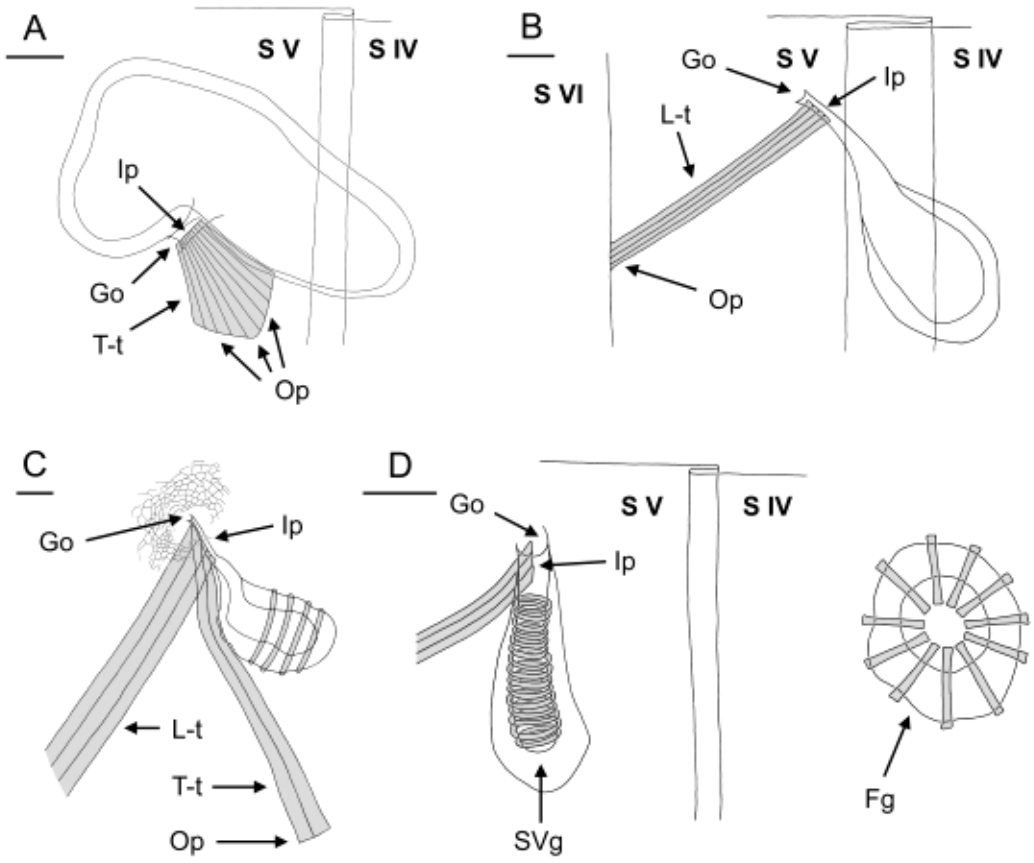
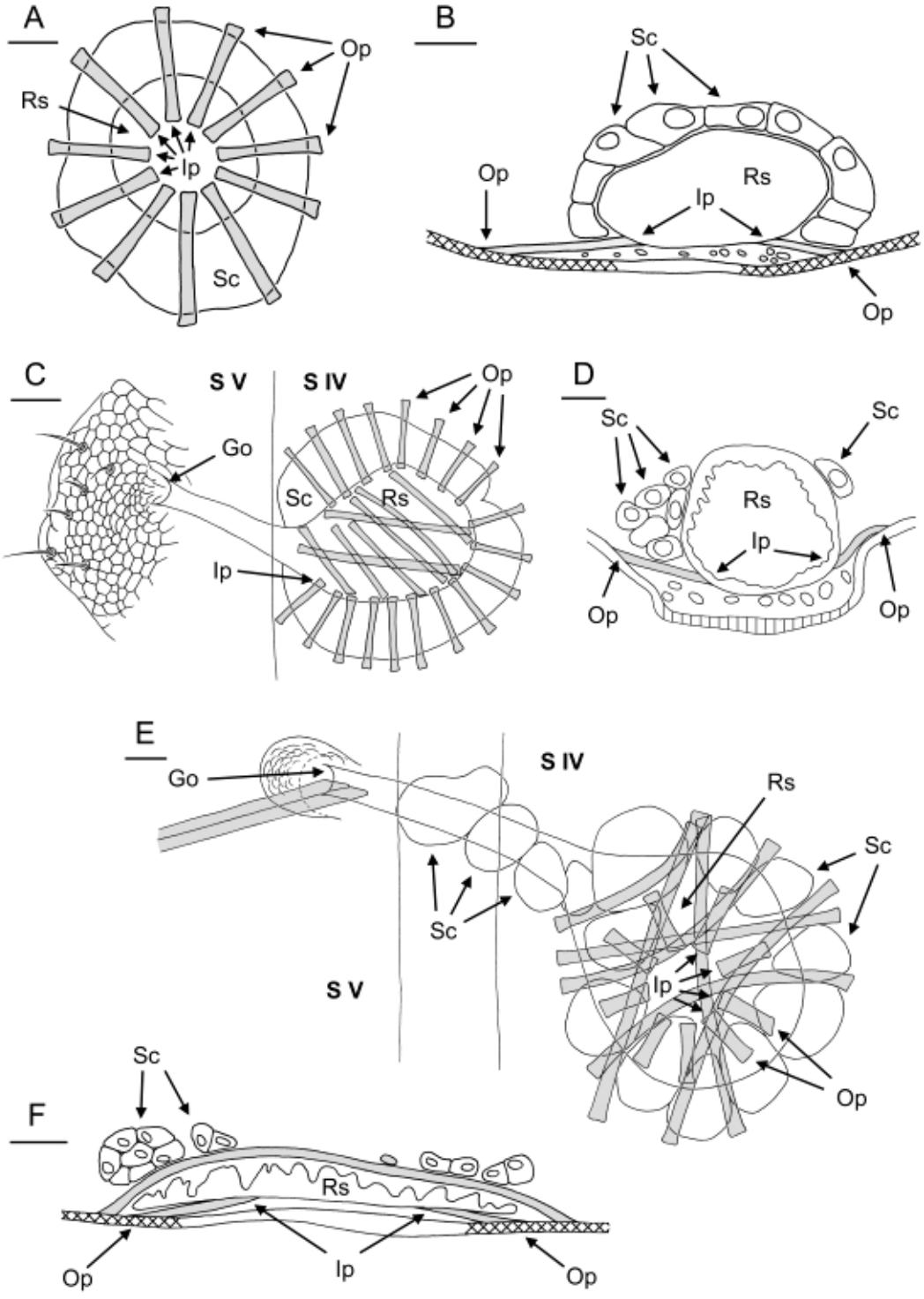

A
S V
S IV
Ip
Go
T-t
Op
B
S VI
Go
S V
Ip
S IV
L-t
Op
C
Go
Ip
L-t
T-t
Op
D
Go
S V
S IV
Ip
SVg
Fg

FIGURE 5-4. Drawings of 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; thus the drawings show the right gland. Muscle fibres are grey and all structures in the wholemounts are drawn as if they were transparent. Transparency of cuticle is only shown in B, F, G and I where cross hatching indicates non-transparent cuticle. Vertical lines in D indicated perforated cuticle. All drawings show the gland or 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* female. C-D: *Tinodes sigodanus* female, note lack of gland-opening muscle. E-F: *Eriocrania cicatricella* female. G-H: *Synempora andesae* female. I: Nepticulidae sp. female. 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. Scale bars: A-C, E-H = 20 μm; D, I = 10 μm


A
Op
Rs
Ip
Sc
B
Sc
Op
Rs
Ip
Op
C
S V
S IV
Op
Go
Sc
Rs
Ip
D
Sc
Sc
Rs
Ip
Op
Op
E
Go
S IV
Rs
Sc
Sc
Ip
S V
Op
F
Sc
Rs
Op
Ip
Op

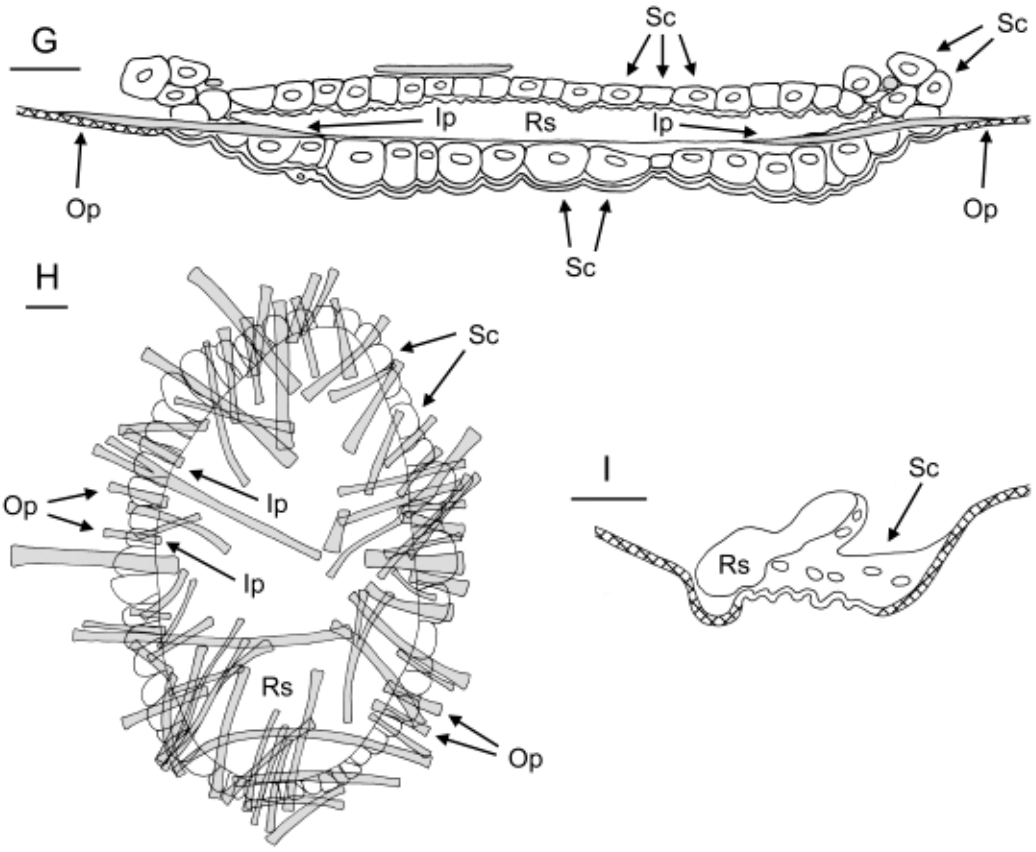
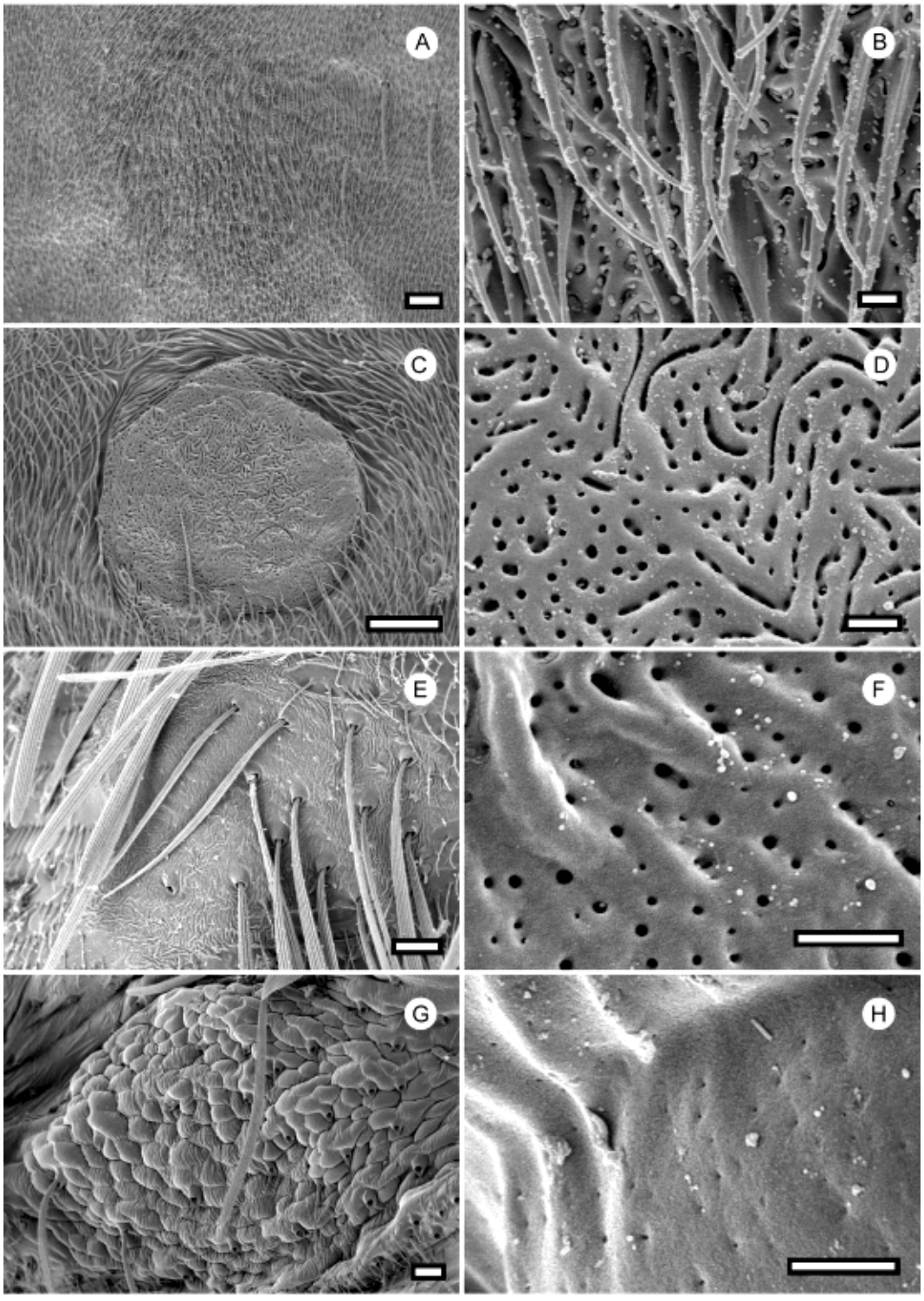

G
Sc
Sc
Ip
Rs
Ip
Op
Op
Sc
H
Sc
Op
Ip
Ip
Rs
Op
I
Sc
Rs

FIGURE 5-5. SEMs of 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* female. C-D: *Psychomyia flavida* female. E-F: *Eriocrania cicatricella* female. G-H: *Synempora andesae* female. I-J: Nepticulidae sp. female. Scale bars: A, C, E, G = 10 μm; B, D, F-J = 1 μm.


A
B
C
D
E
F
G
H

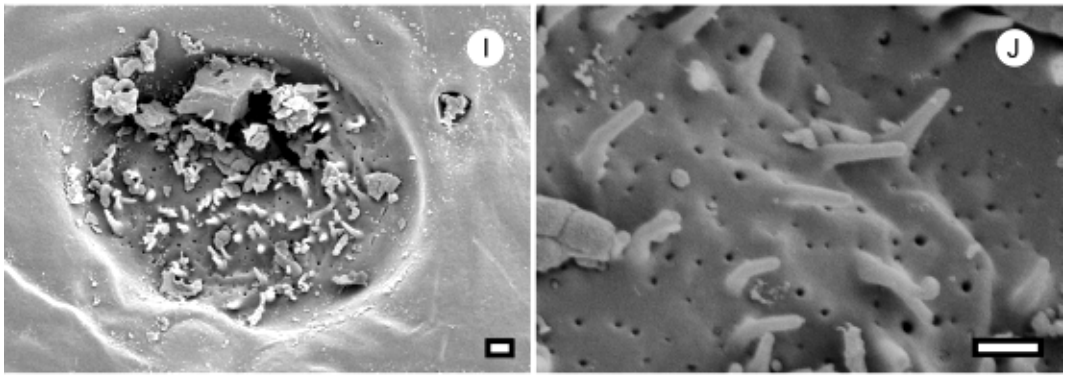

I
J

FIGURE 5-6. 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*, has both types of opening muscles. See text for probability of losses vs. gains under the single origin scenario.

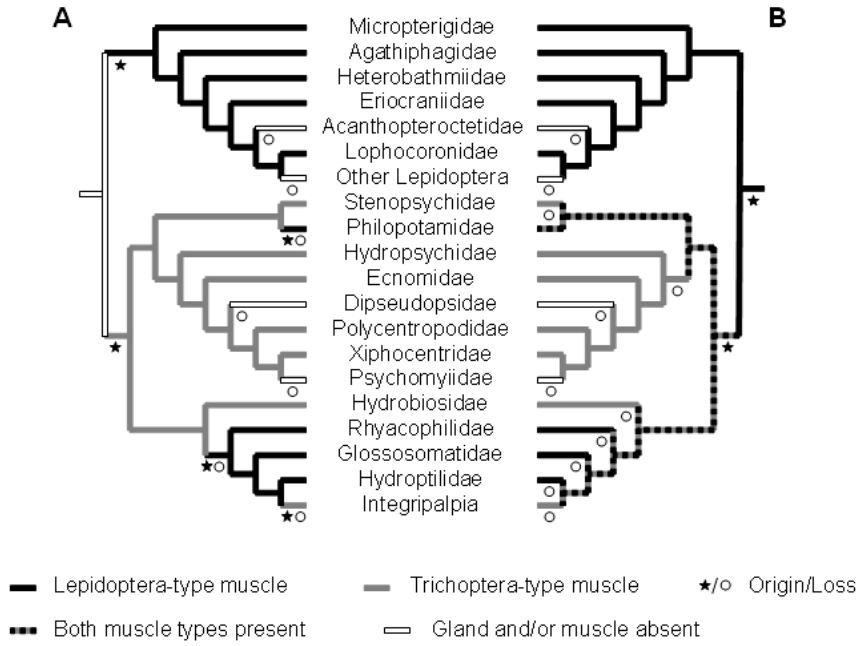

A
B
Micropterigidae
Agathiphagidae
Heterobathmiidae
Eriocraniidae
Acanthopteroctetidae
Lophocoronidae
Other Lepidoptera
Stenopsychidae
Philopotamidae
Hydropsychidae
Ecnomidae
Dipseudopsidae
Polycentropodidae
Xiphocentridae
Psychomyiidae
Hydrobiosidae
Rhyacophilidae
Glossosomatidae
Hydroptilidae
Integripalpia
Lepidoptera-type muscle
Trichoptera-type muscle
★/○ Origin/Loss
Both muscle types present
Gland and/or muscle absent

FIGURE 5-7. Drawings showing proposed derivation of the separate fenestral gland from the sternum V gland. The drawings are the same view as drawings of wholemounts: all internal structures are viewed through the cuticle and the animals are facing right with down being mesad; the drawings thus show the right gland. Muscle fibres are grey and all structures are drawn as if they were transparent. Transparency of cuticle is not indicated and gland-opening muscles are not shown. A: Sternum V gland with fenestra/perforated patch and 'sunburst' musculature associated with the gland reservoir. This configuration is our proposed ancestral condition of the gland in females and is found in female psychomyiids (Trichoptera) and female eriocraniids (Lepidoptera). B: Hypothetical intermediate form showing compartmentalisation of the gland reservoir. The fenestra/perforated patch and the 'sunburst' musculature are associated with the anterior compartment of the reservoir while the regular reservoir musculature surrounds the posterior part of the reservoir. We propose that secretions from the anterior part of the gland were primarily secreted through the reservoir while secretions from the posterior part of the gland were primarily secreted through the gland opening. C: Sternum V gland occurring concurrently with separate fenestral gland. Here the separation of the anterior part of the gland (secreting through the perforated patch) from the posterior part of the gland (secreting through the gland opening) is complete. The 'sunburst' musculature, like the fenestra/perforated patch, is associated with the fenestral gland while the regular gland reservoir musculature is associated with the sternum V gland proper. This configuration is found in female philopotamids (Trichoptera). 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.

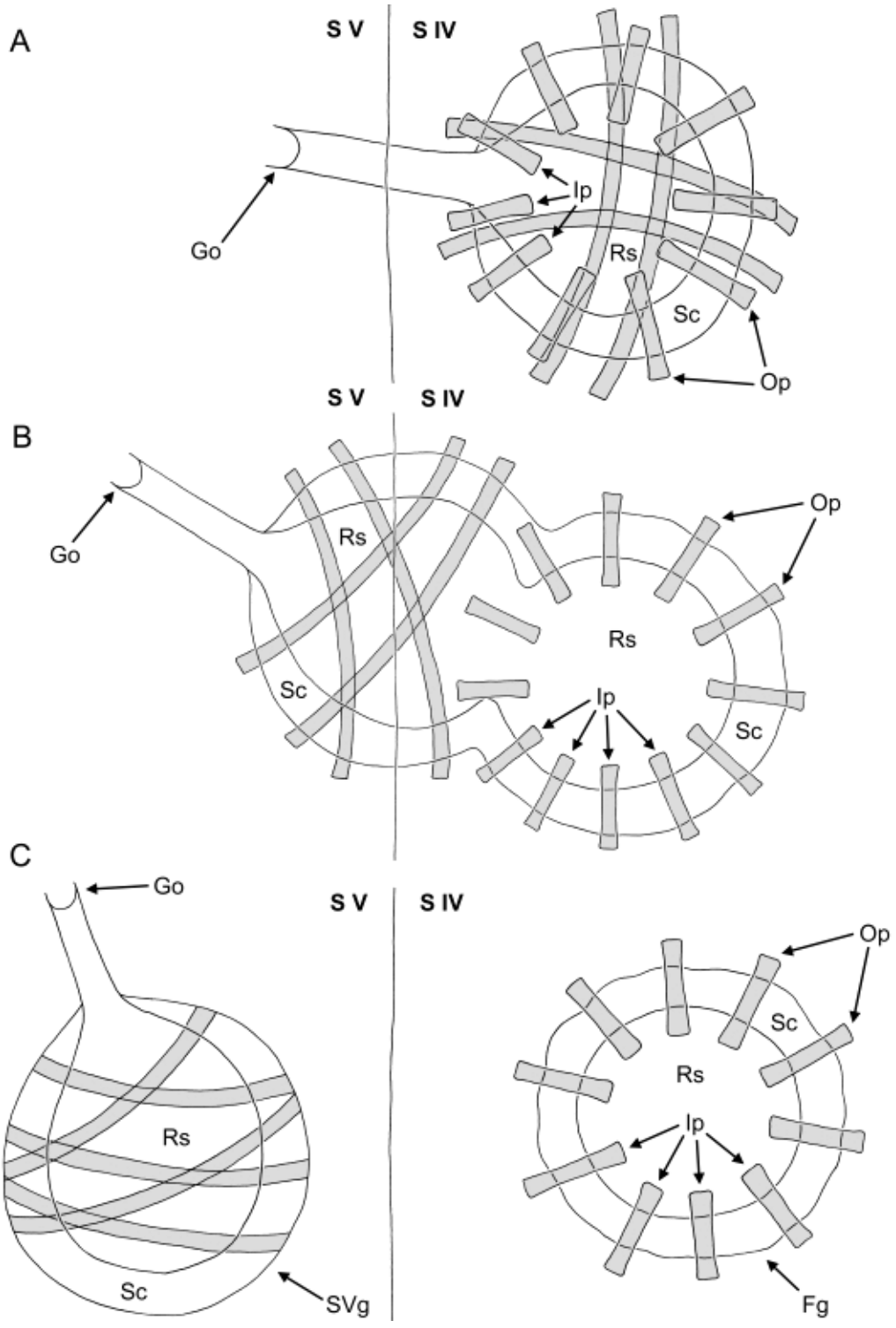
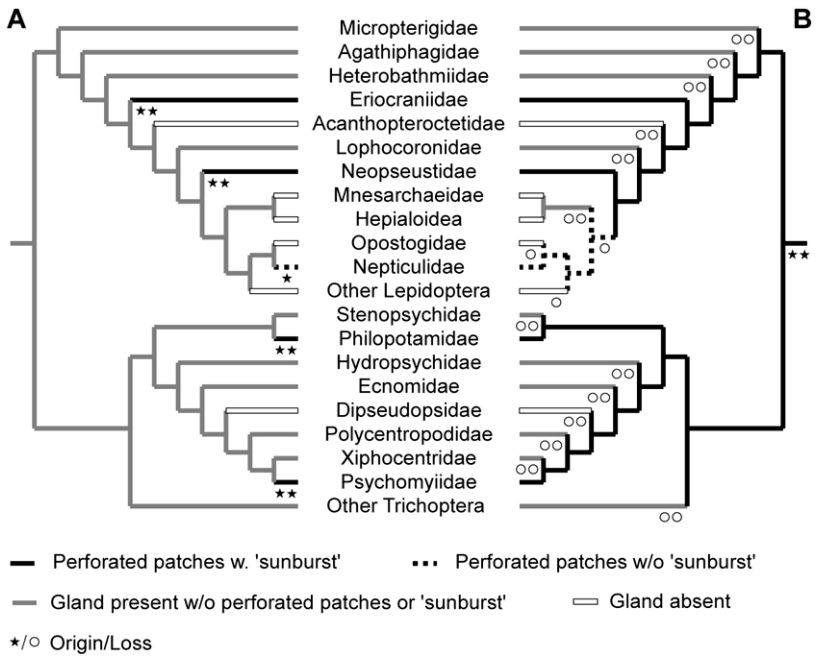

A
S V
S IV
Go
Ip
Rs
Sc
Op
B
S V
S IV
Go
Rs
Op
Rs
Sc
Ip
Sc
C
Go
S V
S IV
Op
Sc
Rs
Ip
Rs
Sc
SVg
Fg

FIGURE 5-8. 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 or 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.


A
B
Micropterigidae
Agathiphagidae
Heterobathmiidae
Eriocraniidae
Acanthopteroctetidae
Lophocoronidae
Neopseustidae
Mnesarchaeidae
Hepialoidea
Opostogidae
Nepticulidae
Other Lepidoptera
Stenopsychidae
Philopotamidae
Hydropsychidae
Ecnomidae
Dipseudopsidae
Polycentropodidae
Xiphocentridae
Psychomyiidae
Other Trichoptera
Perforated patches w. 'sunburst'
Perforated patches w/o 'sunburst'
Gland present w/o perforated patches or 'sunburst'
Gland absent
★/○ Origin/Loss

Chapter 6

Correlations between function and structure in the sternum V glands in Trichoptera[1]

INTRODUCTION

The sternum V glands are one of the autapomorphies of the superorder Amphiesmenoptera which contains the Lepidoptera (Moths and Butterflies) and the Trichoptera (Caddisflies). The glands consist of a pair of glandular invaginations on sternum V. While the gland is present in species throughout Trichoptera, in Lepidoptera it is only present in representatives of a few basal lineages. During the past 25 years, numerous studies have been published on the secretions of this gland, but these results have never been placed in a phylogenetic context nor have they been correlated with the morphology of the gland and associated structures. Löfstedt and Kozlov (1997) produced a phylogenetic analysis of pheromone communication in basal lepidopterans, but did not include details about either the morphology or the products of the sternum V gland (glands scored as present/absent, short chain pheromones scored as present/absent), and treated Trichoptera as a single terminal taxon. Ivanov and Melnitsky (2002) mapped gland shape, but did not attempt to correlate this feature with the gland products.

The female gland products function as sex pheromones in a number of trichopterans in the suborders Spicipalpia (Löfstedt et al., 1994) and Integripalpia (Resh & Wood, 1985; Bjostad et al., 1996; Bergmann, 2002; Löfstedt et al., 2008), as well as in representatives of the lepidopteran family Eriocraniidae (Zhu et al., 1995; Kozlov et al., 1996). Female gland products are known to elicit EAD (electroantennographic detection) and/or EAG (electroantennogram) responses in male antennae in additional species (Bergmann et al., 2001, 2002), and

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compounds similar to those that attract males are found in yet more species (Löfstedt et al., 1994, unpubl.; Bergmann, 2002).

In representatives of the genus *Hydropsyche* (Trichoptera: Hydropsychidae) males produce aggregation pheromones that are chemically similar to sex pheromones produced by female Trichoptera (Löfstedt et al., 1994; Bergmann, 2002). In this genus, males produce much larger quantities of gland product than females (Löfstedt et al., 1994; Bergmann, 2002).

A defensive function of the sternum V gland has been demonstrated in *Pycnopsyche scabripennis* (Trichoptera: Limnephilidae) (Duffield et al., 1977), and has been implicated in several other species (Ansteeg & Dettner, 1991). The defensive compounds found in *P. scabripennis* have not been identified from any other trichopterans whereas most of the compounds identified by Ansteeg and Dettner (1991) are more widespread in trichopterans.

Differences in the sternum V gland secretions between conspecific males and females have been noted in several cases. The largest differences are found in representatives of *Rhyacophila* (Trichoptera: Rhyacophilidae) in which the females produce heptan-2-ol and heptan-2-one, and nonan-2-ol and nonan-2-one while the males produce hexanoic acid, octanoic acid and acetophenone (Ansteeg & Dettner, 1991; Löfstedt et al., 1994; Bergmann, 2002).

In the present study, we combine morphological data about the sternum V gland in representatives of both Trichoptera and Lepidoptera with data on the chemical composition and biological function of gland products. We provide an overview of the published information on chemical substances produced by the sternum V gland. We map the different gland compounds on a current phylogeny, test for correlations between occurrence of different gland compounds, between different morphological features of the gland, and also test for correlations between different gland compounds and morphological features of the gland. We relate gland morphology to defensive function, sex pheromone and aggregation pheromone production. We examine morphological differences between male and female glands in taxa in which there are large differences in gland products between the sexes. In short, we place the current knowledge about sternum V

secretions in a phylogenetic context, and investigate the relationship between form and function.

MATERIALS AND METHODS

Taxon sampling

The taxa for which morphological data are available are from Chapter 2 as are most of the character scorings used for correlation tests. See Appendix 4 for a complete list of characters used, including correspondence to Chapter 2, and changes in character scorings (e.g. from multistate to binary).

Data on chemistry of gland secretions and behavioural observations were derived from a thorough literature survey. The databases Biological Abstracts and Biosis Previews were searched using the terms 'Trichoptera' or 'caddisflies' and 'pheromone' or 'sternum V gland'. Pherobase.com was checked for all included trichopterans and representatives of all Lepidoptera families in which the sternum V gland or associated structures are known to be present. All pertinent literature found in these and other searches was checked for further references. In cases in which the published literature indicated that only parts of a thesis had been published, or that there were further unpublished results, the authors were contacted to enquire about the availability of additional data. To our best knowledge our literature survey encompasses all original research papers in English or German on sternum V gland secretions, as well as a thesis and some unpublished results. Table 6-1 compiles the list of taxa with behavioural observations, EAD/EAG results, and chemical compounds, along with the relevant references.

In almost all reported cases, the extracts used for identification of gland compounds were either obtained from excised glands or from the ventral parts of segment IV and/or V. Toth et al.'s (1995) study of nepticulids (Lepidoptera) was the only exception, as the identified compounds were not associated with any specific body part. The association with the sternum V gland is indirect and based on the similarity between the identified compounds and compounds known to be associated with the sternum V gland in other taxa. Bergmann (2002) reported a

few cases in which extracts were made from the ventral parts of segment IV and V in taxa that do not possess the sternum V gland. In these cases, chemical substances were identified that were different from those found in taxa that do possess the sternum V gland (Bergmann, 2002).

Trees for mapping and analyses

We used a phylogeny primarily based on Holzenthal et al. (2007), the most recent comprehensive trichopteran phylogeny, and followed Kristensen and Skalski (1998) for the generally accepted and well-supported basal splits in Lepidoptera. For the relationships between the subfamilies of Hydropsychidae we followed Geraci (2007), who explicitly explored this. We placed *Diplectrona zealandensis* Mosely (Hydropsychidae, Diplectroninae) (not included by Holzenthal et al. (2007)) as sister to *Asmicridea* and *Smicrophylax* (Hydropsychidae, Smicrideinae) following Schefter (1996) and Geraci (2007). When there was no evidence to the contrary, taxonomic groups were assumed to be monophyletic.

The particular tree chosen to use for character mapping and phylogenetically informed correlation analyses greatly influences the results. We generally followed Holzenthal et al.'s (2007) phylogeny of Trichoptera as we judged it to be the best phylogeny available. However, some of the splits in the presented phylogeny do not have very high support (Holzenthal et al., 2007), and some aspects of the topology might change as more data become available. Redoing the mapping and analyses of the chemical and morphological data with a different tree would most likely produce results that differed in some aspects, especially the specific P-values and R^2-values. The general trends are likely to be more robust.

Character mapping and correlation

We mapped chemical characters in Mesquite 2.6 (build 486) (Maddison & Maddison, 2009a). We treated all characters as unordered and ancestral states were reconstructed using parsimony. If two or more equally parsimonious reconstructions were possible, we chose to minimize the number of gains of a

character.

We used the mirror tree function in Mesquite as a heuristic visual method to look for correlations. We tested potential correlations for significance using Pagel's 1994 Correlation test, part of the Correl package (Midford & Maddison, 2009) in Mesquite. Pagel's 1994 Correlation test employs two models: one in which the changes in the two characters occur independently, and one in which the probability of state change in one character depend on the state of the other character. The test compares the likelihood of the two models, using a number of simulations to calculate the P-value. The null hypothesis for the tests is that the two characters are independently distributed. We used 1000 simulations, each with 10 iterations. In cases in which a P-value between 0.04 and 0.06 was obtained, we ran 10000 simulations. The correlation test can only accept binary characters with no missing data; thus, any multistate characters were scored as presence/absence or converted into two or more binary characters. In the concatenated matrices, some characters were scored as 0/1, in this case tests were run with the character scored as both absent and present and the highest (least significant) P-value was reported.

When mapping chemical characters and in each set of correlation analyses we used three matrices: 1) both males and females with each sex scored separately (conspecific males and females treated as sister taxa, except in cases in which scorings for males and females were identical, in this case the species was treated as a single taxon), 2) one including only females and 3) one including only males. We treated males and females as sister taxa for three reasons: 1) Pagel's correlation analysis does not accept missing data (unknown or inapplicable) which means that the data set would be limited to those species with data available for both. 2) A chemical compound might be produced in females in one species, and in males in a closely related species (and the ability to produce the compound is part of the genetic make-up of both species). Thus if the same compound was scored as different characters depending on whether it occurred in males or females, it would be harder to detect if it was typical of a clade. This might be solved by scoring a trait as a single character, and scoring it as present regardless

of which sex it was present in. 3) However, combining the two sexes might give spurious correlation between compounds and traits. For example hexanoic and octanoic acid occur only in male *Rhyacophila* but could show a spurious correlation with a chemical or morphological trait present in female *Rhyacophila* if both sexes were treated as a single taxon.

We investigated correlations between chemical compounds using the same three matrices that were used for character mapping with adjustments for multistate characters and uncertain scorings.

Correlations between morphological characters were investigated using data from Chapter 2. Appendix 4 contains details of characters scorings. In some cases, missing morphological data (opening muscles, perforated patches on SIV, arrangement of secretory cells) were filled in based on the expected character state if these characters were non-labile. Otherwise missing data were treated as an absence (e.g. presence or absence of reservoir musculature). These correlation analyses were performed using Pagel's 1994 Correlation test in Mesquite 2.71 (build 514) (Maddison & Maddison, 2009b).

Correlations between chemical compounds and morphological traits were investigated using a concatenated combined matrix, as there were few species in which both morphological and chemical data were available. We created the combined matrix based on ancestral state reconstructions from the separate morphological and chemical matrices. The taxa in the concatenated matrix are given the lowest taxonomic rank that encompasses all the species used for the concatenated taxon. Correlation tests that included both sexes were performed in Mesquite 2.71 (build 514).

Appendix 4 shows the matrices, with both sexes included, and the trees used for correlation analyses.

Gland measurements

The gland reservoir contains a volume of gland compounds, and the secretory tissue surrounding the gland also has a volume. However, in museum specimens (preserved either in 70% ethanol or dried on pins) these are not practical or

sometimes even possible to measure. Observations of histological sections of alcohol preserved material showed that the gland reservoir was generally flat rather than balloon-like in cross section (e.g. Chapter 2, figure 2-5 C). Furthermore, in wholemounts, which is the single preparation that shows most gland structures, the specimen (here the ventral mid-abdomen) is placed between two glass slides, and thus the whole preparation is flattened. Based on this, we decided to use the measurable area of the flattened gland reservoir as a proxy for gland volume, and use the area of secretory tissue surrounding the gland reservoir as a proxy for volume of the secretory tissue. As most reservoirs had a round to ovoid shape, we used the formula for calculating the area of an oval. The details of calculation are given below. The measurements for each species are based one specimen of each sex.

Gland reservoir size was calculated by measuring the length (rl) and width (rw) of the gland reservoir in μm and using the following formula: 0.5 rl x 0.5 rw x π. Amount of secretory tissue was calculated by measuring length (gl) and the width (gw) of the gland reservoir including secretory tissue in μm and using the following formula: 0.5 gl x 0.5 gw x π, then subtracting the area of the gland reservoir. Exceptions to this procedure were employed for the secretory tissue in cases in which the secretory tissue only surrounded part of the reservoir: Female *Parapsyche elsis* Milne in which the secretory tissue (including the part of the reservoir surrounded by it) was measured, area calculated as above, then calculating (as above) and subtracting the area of the gland reservoir surrounded by secretory tissue. Male *Atopsyche callosa* Navas in which the secretory tissue (including the part of the reservoir surrounded by it) was measured, area calculated as above, then subtracting 0.3 x area of gland reservoir instead of the area of the entire gland reservoir.

As only the ventral abdomen was included in the wholemount preparations used for measurements, we used the length of the fifth sternite as a proxy for body size. This measure is not affected by the degree of distension of the abdomen (e.g. females before and after depositing eggs). As the gland size was calculated as an area we squared the sternite length so that the body size measure would increase

by the same rate as the gland size measures (e.g. if all measured lengths were doubled, the relationship between gland size and body size would stay the same). The values for gland reservoir size, size of secretory tissue and body size were log10 transformed. Raw values for reservoir size, size of secretory tissue and body size are shown in Appendix 5.

Phylogenetic trends in gland reservoir size and amount of secretory tissue were explored in Mesquite. Log10 values for reservoir size and amount of secretory tissue were regressed on Log10 values for body size using Microsoft Excel 2003 (Microsoft Corporation, Redmond, Washington, USA) and the residuals were mapped in Mesquite. Males and females were treated separately.

We used the PDAP: PDTREE, version 1.14 module (Midford et al., 2008) in Mesquite to investigate trends and outliers, and to calculate correlations. Chart 9 calculates the independent contrast Pearson product-moment correlation coefficient of which we reported the associated P-value. Chart 9 also calculates the independent contrast Least Squares Regression of which we reported the R^2-value. Chart 9B calculates confidence intervals for the independent contrast Least Squares Regression and illustrates these as mapped onto the original tip data. We used this screen to identify species with very large or small values (e.g. very small or large gland reservoirs). Residuals were calculated in Excel separately for each data set. Each data set included only the species that had no missing data for the traits investigated. Based on diagnostic plotting of absolute values of standardized contrasts versus their standard deviations, we used the branch lengths method of Nee (described by Purvis, 1995), and subtracted 1 degree of freedom for soft polytomies. We plotted reservoir size versus body size and size of secretory tissue versus body size using the Log10 values; males and females were treated separately. The relationship between the amount of secretory tissue and the size of the gland reservoir was investigated using residuals, treating males and females separately. We also used residuals to investigate the relationship between female and male gland size, and between the amount of secretory tissue in females and males, in the former case using the branch lengths method of Nee with Grafen's Rho transform (Rho value = 0.62).

RESULTS

Phylogenetic distribution of gland compounds

The most widespread sternum V gland compounds are heptan-2-one, nonan-2-one and their corresponding alcohols. The only large group in which they are absent is the included representatives of Apataniidae + Limnephilidae.

Methylated 3-ketones and their corresponding alcohols (4-methyl-3-hexanone/ol, 4-methyl-3-heptanone/ol, 4-methyl-3-octanone/ol, (4, 6)-dimethyl-3-octanone, (4, 6)-dimethyl-3-nonanone, 6-methyl-3-nonanone/ol) are only found in representatives of Apataniidae + Limnephilidae. 4-methyl-3-heptanol is more widespread in females than is the corresponding ketone, while the ketone is more widespread than the alcohol in males. Non-methylated 3-ketones and their corresponding alcohols (octan-3-one/ol, nonan-3-one/ol) are uncommon, only found in *Philopotamus montanus* (Philopotamidae), *Cailloma pumida* (Hydrobiosidae), *Apatania fimbriata* (Apataniidae) and *Monocosmoecus pulcherrimus* (Limnephilidae). Furthermore, octan-3-one/ol has only been reported from females.

In addition to the methylated 3-ketones and their corresponding alcohols, some other substances are also only reported in representatives of Apataniidae + Limnephilidae. These are 1,8-cineol, 3-hydroxy-2-butanone, 2,3-butanediol and 7-episordidin, the latter has only been reported from representatives of the genus *Potamophylax*.

Various organic acids as well as acetophenone and derivatives are only found in representatives of Annulipalpia and Spicipalpia. Acetic acid is the most common acid, while butanoic and methylbutanoic acid only have been reported from representatives of Annulipalpia; hexanoic and octanoic acid have only been reported from male *Rhyacophila* (Rhyacophilidae).

Nonanal is known from some annulipalpians and spicipalpians; it is not widespread and is only known from females, including all polycentropodid females investigated.

Correlations between gland compounds

Potential correlations between gland compounds were tested using Pagel's 1994 Correlation test in Mesquite.

The co-occurrence of several common gland compounds was significant across all three data sets: nonan-2-one and nonan-2-ol (M+F: p = 0.000; M: p = 0.003; F: p = 0.022); heptan-2-one/ol and nonan-2-one/ol (M+F: p = 0.000; M: p = 0.001; F: p = 0.000); heptan-2-one and nonan-2-one (M+F: p = 0.001; M: p = 0.014; F: p = 0.000); heptan-2-ol and nonan-2-ol (M+F: p = 0.000; M: p = 0.015; F: p = 0.000). Heptan-2-one and heptan-2-ol were significantly correlated when both sexes were included and in females, but not in males (M+F: p = 0.000; F: p = 0.000).

In females, the main types of methylated 3-ketones and the corresponding alcohols were all significantly correlated with each other, and all except one set was significantly correlated when both sexes were included in the data set; some were also significantly correlated in males (6-methyl-3-nonanol/one not present in M). See Table 6-2 for probabilities.

The correlation between presence of heptan-2-one/ol and/or nonan-2-one/ol and absence of methylated 3-ketones and corresponding alcohols and vice versa was highly significant in all three data sets (M+F: p = 0.000; M: p = 0.031; F: p = 0.000).

The co-occurrence of several uncommon gland compounds was significant across all three data sets: 4-hepten-2-ol/one and 3-hepten-2-one (M+F: p = 0.000; F: p = 0.000), (neither present in M); octan-2-ol/one and decan-2-ol/one (M+F: p = 0.000; M: p = 0.005; F: p = 0.009); octan-2-ol/one and undecan-2-ol/one (M+F: p = 0.000; M: p = 0.000; F: p = 0.001); *Z*6-nonen-2-ol and *Z*6-nonen-2-one (M+F: p = 0.000; M: p = 0.037; F: p = 0.000); any organic acids and undecan-2-ol/one (M+F: p = 0.018; M: p = 0.023; F: p = 0.000); decan-2-ol/one and undecan-2-ol/one (M+F: p = 0.000; M: p = 0.002; F: p = 0.013); 3-hydroxy-2-butanon and 2,3-butanediol (M+F: p = 0.000; M: p = 0.000) (latter not present in F).

The co-occurrence of Nonan-2-ol/one and nonen/nonadien-2-ol/ones was significant across all three data sets (M+F: p = 0.000; M: p = 0.026; F: p = 0.030).

The occurrence of acetic acid and butanoic and/or methylbutanoic acid was significantly correlated across all three data sets (M+F: p = 0.000; M: p = 0.000; F: p = 0.003) as was the occurrence of any organic acids and acetophenone + derivatives (M+F: p = 0.000; M: p = 0.038; F: p = 0.013). The occurrence of hexanoic and/or octanoic acid and acetophenone was significantly correlated when both sexes were included (M+F: p = 0.000) and in males (M: p = 0.000) (hexanoic or octanoic acid not present in females).

Correlations between morphological traits

Potential correlations between morphological traits were tested using Pagel's 1994 Correlation test in Mesquite.

The presence of perforated patches on sternum IV in females (Chapter 2, figures 2-17 C, D; 2-18 C, D) was significantly correlated with a scaly patch around the gland opening (Chapter 2, figures 2-2 B-E; 2-3 A, B; 2-4 C, D; 2-5 D, E; 2-17 A, F; 2-18 A) , both when only females were included in the analysis and when both sexes were included (M+F: p = 0.000; F: p = 0.000). The same was the case for the presence of perforated patches on sternum IV in females and absence of the Trichoptera-type opening muscle (Chapter 2, figures 2-2 D, E; 2-3 A-C; 2-5 B, D, E) (M+F: p = 0.003; F: p = 0.000). Presence of a scaly patch around the gland opening and absence of the Trichoptera-type opening muscle was significantly correlated when both sexes were included (p = 0.010) and in females (p = 0.012, but not in males. We found a significant correlation between presence of an enlarged evaporative surface (Chapter 2, e.g. figures 2-10 D; 2-17 A, F; 2-18 A, E-H; 2-19 A, D-F; 2-20; 2-21 A-C; 2-22 A, B, E, F; 2-23 A, B) and absence of Trichoptera-type opening muscle in all three data sets (M+F: p = 0.017; M: p = 0.046; F: p = 0.007).

Presence of hexagons with grooves between them on sternum V (Chapter 2, figures 2-23 A, B; 2-24 A) was correlated with presence of a reniform reservoir (Chapter 2, figures 2-13 A-G; 2-26 C) in all three data sets (M+F: p = 0.000; M: p = 0.000; F: p = 0.031). Presence of reniform reservoir and lack of reservoir musculature (Chapter 2, e.g. figures 2-11 C, D, F; 2-13; 2-14; 2-15 A-C) were

correlated across all three data sets (M+F: p = 0.003; M: p = 0.003; F: p = 0.026). Presence of a periform, ovoid or round reservoir (Chapter 2, e.g. figures 2-2; 2-4; 2-5 A, B, D, E; 2-6; 2-8 A-D) was significantly correlated with presence of reservoir musculature (Chapter 2, e.g. figures 2-2; 2-6 A, B, D; 2-7; 2-10; 2-11 A, B, E, G; 2-12 C, E; 2-16 B-D) in all three data sets (M+F: p = 0.000; M: p = 0.010; F: p = 0.002). The presence of a protuberance with groove (Chapter 2, figures 2-18 G-H; 2-19 A; 2-20) was significantly correlated with presence of detached secretory cells (Chapter 2, figures 2-7; 2-10 C) across all three data sets (M+F: p = 0.000; M: p = 0.000; F: p = 0.000). The presence of an area devoid of microtrichia that extend from the front edge of sternum V (Chapter 2, figure 2-24 F-H) and a cuticular ridge around the gland opening (Chapter 2, figures 2-15; 2-16; 2-26 G) was significantly correlated across all three data sets (M+F: p = 0.009; M: p = 0.010; F: p = 0.000).

Correlations of chemistry and morphology

Potential correlations between gland compounds and morphological traits were tested using Pagel's 1994 Correlation test in Mesquite.

Various gland compounds were significantly correlated with the presence or absence of gland reservoir musculature and the shape of the gland reservoir. Presence of methylated 3-ketones and/or corresponding alcohols and absence of gland reservoir musculature (Chapter 2, e.g. figures 2-11 C, D, F; 2-13; 2-14; 2-15 A-C) were significantly correlated when both sexes were included in the dataset and in females (M+F: p = 0.007; F: p = 0.003), but not in males. Presence of methylated 3-ketones and/or corresponding alcohols was significantly correlated with the presence of reniform reservoir (Chapter 2, figures 2-13 A-G; 2-26 C) in all three data sets (M+F: p = 0.009; M: p = 0.047; F: p = 0.005). Presence of heptan-2-one/ol and gland reservoir musculature (Chapter 2, e.g. figures 2-2; 2-6 A, B, D; 2-7; 2-10; 2-11 A, B, E, G; 2-12 C, E; 2-16 B-D) were significantly correlated when both sexes were included in the dataset (M+F: p = 0.001), but not when each sex was analyzed separately as was presence of nonan-2-one/ol and gland reservoir musculature (M+F: p = 0.010). Presence of heptan-2-one/ol and

periform, ovoid or round reservoir (Chapter 2, e.g. figures 2-2; 2-4; 2-5 A, B, D, E; 2-6; 2-8 A-D) was significantly correlated when both sexes were included in the dataset (M+F: p = 0.002), but not when analysing each sex separately as was presence of nonan-2-one/ol and periform/ovoid gland reservoir (M+F: p = 0.019). Acetophenone + derivatives were significantly correlated with the presence of ovoid reservoir (Chapter 2, e.g. figures 2-12; 2-15 C-E; 2-16) when both sexes were included and in males, but not in females (M+F: p = 0.011; M: p = 0.002).

Other gland compounds were significantly correlated with the presence of perforated patches on sternum IV in females (Chapter 2, figures 2-17 C, D; 2-18 C, D). The occurrence of *Z*4-nonen-2-one and perforated patches associated with the gland reservoir (Chapter 2, figure 2-25 E) was significantly correlated when both sexes were included in the dataset and in females (M+F: p = 0.008; F: p = 0.007) as was methyl-2-hexanol (M+F: p = 0.039; F: p = 0.038). The occurrence of 3-nonen-2-one and perforated patches associated with the gland reservoir was significantly correlated in both data sets (M+F: p = 0.042; F: p = 0.042) while 3-hepten-2-one and separate fenestral glands (Chapter 2, figure 2-3 C, D) were significantly correlated in both data sets (M+F: p = 0.010; F: p = 0.020).

Other significant correlations were the presence of octan-2-one/ol and a grooved protuberance (Chapter 2, figures 2-18 G-H; 2-19 A; 2-20) in all three data sets (M+F: p = 0.025; M: p = 0.033; F: p = 0.006). Octan-2-one/ol was also significantly correlated with detached secretory cells (Chapter 2, figures 2-7; 2-10 C) in all three data sets (M+F: p = 0.001; M: p = 0.004; F: p = 0.0). Undecan-2-ol/one was significantly correlated with the presence of detached secretory cells when both sexes were included in the dataset (M+F: p = 0.001), but not in either of the two separate data sets. We found a significant correlation between presence of 6-methyl-3-octanone and hexagons covering sternum V (Chapter 2, figures 2-23 A, B; 2-24 A) when both sexes were included in the dataset and in females (M+F: p = 0.005; F: p = 0.003), but not in males (no taxa with hexagons present in the concatenated data set for males). Acetophenone + derivatives were significantly correlated with the presence of a protuberance (Chapter 2, e.g. figures 2-4 E; 2-18 E; 2-19 A, B; 2-20 A, D; 2-21 D; 2-22 A, D, E; 2-23 E) in the

combined data set and in males, but not in females (M+F: $p = 0.031$; M: $p = 0.014$).

Many of the correlations between different characters, whether chemistry, morphology or a combination, are related to the presence or absence of these characters in representatives of Limnephilidae sensu lato. Correlations between characters not related to presence or absence in representatives of Limnephilidae sensu lato, are generally found between characters present in just a few of the included taxa.

Gland measurements

Correlations between various gland measurements were calculated using the PDAP: PDTREE module in Mesquite.

Gland reservoir size

As can be seen in Figure 6-1, the most extreme and basically only outlier among female trichopterans was *Wormaldia arizonensis* (Ling) (Philopotamidae), with very small relative gland size.

Figure 6-2 shows *Leptonema albovirens* (Walker) (Hydropsychidae), with extremely large relative gland size, as the most extreme outlier among male trichopterans. *Limnephilus externus* Hagen (Limnephilidae) has the smallest relative gland size. *Pycnopsyche* (Limnephilidae) males and most *Hydropsyche* (Hydropsychidae) males have relatively large glands.

Male and female gland size was not significantly correlated when corrected for body size ($p = 0.105$), and there was no strong trend ($R^2 = 0.070$) (Figure 6-3). Relatively large male glands compared to conspecific females were found in *Hydropysche* (Hydropsychidae), *Chimarra obscura* (Walker) (Philopotamidae), *Himalopsyche phryganea* (Ross) (Rhyacophilidae), the two included species of *Pycnopsyche* (Limnephilidae) and especially *Leptonema albovirens* (Hydropsychidae). Male glands were relatively smaller than those of conspecific females in *Limnephilus externus* (Limnephilidae), *Neothremma alicia* Dodds annd Hisaw (Uenoidae), *Dolophilodes novusamericanus* (Ling)

(Philopotamidae), *Xiphocentron haitiense* (Banks) (Xiphocentronidae) and *Parapsyche elsis* (Hydropsychidae).

Secretory tissue size

In females, *Anagapetus debilis* (Ross) (Glossosomatidae) had the relatively largest amount of secretory tissue compared to body size, while *Pycnopsyche scabripennis* (Limnephilidae) also had a relatively large amount. The most extreme outlier was *Hydropsyche tana* Ross (Hydropsychidae) with little secretory tissue closely followed by *Wormaldia arizonensis* (Philopotamidae). Others with little secretory tissue for their size were *Philanisus plebeius* Walker (Chathamiidae), *Austrocentrus griseus* Schmid (Helicophidae) and *Drusus annulatus* (Stephens) (Limnephilidae).

Gland reservoir size and size of the secretory tissue was significantly correlated when corrected for body size ($p = 0.0001$) in females, but reservoir size does not explain the majority of the variance in amount of secretory tissue ($R^2 = 0.286$). The most extreme outlier was *Hydropsyche tana* (Hydropsychidae) with little secretory tissue for its gland size, others with little secretory tissue compared to their reservoir were *Drusus annulatus* and *Onocosmoecus unicolor* (Banks) (both Limnephilidae). *Limnephilus externus* and *Pseudostenophylax sparsus* (Banks) (both Limnephilidae) had relatively large amounts of secretory tissue when compared to their reservoirs.

In males, *Anagapetus debilis* (Glossosomatidae), *Hydropysche bronta* Ross, *H. confusa* (Walker), *H. placoda* Ross, *H. cockerelli* Banks (Hydropsychidae) and both species of *Pycnopsyche* (Limnephilidae) had relatively large amounts of secretory tissue compared to body size. *Neothremma alicia* (Uenoidae), *Atopsyche callosa* (Hydrobiosidae), *Wormaldia gabriella* (Banks) (Philopotamidae) and *Drusus annulatus* (Limnephilidae) had relatively little secretory tissue for their body size.

Gland reservoir size and size of the secretory tissue was significantly correlated in males when corrected for body size ($p = 0.0048$), but reservoir size does not explain the majority of the variance in amount of secretory tissue ($R^2 =$

0.200). The most extreme outlier was *Atopsyche callosa* (Hydrobiosidae) with little secretory tissue for its gland size. Others with little secretory tissue compared to their reservoirs were *Wormaldia gabriella* (Philopotamidae), *Drusus annulatus* and *Onocosmoecus unicolor* (both Limnephilidae). *Polycentropus cinereus* Hagen (Polycentropodidae), *Anagapetus debilis* (Glossosomatidae), *Hydropsyche cockerelli* (Hydropsychidae), *Pseudostenophylax sparsus* and *Pycnopsyche lepida* (Hagen) (both Limnephilidae) had relatively large amounts of secretory tissue compared to their reservoirs.

Amounts of secretory tissue in males and females were significantly correlated when corrected for body size ($p = 0.0041$, $R^2 = 0.269$). Relatively large amounts of secretory tissue in males compared to conspecific females was found in *Polycentropus cinereus* (Polycentropodidae), *Hydropsyche placoda* (Hydropsychidae), *Pseudostenophylax sparsus* and both species of *Pycnopsyche* (both Limnephilidae). Relatively small amounts of secretory tissue in males compared to conspecific females were found in *Atopsyche callosa* (Hydrobiosidae) and *Wormaldia gabriella* (Philopotamidae).

Phylogenetic trends

Females did not show any obvious phylogenetic trends in gland size; small and large glands were scattered through the tree. Males showed several trends: smaller glands in philopotamids except *Chimarra obscura* and small glands in psychomyoids. In hydropsychids, glands were large in representatives of *Hydropsyche*, slightly enlarged in representatives of *Cheumatopsyche*, and very large in *Leptonema albovirens*, while arctopsychines had small glands. rhyacophilids had larger glands while limnephilids sensu lato had small glands, with exceptions in the two *Pycnopsyche* species and *Limnephilus secludens* Banks (both Limnephilidae).

In females, representatives of Hydrobiosidae and Sericostomatoidea except *Contulma talamanca* Holzenthal and Flint (Anomalopsychidae) had little secretory tissue for their body size. In males, representatives of *Hydropsyche* (Hydropsychidae) had relatively large amounts of secretory tissue compared to

body size as did the two *Pycnopsyche* species (Limnephilidae). Figure 6-4 illustrates trends in gland size and amount of secretory tissue in both females and males.

DISCUSSION

Mapping of gland compounds

Gland compounds that are present in both trichopterans and lepidopterans are the best candidates for ancestral gland compounds. Such compounds are heptan-2-ol, 4-hepten-2-one and –ol, nonan-2-one, 6-nonen-2-one and –ol. These compounds are known attractants or elicit a response in male antenna (Löfstedt et al., 1994, 2008; Zhu et al., 1995; Kozlov et al., 1996; Bergmann et al., 2002); thus, pheromone production is probably an ancestral function of the sternum V gland.

The restricted and overlapping distribution of the various methylated 3-ketones and their corresponding alcohols strongly suggests that they share common or similar biosynthetic pathways, and that this pathway either originated or was recruited for the sternum V gland on the line leading to Apatanidae + Limnephilidae after the split from the line leading to Phryganeidae. In this context, it would be interesting to know the chemical composition of the gland products in representatives of Oeconesidae and Uenoidae, as well as representatives of Rossianidae if members of the latter family possess the sternum V gland.

The non-overlapping distribution of the methylated 3-ketones and their corresponding alcohols and the widespread ketones heptan-2-one and nonan-2-one and their corresponding alcohols respectively, suggest that the methylated 3-ketone/ols have taken on the function of heptan-2-one/ol and nonan-2-one/ol. This is supported by male antenna reacting to and males being attracted to methylated 3-ketone/ols produced by conspecific females in representatives of Apataniidae + Limnephilidae, and to heptan-2-one/ol and nonan-2-one/ol produced by conspecific females in other representatives of Amphiesmenoptera (Löfstedt et al., 1994, 2008; Zhu et al., 1995; Bjostad et al., 1996; Jewett et al, 1996; Kozlov et al.,

1996; Bergmann et al., 2001, 2002; Bergmann, 2002). Mapping of selected traits is shown in Figure 6-5.

Correlations between gland compounds

A number of gland compounds were significantly correlated with one another. A significant co-occurrence of two gland compounds might be explained by shared biosynthetic pathways, as is the case for ketones and their corresponding alcohols (Löfstedt et al., 2008). This explains the co-occurrence of heptan-2-one and heptan-2-ol, nonan-2-one and nonan-2-ol, *Z*6-nonen-2-one and *Z*6-nonen-2-ol, 3-hydroxy-2-butanon and 2,3-butanediol. Shared biosynthetic pathways might also explain the completely overlapping distribution of (4*S*,6*S*)-dimethyl-3-octanone and (4*S*,6*S*)-dimethyl-3-nonanone as well as the generally overlapping distribution of methylated 3-ketones and their corresponding alcohols as discussed above. Bergmann et al. (2004) presumed that 4,6-dimethyl-6-nonen-3-one could easily be transformed to 1,3-diethyl-4,6-dimethyl-2,7-dioxabicyclo[2.2.1]heptane (both compounds only known from *Glyphotaelius pellucidus* (Limnephilidae)), and thus that the compounds shared most of their biosynthetic pathway.

Another possible explanation is that the compounds serve a similar function and thus have been subjected to similar selective pressures. This might be the case for the co-occurrence of acetophenone and organic acids as both are possible defensive substances as discussed below

A third possible explanation for co-occurrence is simple phylogenetic happenstance. However, Pagel's 1994 correlation method specifically test the difference in likelihood between independent changes in two characters and the likelihood of dependent changes in the two characters. Thus, significant correlation between two traits is unlikely to be caused by phylogenetic happenstance. It is also possible that more data will show that some correlations between compounds and/or traits were an effect of the taxa available for the present study. This is more likely to be the case for compounds and/or traits only present in a few species (e.g. octan-2-ol and detached secretory cells) as there were few data points.

Correlations between morphological traits

A tree showing distribution of and illustrating selected traits can be seen in Figure 6-5.

Scale-like structures around the gland opening and perforated patches

The presence of a scaly patch around the gland opening (Chapter 2, figures 2-17 A, F; 2-18 A) and perforated patches on sternum IV in females (Chapter 2, figures 2-17 C, D; 2-18 C, D) is strongly correlated among representatives of Trichoptera. Representatives of Eriocraniidae, the only lepidopteran family in which females have perforated patches on sternum IV and a gland opening on sternum V, have the gland opening surrounded by scale-like structures/domes with grooves between them, although the scale-like structures/domes are smaller than the scale-like structures in Trichoptera (Chapter 2; 3, figure 3-2 D-F). A possible explanation for the co-occurrence of scale-like structures/domes and perforated patches is that the same type of chemical compounds work well with both structures. Compounds that can be secreted through the tiny pores of the perforated patches (see Chapter 5 for pore size) might also be well suited for distribution through the tiny channels between the scale-like structures/domes. However, in philopotamid females, the perforated patches are associated with the separate fenestral gland complex (Chapter 2, figure 2-3 C, D), so here different compounds might be secreted through the fenestra and the gland opening. Representatives of the philopotamid genus *Chimarra* do not have scale-like structures around the gland opening; in these species there is a tiny smooth area around the gland opening, then the dense cover of microtrichia common to all the sternites (Chapter 2). It is possible that the sternum V gland proper in representatives of *Chimarra* produce different compounds from those produced by the separate fenestral gland.

Both the presence of a scaly patch around the gland opening and perforated patches on sternum IV in females were significantly correlated with the absence of Trichoptera-type gland-opening muscle. Philopotamids have

Lepidoptera-type opening muscles, and philopotamid females have perforated patches on sternum IV, both traits identified as part of the groundplan for the sternum V gland (Chapter 5). Thus the condition in representatives of Philopotamidae might be regarded as simple retention of ancestral traits. However, in representatives of Psychomyiidae in which perforated patches are also present, gland-opening muscles are completely absent, and representatives of other families of Psychomyioidea (except dipseudopsids in which the sternum V gland is absent) possess Trichoptera-type opening muscles (Chapter 2). It is possible that the presence of perforated patches, by providing an alternative route of gland-product secretion, lessens the importance of the gland-opening muscles. Furthermore, Trichoptera-type opening muscles might be more efficient than Lepidoptera-type opening muscles. The latter hypothesis is consistent with the evolutionary scenario proposed in Chapter 5: Trichoptera-type opening muscles originated in the trichopteran ancestor, which already possessed Lepidoptera-type opening muscles. If Trichoptera-type opening muscles are more efficient, this would explain why the Trichoptera-type became prevalent in Trichoptera while the lepidopteran type generally was lost.

Enlarged evaporative surface and absence of Trichoptera-type opening muscles

The correlation of a scaly patch around the gland opening and absence of Trichoptera-type opening muscles seems curious at first. However, it can be viewed as a subset of the correlation between enlarged evaporative surface and lack of Trichoptera-type opening muscle. An enlarged evaporative surface causes a given amount of gland product to evaporate faster. However, the various forms of enlarged evaporative surfaces (e.g. scaly patches, grooves, elongated setae, eversible sac) also retain a certain amount of gland product. Thus the overall effect of enlarged evaporative structures might mainly be that larger amounts of gland product are dispersed rather than causing gland products to be dispersed during a shorter time interval.

If there is a functional connection between lack of Trichoptera-type opening muscles and elaborate evaporative structures around the gland opening,

then elaborate evaporative structures should be widespread in Lepidoptera in which all taxa with a proper sternum V gland have Lepidoptera-type opening muscles. Indeed, most of these have evaporative structures associated with the gland opening; the exceptions are some micropterigids and all agathiphagids (Kristensen, 1984; Chapter 3, 4).

Grooved protuberance and detached secretory cells

There is a significant correlation between the presence of a grooved protuberance and separation of the secretory cells from the gland reservoir. Grooved protuberances and detached secretory cells are found in representatives of Polycentropodidae and two hydropsychid subfamilies, Diplectroninae and Smicrideinae. One possible explanation for the co-occurrence of the two traits is that they are retained ancestral traits from the ancestor of Psychomyiiodea and Hydropsychidae. However, this would require many losses and does not explain why the two traits are not found separately. Furthermore, apart from having a groove leading from the gland opening to the apex of the protuberance, the protuberances in polycentropodids are quite different from those in diplectronines and smicrideines (Chapter 2, figures 2-18 E-F, 2-20; See also Figure 6-5 A). Neither does this scenario explain the presence of detached secretory cells in representatives of *Agapetus* (Glossosomatidae).

Another possible explanation is that chemical compounds suitable for distribution along a groove, or between folded cuticle in male *Agapetus*, can easily travel through the long ductules between the secretory cells and the reservoir, allowing the secretory cells to be placed some distance from the reservoir. However, that raises the question of what possible advantage there is in separating the secretory cells from the gland reservoir. The immediate effect of the separation would be that it takes longer from the time gland compounds are synthesized until they reach the reservoir. This could be an advantage if the gland compounds produced by the secretory cells underwent further modification in the reservoir itself, and the resulting compounds and/or intermediates were deleterious to the secretory cells.

The only compound found exclusively in taxa with detached secretory cells is octan-2-ol, but this compound is not present in all taxa with the secretory cells separate from the gland reservoir. Neither is it clear that octan-2-ol would require a different gland configuration than, e.g., heptan-2-ol and nonan-2-ol, which are found in taxa with detached secretory cells as well as being widespread in other trichopterans. Thus, at present, the chemical composition of gland products in taxa with detached secretory cells does not seem to explain this unusual configuration of the sternum V gland.

Reniform glands and absence of reservoir musculature

Reniform glands are significantly correlated with lack of reservoir musculature. In addition to the reniform shape, reniform glands are characterized by very short gland ducts, except in *Molanna flavicornis* Banks (Molannidae), the only reniform gland found outside Limnephilidae sensu lato (Chapter 2). The gland opening muscle inserts along a large part of the length of the duct, sometimes even along the entire length (Chapter 2). Thus the gland duct can be opened much more effectively than in taxa with a long narrow duct (e.g. *Anagapetus debilis* (Glossosomatidae), see figure 2-10 B in Chapter 2) and reservoir musculature might not be required for efficient release of the gland products. If the reniform gland reservoir renders gland reservoir musculature superfluous, this could also explain the correlation between a periform/ovoid reservoir, which tends to have a longer gland duct, and the presence of gland reservoir musculature.

Cuticular ridge and bald area around gland opening

We found a strong correlation between presence of a thickened cuticular ridge around the gland opening and presence of an area devoid of microtrichia extending from the front edge of the sternite. The cuticular ridge is connected to the antecosta on both sides of the gland opening and reinforces the cuticle around the gland opening. This allows a stronger pull to be exerted on the gland duct without distortion of the body wall. The completely smooth area around the gland opening provides a smaller evaporative area compared to unmodified sternite with

microtrichia, and also does not retain gland products as a sculpted surface would. The reinforcing ridge around the gland opening indicates that the gland opening muscle can exert considerable force on the gland duct. Together with the lack of structures that could retain gland products, this suggests that the brevitentorian trichopterans employ short term releases of gland products.

Correlations between chemistry and morphology

We found significant correlations between the presence of several gland compounds and the presence of perforated patches on sternum IV in females. Apart from methyl-2-hexanol (which was not conclusively identified, Löfstedt et al., unpubl.), all these compounds had double bonds between carbon atoms. This raises the possibility that there is a functional correlation between secreting through the tiny pores of the perforated patches and presence of double bonds between carbon atoms. It is possible that double bonds lower the viscosity of the compounds, making it easier to secrete them through the tiny pores of the perforated patches. While we were not able to find data on the viscosity of the particular gland compounds with double bonds, short-chained alkenes do have a lower viscosity than the corresponding alkanes (Viswanath et al., 2006). However, presence of the most widespread gland compound with a double bond between carbon atoms, 6-nonen-2-one, was not significantly correlated with the presence of perforated patches on sternum IV.

We found that the presence of methylated 3-ketones and corresponding alcohols and absence of gland reservoir musculature was significantly correlated. Methylated 3-ones and –ols are only known from representatives of Apatanidae + Limnephilidae while gland reservoir musculature is not known in representatives of this clade nor in *Molanna flavicornis* (Molannidae). Other members of Brevitentoria in which the sternum V gland is present often possess gland reservoir musculature (Chapter 2), so the lack of reservoir musculature is likely a relatively recent, parallel development in *M. flavicornis*, and is not related to presence of methylated 3-ones and –ols as *Molanna angustata* produce heptan-2-one/ol and nonan-2-one/ol (Löfstedt et al., 2008).

Presence of methylated 3-ketones and corresponding alcohols and a reniform reservoir was also significantly correlated; as with the lack of reservoir musculature, the reniform reservoir in *M. flavicornis* is probably a parallel development. The presence of heptan-2-one/ol or nonan-2-one/ol and gland reservoir musculature was significantly correlated when both sexes were included. As heptan-2-one/ol and nonan-2-one/ol are absent from representatives of Apataniidae + Limnephilidae, as is reservoir musculature, this is to some degree the twin to the correlation between methylated 3-ketones and corresponding alcohols and absence of gland reservoir musculature. Whether methylated ketones and the corresponding alcohols works better with a non-muscled, reniform reservoir, or if the production of methylated 3-ketones and corresponding alcohols and the non-muscled, reniform reservoir arose independently between the phryganeids and Apataniidae + Limnephilidae is unclear. As methylated 3-ketones and corresponding alcohols and the typical reniform non-muscled reservoir are only known from representatives of Limnephilidae sensu lato, there is currently only one data point.

The presence of 6-methyl-3-octanone and hexagons with grooves between them was strongly correlated in the concatenated data sets. In addition to *Apatania fimbriata*, *Anomalopterygella chauviniana* (Drusinae) and some representatives of *Limnephilus*, 6-methyl-3-octanone is also known from *Hesperophylax occidentalis* females (Bjostad et al., 1996; Bergmann, 2002), and at least males of one *Hesperophylax* species have hexagons around the gland opening (Chapter 2), supporting a link between 6-methyl-3-octanone and hexagons around the gland opening. However, hexagons also surround the gland opening in *Brachycentrus occidentalis* (Chapter 2), and methylated 3-ketones are not known outside Apataniidae + Limnephilidae. Thus, either representatives of *Brachycentrus* unexpectedly produce 6-methyl-3-octanone or presence of hexagons around the gland opening does not necessitate the presence of 6-methyl-3-octanone among the gland compounds.

Gland size

We have presumed that size of the gland reservoirs is connected to amounts of gland product produced, or more specifically, to amounts of gland product stored. If the actual production of gland compounds could be measured (e.g. as ng per hour), it would more likely be related to the amount of secretory tissue. Actual amounts of the major compound and not just relative ratios have been measured in both sexes in *Hydropsyche angustipennis* (Hydropsychidae) (Löfstedt et al., 1994). Male *H. angustipennis* store 10 µg of nonan-2-one (the major compound in both sexes) while females store 500 ng. Our measurement of various *Hydropsyche* species shows that the area of the glands in the males is more than an order of magnitude larger than in conspecific females. Albeit limited, these data indicates that there is a strong relationship between area of the gland reservoir and amounts of stored gland products.

Small and large glands

It might have been expected that there was a connection between gland size and function. However, gland size in females does not seem to be directly related to function as congeners of species with relatively large glands (*Rhyacophila arnaudi* Denning and *R. fasciata*; *Molanna flavicornis* and *M. angustata*) and a species with relatively small glands (*Gumaga griseola*) have been demonstrated to use the sternum V gland for pheromone production (Resh & Wood, 1985; Löfstedt et al., 1994, 2008).

Gland function in males is only known in *Hydropsyche angustipennis* (Hydropsychidae) and *Pycnopsyche scabripennis* (Limnephilidae); in the former it produces aggregation pheromones, in the latter defensive substances (Duffield et al., 1977; Löfstedt et al., 1994). In both representatives of *Hydropsyche* and *P. scabripennis*, the glands are relatively large compared to those of other males as well as to conspecific females. Based on this scarce information, little can be deduced about gland size except support for the general assumption that there is a high probability that the male glands are functional when they are relatively larger than in conspecific females.

Small glands in female *Wormaldia arizonensis*

Female *W. arizonensis* have very small sternum V glands relative to their size, as well as compared to other *Wormaldia* species. However, in philopotamid females, the sternum V gland proper exist alongside a fenestral gland complex on sternum IV (Chapter 2, figure 2-3 C, D), and the fenestral gland reservoirs in female *W. arizonensis* are the largest compared to body size of the measured philopotamids. In another *Wormaldia* species, *W. planae* Ross and King, females do not have fenestral glands at all, but have the largest sternum V gland reservoirs compared to body size of all female philopotamids. This suggests a complementary function of the sternum V gland proper and the fenestral glands.

In Chapter 5, it is proposed that the fenestral glands were derived from the sternum V glands; a complementary function of the two structures is consistent with this scenario. Both studies that investigated the chemical composition of the gland secretions in female representatives of Philopotamidae (Bergmann, 2002; Bergmann et al., 2002) made extracts of sternite IV and V together, and thus would have obtained a mixture of compounds from the sternum V gland proper and the fenestral gland. This procedure does not allow for a distinction between the compounds produced by the two glands, but it is worth noting that no compounds were identified that have not also been identified from the sternum V glands in other species. Hence the fenestral gland seems to produce the same type of compounds as the sternum V gland. However, it would be interesting to know whether the two structures produce virtually identical secretions in a species or if there is a division of labour between the two structures. Future studies on the secretions of the sternum V gland in female Philopotamidae would be enhanced by analysing the two structures separately. It is possible that a mixture of volatiles from the two structures is used to attract males, so in field studies the two structures might need to be considered together, even if they produce different compounds or different ratios of compounds.

Large glands in male *Hydropsyche*

In examined *Hydropsyche* (Hydropsychidae) males, several have large glands, and all have larger than average glands. *Hydropsyche* females, on the other hand, have glands that are average to small in size. *Hydropsyche angustipennis* males produce aggregation pheromones that attract other males and, to some degree, females (Löfstedt et al., 1994). In this genus, males form swarms, a behaviour thought to be facilitated by the male aggregation pheromone; females arrive at the swarm and then mate (Gruhl, 1960; Schumacher, 1969; Benz, 1975; Löfstedt et al., 1994).

A male that secretes aggregation pheromone in order to initiate swarming presumably would need to attract several males in a short period of time to create a sufficiently large swarm; thus, his signal needs to reach a much larger area than the female's signal. A male initiating swarming consequently needs a 'louder' signal than a calling female. As the main compound of the male *Hydropsyche angustipennis* aggregation pheromone has also been identified from many female Trichoptera, including conspecific females (Löfstedt et al., 1994, 2008; Bergmann, 2002; Bergmann et al., 2002), it must be assumed to have a similar range to the female sex pheromones. Thus to reach more individuals than a calling female, a calling male will have to produce a larger amount of pheromone. Males of *Hydropsyche angustipennis* and *H. siltalai* do indeed produce much larger amounts (e.g. >20 times) of pheromonal substances than do conspecific females (Löfstedt et al., 1994; Bergmann, 2002).

If there is a connection between large gland size in males and the production of aggregation pheromones, *Leptonema albovirens* would be very interesting in this regard as males of this species possess by far the largest glands found in this study.

Pycnopsyche

Males of *Pycnopsyche scabripennis* and *P. lepida* have relatively large glands, as do female *P. scabripennis*. They also share an unusual gland configuration; the gland reservoir is partially divided into compartments. Female *P. lepida* have

normally sized glands and do not have the gland reservoir divided into compartments. Whole body extracts of female *P. lepida* attract conspecific males (Houghton, 2002), so here the sternum V gland is likely involved in pheromone production. Whole body extracts of male *P. lepida* are not attractive to either sex (Houghton, 2002), so here the sternum V gland is presumably not involved in long distance pheromone production. Furthermore, Duffield et al. (1977) demonstrated that the sternum V gland in both sexes in *P. scabripennis* produce defensive substances. Hence it is reasonable to suppose that male *P. lepida* produce defensive substances, and it is plausible that the large, compartmentalised gland reservoirs in *P. scabripennis* and male *P. lepida* are linked to the production of defensive substances.

The compartmentalised gland reservoirs could indicate that different parts of the secretory tissue produce different compounds, with these being stored in the different compartments of the gland. Thus the expelled mixture of compounds would be a blend of compounds from different parts of the gland. If the combination of compounds is more toxic and/or irritating than the constituent compounds, the compartmentalised storage would facilitate safe storage of these compounds in the gland reservoir.

Limnephilus externus

Male *Limnephilus externus* (Limnephilidae) had the smallest relative gland size of all measured males. As the sternum V gland has been lost repeatedly through the evolution of Trichoptera, especially in males, it is possible that the sternum V gland in male *L. externus* is non-functional, or of reduced importance, and is showing an intermediate stage between full development and complete loss. On the other hand, female *L. externus* also have relatively small glands, so the small gland size in males might simply reflect the condition in conspecific females. This, however, does not explain why the females have small glands. The sternum V glands in female *L. externus* are of about the same relative size as the glands in female *Gumaga griseola* (Sericostomatidae), which are known to be functional (Resh & Wood, 1985). Furthermore, the layer of secretory cells that surrounds the

reservoir in both male and female *L. externus* is very thick, arguing against the glands being non-functional.

Function of gland in males and loss of gland in male Brevitentoria

In addition to the pheromonal compounds, many trichopterans also produce organic acids, acetophenone and derivatives of the latter (Ansteeg & Dettner, 1991; Löfstedt et al., 1994, unpubl.; Bergmann, 2002; Bergmann et al., 2002). These do generally not cause reactions in conspecific antenna (no reaction in five out of six species in which these compounds were found and EAG studies were conducted) (Löfstedt et al., 1994; Bergmann et al., 2002). This strongly suggests that these substances are not used as pheromones.

In species in which these compounds are found and both male and female gland contents are known (*Wormaldia subnigra* (Philopotamidae), *Polycentropus flavomaculatus* (Polycentropodidae), *Rhyacophila fasciata*, *R. nubila*, *R. oblitarata* (Rhyacophilidae), *Agapetus fuscipes* (Glossosomatidae)), these compounds are either produced in larger amounts in the male or only in the male (Ansteeg & Dettner, 1991; Löfstedt et al., 1994, unpubl.; Bergmann, 2002; Bergmann et al., 2002). The only exception is *Hydropsyche siltalai* (Hydropsychidae) in which organic acids are produced in larger amounts in the female (Bergmann, 2002). This is consistent with no pheromonal activity for these substances. Generally, females produce pheromones, but as the congener *Hydropsyche angustipennis* (Löfstedt et al., 1994), male *H. siltalai* probably produces aggregation pheromones: male *H. siltalai* produce very large amounts of the main component of the aggregation pheromone produced by male *H. angustipennis* (Löfstedt et al., 1994, Bergmann, 2002). Thus in *Hydropsyche* the roles of the sexes seem to be reversed in this respect. Generally in representatives of Trichoptera, males produce smaller amounts (or none) of the pheromonal substances produced by the females, but instead produce substances that do not cause a reaction in conspecifics. In *Hydropsyche* the males produce pheromonal substances in large amounts while the females produce much less gland product overall and may produce non-pheromonal compounds.

Ansteeg and Dettner (1991) proposed that the sternum V gland was originally a defensive structure. However, the only function of the sternum V gland known from representatives of both Lepidoptera and Trichoptera is sex pheromone production by females, so presumably this is (part of) the original function of the sternum V gland. On the other hand, the organic acids and acetophenone are irritants and/or toxic to vertebrates (Lewis, 2000; Pohanish, 2002); organic acids are known as components of defensive secretions in Hemiptera, Hymenoptera, Coleoptera and Lepidoptera (Blum, 1981) and acetophenone is known to be toxic to insects (Ansteeg & Dettner, 1991; Mohsen et al., 1995). Furthermore, apart from male *Hydropsyche angustipennis*, male trichopterans have not been found to use the sternum V gland to produce pheromones (Resh & Wood, 1985; Solem, 1985; Löfstedt et al., 1994; Bjostad et al., 1996; Bergman et al., 2002; Houghton, 2002) and the production of possible defensive substances (organic acids and acetophenone) is more prevalent in male trichopterans.

The function of the sternum V gland as well as the composition of gland products is unknown in male lepidopterans; the only study that investigated function showed that male *Sabatinca chalcophanes* Meyrick and *S. demissa* Philpott (Micropterigidae) do not produce long-distance pheromones (Kozlov and Zvereva, 1999). However, the sternum V gland is generally well developed in males in basal lepidopterans, as it is in male trichopterans except representatives of Brevitentoria, and is only present in males in the two known species of Agathiphagidae (Chapter 2, 3, 4). Thus it is possible that the sternum V gland serves a defensive function in some amphiesmenopterans, especially males.

Loss of the sternum V gland has occurred in several lineages of Trichoptera, and is especially prevalent in representatives of Brevitentoria. Furthermore, while the gland is present in representatives of nine out of 16 investigated families in Brevitentoria, it is only present in both sexes in representatives of Sericostomatidae and possibly Chathamiidae (Chapter 2). This suggests that the sternum V gland in male brevitentorians has largely become superfluous. Unfortunately, nothing is known of either the function or the

chemical composition of the gland compounds in the few male brevitentorians that do possess the sternum V gland. To the extent that the function and chemical composition of the gland compounds in female representatives of Brevitentoria are known, it is pheromone production utilizing very widespread sternum V gland products (Resh & Wood, 1985; Löfstedt et al., 2008).

In representatives of Plenitentoria, the sistergroup of Brevitentoria, the gland is generally present in both sexes. While the function of the gland in males is only known in a single species (*Pycnopsyche scabripennis*, Duffield et al., 1977), the chemical composition of the male gland products is known from several species. The gland compounds found in male plenitentorians are generally the same or a subset of those found in conspecific females; the exception is 2,3-butanediol which is only found in males (Bergmann, 2002). Compounds that have not been found in representatives of Integripalpia include the various organic acids and acetophenone and derivatives found in various representatives of Annulipalpia and Spicipalpia (Ansteeg & Dettner, 1991; Löfstedt et al., 1994, unpubl.; Bergmann, 2002; Bergmann et al., 2002). These compounds are relatively widespread in males, and are the only compounds found in the glands of male *Rhyacophila* (Rhyacophilidae) (Ansteeg & Dettner, 1991; Löfstedt et al., 1994; Bergmann, 2002), thus it is possible that the loss of these compounds is related to the loss of male glands in representatives of Brevitentoria. However, these compounds are also absent in plenitentorians in which male glands are generally present.

Conclusion

We propose pheromone production as a likely ancestral function of the sternum V gland, contrary to Ansteeg and Dettner (1991) who proposed that production of defensive compounds was the ancestral function. The best candidates for ancestral gland compounds are heptan-2-ol, 4-hepten-2-one and -ol, nonan-2-one, 6-nonen-2-one and –ol, and the most widespread gland compounds are heptan-2-one and -ol and nonan-2-one and –ol. All these compounds serve as attractants and/or elicit a response in conspecific antenna (Löfstedt et al., 1994, 2008; Zhu et al., 1995;

Kozlov et al., 1996; Bergmann et al., 2002). These widespread compounds are not known from representatives of Apataniidae + Limnephilidae, the only larger group in which these compounds have not been found, despite several studies. However, representatives of Apataniidae + Limnephilidae produce a number of methylated 3-ketones and their corresponding alcohols, which have not been associated with the sternum V gland in any other trichopterans or lepidopterans. The methylated 3-ketones and their corresponding alcohols serve as attractants of males and/or elicit a response in male antennae (Bjosted et al., 1996; Jewett et al., 1996; Bergmann et al., 2001; Bergmann, 2002).

Based on correlations between morphological traits we propose a functional connection between perforated patches on sternum IV in females and scale-like structures/domes around the gland opening and between perforated patches and absence of Trichoptera-type gland-opening muscles. We also propose a functional connection between the presence of elaborate evaporative structures around the gland opening and absence of Trichoptera-type opening muscles. We propose a functional connection between a reniform reservoir with a short gland duct and the absence of gland reservoir musculature as well as between a periform or ovoid reservoir, which tends to have a longer gland duct, and the presence of gland reservoir musculature.

The presence of perforated patches in females was significantly correlated with several gland compounds, most of which have double bonds between carbon atoms. The pores in the perforated patches are tiny, and the presence of double bonds may lower the viscosity of the compounds, making secretion through the perforated patches easier. Some *Pycnopsyche* (Limnephilidae) produce defensive substances (Duffield et al., 1977) and this production is likely linked to the presence of the large, compartmentalized gland reservoirs.

We did not find any direct correlation between function and size in female trichopterans as both large and small glands can function in sex pheromone production. However, production of male aggregation pheromone in representatives of *Hydropsyche* (Hydropsychidae) is likely connected to the presence of large male glands. The sternum V gland proper and the fenestral

glands in philopotamid females seem to function in a complementary function as the largest sternum V gland reservoirs occur with absent fenestral glands while the smallest sternum V gland reservoirs occur with the largest fenestral gland reservoirs. This indicates that either the sternum V gland proper or the fenestral gland can execute the function, presumably pheromone production, of the sternum V gland in philopotamid females.

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TABLE 6-1. List of taxa for which behavioural or chemical data relating to the function of the sternum V glands are available. The table lists behavioural observations, EAD (electroantennographic detection) or EAG (electroantennogram) responses, identified gland compounds and the relevant references. F, female; M, male; S IV, sternum IV or ventral part of segment IV; S V, sternum V or ventral part of segment V.

Taxa	Reactions	Gland compounds	References
Lepidoptera			
Eriocraniidae			
Eriocrania cicatricella (Zetterstedt)	**F:** no EAG response to M extracts **M:** EAG response to extracts of F S V/ underlined compounds, attracted to (2*R*)-(*Z*)-4-hepten-2-ol	**F:** (2*R*)-(*Z*)-4-hepten-2-ol (110, 126 ng/F); (2*R*)-heptan-2-ol (20, 23 ng/F); (*Z*)-4-hepten-2-one (1, 1,1 ng/F)	Zhu et al., 1995
E. sangii (Wood)	**M:** EAD response to extracts of F S V/ underlined compounds, attracted to (2*S*, 6*Z*)-nonen-2-ol	**F:** nonan-2-one; (2*S*, 6*Z*)-nonen-2-ol (attractant); (*Z*)-6-nonen-2-one (repellant)	Kozlov et al., 1996
E. semipurpurella (Stephens)	**M:** EAD response to extracts of F S V/ underlined compounds, attracted to mix of the two alcohols	**F:** nonan-2-one; (2*S*, 6*Z*)-nonen-2-ol (attractant); (2*R*, 6*Z*)-nonen-2-ol (attractant) (*S*, *R* ratio: 2:1); (*Z*)-6-nonen-2-one (repellant)	Kozlov et al., 1996
E. sparrmanella (Bosc)	**M:** attracted to (2*S*)-(*Z*)-4-hepten-2-ol in field trials		Zhu et al., 1995
Nepticulidae			
Stigmella malella (Stainton)	**M:** attracted to underlined compounds Other species attracted to similar blends	**F:** (*S*)-(*E*)-6,8-nonadien-2-ol; (*S*)-(*Z*)-6,8-nonadien-2-ol (*E*, *Z* ratio 10:3 most attractive; production not linked to a specific body part)	Toth et al., 1995
Trichoptera			
Annulipalpia			
Philopotamidae			
Philopotamus montanus (Donovan)	**F:** no response to M extracts **M:** EAD response to underlined compounds, no response to M extracts	**F:** heptan-2-ol +; octan-3-ol +; (*E*)-6-nonen-2-one +++; (*E*)-6-nonen-2-ol +++; (*Z*)-6-nonen-2-one +; (*Z*)-6-nonen-2-ol +; nonan-2-one ++; nonan-2-ol +; acetic acid ++	Bergmann et al., 2002
Wormaldia occipitalis (Pictet)		**M:** methyl-2-hexanol; heptan-2-one; acetic acid; 2-methylpropanoic acid; butanoic acid	Löfstedt et al., unpubl.

W. subnigra McLachlan		**F:** heptan-2-one 100; 4-hepten-2-one 5; 3-hepten-2-one 1; heptan-2-ol 2; octan-2-one 1; acetic acid 6 **M:** acetic acid ++; 2-methyl-butanoic acid ++	Bergmann, 2002
Psychomyiidae			
Lype phaeopa (Stephens)	**M:** attracted to middle of F abomen	**F:** heptan-2-one ++; (*E*)-6-nonen-2-one +; (*Z*)-6-nonen-2-one ++; (*Z*)-4-nonen-2-one +++; (*E*)-3-nonen-2-one ++; nonan-2-one ++; acetic acid ++; formic acid +	Bergmann, 2002; Ivanov, 1993
Psychomyia pusilla (Fabricius)		**F+M:** methyl-2-hexanol 100; heptan-2-one 80	Löfstedt et al., unpubl.
Tinodes pallidulus McLachlan		**F:** methyl-2-hexanol 37; heptan-2-one 100; nonan-2-one 23; (*Z*)-4-nonen-2-on 38; nonanal 50	Bergmann, 2002; Löfstedt et al., unpubl.
T. rostocki McLachlan		**F:** heptan-2-one ++; nonan-2-one ++; (*Z*)-4-nonen-2-on +++; menthon + **M:** heptan-2-one ++; nonan-2-one ++; menthon +	Bergmann, 2002
T. waeneri (Linnaeus)		**F:** heptan-2-one 61; 3-nonen-2-one 38 (probably rearranged 4-nonen-2-one); nonan-2-one 1	Löfstedt et al., unpubl.
Polycentropodidae			
Neureclipsis bimaculata (Linnaeus)	**M:** EAD response to underlined compounds, follows track of F	**F:** heptan-2-one +++; nonan-2-one +; nonanal +; (*Z*)-6-nonen-2-one +; 2-methylbutanoic acid +++; acetic acid ++; 2-methylpropanioc acid ++; propanoic acid +; acetophenone ++	Ivanov, 1993; Bergmann et al., 2002
Plectrocnemia conspersa (Curtis)	**M:** EAD response to underlined compounds	**F:** (*S*)-heptan-2-ol +++; 6-methyl-5-hepten-2-ol +; octanal +; (2*S*,6*Z*)-6-nonen-2-ol +++; nonanal +; 2-methylbutanoic acid +; acetophenone +; 1-phenylethanol +	Bergmann et al., 2002
Polycentropus flavomaculatus (Pictet)	**M:** not attracted to F	**F:** heptan-2-ol ++; octan-2-ol +++; nonan-2-one +; nonanal +; nonan-2-ol ++; acetic acid +++; 2-undecanone ++; 2-decanol ++; 3,6,9-heptadecatrien +++; nonadecane ++ **M:** 2-methyl-butanoic-acid +++; acetic acid +++; 2 hydroxymethylacetophenones	Solem & Petersson, 1987; Löfstedt et al., unpubl.
		F: gland contain very little, 2 acetophenone derivatives and an alkane **M:** 2-methyl-butanoic-acid; 4 additional compounds, prob. derivatives of acetophenone	Ansteeg & Dettner, 1991
Hydropsychidae			
Cheumatopsyche lepida (Pictet)		**F:** heptan-2-one +++; acetophenone +; benzaldehyde +; methyldecanoat +; ethyldecanoat +; methyldodecanoat +; ethyldodecanoat +; methyltetradecanoat +	Bergmann, 2002

Hydropsyche angustipennis (Curtis)	**F:** attracted to M extracts/nonan-2-one **M:** EAD response to underlined compounds, more to M, M extracts attract F and M in the field, more M	**F:** nonan-2-one (500 ng/F) **M:** heptan-2-one; octan-2-one; nonan-2-one (10 µg/M, major compound); (*Z*)-6-nonen-2-one; nonan-2-ol; decan-2-one; methylated decan-2-one; 2-undecanone	Löfstedt et al., 1994
H. siltalai Doehler		**F:** heptan-2-one 5; heptan-2-ol 11; nonan-2-one 100; nonan-2-ol 14; acetic acid 37; 2-methylbutanoic acid 10; other carboxylic acids; 2-undecanone 14 **M:** heptan-2-one 14; heptan-2-ol 6; nonan-2-one 1000; (*Z*)-6-nonen-2-one 26; nonan-2-ol 18; acetic acid 2; 2-methylbutanoic acid 3; other carboxylic acids; 2-undecanone 4; small amounts (<2) of more substances	Bergmann, 2002
Smicridea annulicornis (Blanchard)		**F:** 2-pentanol ++; heptan-2-one +++;, 6-methyl-heptan-2-one +; 3-hepten-2-on +;, heptan-2-ol ++;, 4-hepten-2-ol +; octan-2-one +; nonan-2-one ++; 6-nonen-2-one ++; nonan-2-ol +; acetic acid +; 3-methylbutanoic acid +; acetophenone +; docenaoic acid ++; 3,6,9-hexadecadien ++; nonadecan++ [a]	Bergmann, 2002
S. frequens (Navas)		**F+M:** heptan-2-one +; 2-octanol +; nonan-2-one +; 2-undecanon ++	Bergmann, 2002
Spicipalpia			
Hydrobiosidae			
Cailloma pumida Ross		**F:** octan-3-ol +++; 1-octen-3-ol + [b]	Bergmann, 2002
Rhyacophilidae			
Rhyacophila fasciata Hagen	**F:** no EAD response to M extracts, not attracted to M extracts **M:** attracted/EAD response to F extracts, no response to M extracts	**F (reared):** heptan-2-one (100); heptan-2-ol (338); nonan-2-one (40); nonan-2-ol (12) **F (caught):** heptan-2-one (100); heptan-2-ol (10); nonan-2-one (10); nonan-2-ol (1.5) **M:** hexanoic acid; octanoic acid; acetophenone	Ansteeg & Dettner, 1991; Löfstedt et al., 1994
R. fuscula (Walker)		**F:** nonan-2-ol (95% of gland exudate)	Duffield, 1981
R. nubila Zetterstedt	**F:** not attracted to M, receptor neurons respond to F gland compounds **M:** attracted to F, receptor neurons respond to F gland compounds	**F:** heptan-2-one (100); heptan-2-ol (120); nonan-2-one (119); nonan-2-ol (12) **M:** hexanoic acid; octanoic acid; acetophenone	Solem, 1985; Ansteeg & Dettner, 1991; Löfstedt et al., 1994; Larsson & Hansson, 1998
R. obliterata McLachlan		**F:** heptan-2-one 67; heptan-2-ol 100 **M:** hexanoic acid +++; octanoic acid ++; acetophenone +++	Bergmann, 2002

Glossosomatidae			
Agapetus fuscipes Curtis	**M:** follows track of F, attracted to middle of F abdomen, EAD response to extracts of F S IV-V/underlined compounds	**F:** heptan-2-one +; heptan-2-ol +; octan-2-one +; (*Z*)-6-nonen-2-one +++; nonan-2-one ++; (*Z*)-6-nonen-2-ol +; nonan-2-ol +; nonanal +; acetic acid + **M:** heptan-2-ol +; octan-2-one +; octan-2-ol +; (*Z*)-6-nonen-2-one +; (*Z*)-6-nonen-2-ol +; nonan-2-one +; nonan-2-ol +; acetic acid ++; 2-methyl propanoic acid +; propanoic acid +; decan-2-one +; decan-2-ol +; undecan-2-one +	Ivanov, 1993; Bergmann et al., 2002
A. ochripes Curtis	**F:** EAD response to M extracts **M:** EAD response to underlined compounds	**F:** nonan-2-one +++; (*Z*)-6-nonen-2-one ++; acetic acid +++; undecan-2-one +	Ivanov & Löfstedt, 1998; Bergman et al., 2002
Mastigoptila longicornuta (Schmid)		**F:** heptan-2-one +++; heptan-2-ol +++; hexanoic acid-2-heptylester + **M:** heptan-2-one ++; heptan-2-ol ++; nonan-2-one ++; hexanoic acid-2-heptylester +++	Bergmann, 2002
Hydroptilidae			
Hydroptila forcipata (Eaton)		**F:** heptan-2-one 34; 6-methyl-5-hepten-2-ol 4; nonan-2-one 100	Bergmann, 2002
Integripalpia			
Brachycentridae			
Brachycentrus sp.	**M:** EAD response to M extract		Ivanov & Löfstedt, 1998
Phryganeidae			
Agrypnia pagetana Curtis	**M:** EAD response to underlined compounds	**F:** (*S*)-heptan-2-ol +; (*R*)-6-methyl-5-hepten-2-ol ++; heptanal +; (2*S*,6*Z*)-6-nonen-2-ol ++; (*S*)-nonan-2-ol +; (Z)-6-nonen-2-one ++; nonanal +	Bergmann et al., 2002
Phryganea grandis Linnaeus		**F:** 1-pentanol; 3-methyl-heptan-2-one; 1-octanol; 2-phenylethanol; 1 additional compound	Ansteeg & Dettner, 1991
Apataniidae			
Apatania fimbriata (Pictet)	**M:** attracted to F, F extracts, underlined compounds	**F:** octan-3-one +++; 6-methyl-3-octanon ++; 1,8-cineol +	Solem & Solem, 1991; Bergmann, 2002
Limnephilidae			
Dicosmoecus gilvipes (Hagen)	**F:** not attracted to M **M:** attracted to F/F S VI extracts		Resh & Wood, 1985
Monocosmoecus pulcher Ulmer (syn. *pulcherrimus*)		**F:** hexan-2-ol +; nonan-3-one +; nonan-3-ol +; 3-hydroxy-2-butanon +; 2-phenylethanol +; benzoic acid ++; verbenone +++ **M:** 3-hydroxy-2-butanon +; 2,3-Butandiol +, ++; 2-phenylethanol +	Bergmann, 2002
Anomalopterygella chauviniana (Stein)		**F:** (3*R*,4*S*)-4-methyl-3-hexanol +; (3*R*,4*S*)-4-methyl-3-heptanol +; 6-methyl-3-octanon +++; 6-methyl-octan-3-ol ++; 6-methyl-nonan-3-one +	Bergmann, 2002

Anabolia laevis (Zetterstedt)	**M:** attracted to underlined compounds	**F:** (3*S*,4*S*)-4-methyl-3-hexanol 100; (3*S*,4*S*)-4-methyl-3-heptanol 28; 6-Nonen-2-one 19; 6-Nonen-2-ol 25; 3-hydroxy-2-butanon 45	Bergmann, 2002
Chaetopteryx villosa (Fabricius)		**F:** (3*S*,4*S*)-4-methyl-3-hexanol +++; 4-methyl-3-hexanone ++; 4-methyl-3-heptanone +++; (3*S*,4*S*)-4-methyl-3-heptanol + **M:** 4-methyl-3-hexanon ++; 4-methyl-3-heptanon +++	Bergmann, 2002
Glyphotaelius pellucidus (Retzius)	**F+M:** EAG response to underlined compounds/F and M extracts	**F+M:** (4*S*,6*S*)-dimethyl-3-octanone; (4*S*,6*S*)-dimethyl-nonan-3-one); 4,6-dimethyl-6-nonen-3-one; 1,3-diethyl-4,6-dimethyl-2,7-dioxabicyclo[2.2.1]heptane	Bergmann et al., 2001, 2004
Halesus radiatus (Curtis)	**F:** do not attract M		Solem & Petersson, 1987
Hesperophylax occidentalis (Banks)	**F:** weak EAG response to underlined compound/F S IV-V extracts, no response to M extracts **M:** strong EAG response/ attracted to underlined compound, strong EAG response to F S IV-V extracts, no response to extracts of M	**F:** 6-methylheptan-3-one; 6-methylheptan-3-ol; 6-methyloctan-3-one; 6-methyloctan-3-ol (minor compounds); 6-methylnonan-3-one (main compound); 6-methylnonan-3-ol 6-methyldecan-one; 6-methylundecan-one; indole present in some) (minor compounds)	Bjostad et al., 1996; Jewett et al., 1996
Limnephilus nigriceps (Zetterstedt)		**F:** 4-methyl-3-hexanol +; 4-methyl-3-heptanon +; 4-methyl-3-heptanol +; 3-hydroxy-2-butanon +++ **M:** 4-methyl-3-hexanol 20 [(3*S*,4*S*)/(3*R*,4*S*) = 90:10]; 4-methyl-3-heptanon 100; 4-methyl-3-heptanol 20 [(3*S*,4*S*)/(3*R*,4*S*) = 90:10]	Bergmann, 2002
L. politus McLachlan		**F:** 4-methyl-3-hexanol 100 [(3*S*,4*S*)/(3*R*,4*S*) = 70:30]; 4-methyl-3-heptanone 50; 4-methyl-3-heptanol 30 [(3*S*,4*S*)/(3*R*,4*S*) = 85:15]; 6-methyl-3-octanone 90 **M:** 4-methyl-3-hexanol 100 [(3*S*,4*S*)/(3*R*,4*S*) = 70:30]; 4-methyl-3-heptanone 100; 4-methyl-3-heptanol 60 [(3*S*,4*S*)/(3*R*,4*S*) = 85:15]; 6-methyl-3-octanon 1; 3-hydroxy-2-butanon 1, 2,3-butandiol 1	
Potamophylax cingulatus (Stephens)	**F:** EAD response to 7-Episordidin **M:** not attracted to F, EAD response to underlined compounds/extracts of F S IV-V	**F:** (*S*)-4-methyl-3-heptanone +++; 4-methyl-3-heptanol +; (4*S*,6*S*)-dimethyl-3-octanone +; (4*S*,6*S*)-dimethyl-nonan-3-one +++; 7-Episordidin ++ **M:** 7-Episordidin	Solem & Petersson, 1987; Bergmann et al., 2001, Bergmann, 2002

P. latipennis (Curtis)	**F:** EAD response to 7-Episordidin **M:** weakly attracted to F, EAD response to underlined compounds/ extracts of F	**F:** (*S*)-4-methyl-3-heptanone +++; 4*S*,6*S*)-dimethyl-3-octanone +; (4*S*,6*S*)-dimethyl-nonan-3-one +++; 7-Episordidin ++ **M:** (*S*)-4-methyl-3-heptanone; 7-Episordidin	Solem & Petersson, 1987; Bergmann et al., 2001, 2004; Bergmann, 2002
P. luctuosus (Piller & Mitterpacher)		**F:** 4-methyl-3-heptanone +; 4-methyl-3-heptanol +; 7-Episordidin +; 1,8-cineol + **M:** 4-methyl-3-heptanone +; 7-Episordidin +; 1,8-cineol +	Bergmann, 2002
Pycnopsyche scabripennis (Rambur)	**F+M:** gland extracts repel ants	p-cresol; indole; scatole	Duffield et al., 1977
Molannidae			
Molanna angustata Curtis	**M:** attracted to extracts of F/underlined compounds, other compounds repel	**F:** *S*)-heptan-2-ol 1; heptan-2-one 1; *S*)-nonan-2-ol 10; nonan-2-one 4	Solem & Petersson, 1987; Löfstedt et al., 2008
Sericostomatidae			
Gumaga griseola (McLachlan)	**M:** attracted to F/extracts of F/F S IV-V		Wood & Resh, 1984; Resh & Wood, 1985
G. nigricula (McLachlan)	**M:** attracted to F/extracts of F/F S IV-V		Resh & Wood, 1985

[a] Various –cosans and small amounts of many other substances were also found in this species (Bergmann, 2002), However since various –cosans were also found in male *Cailloma pumida* in which the sternum V gland is absent and in *Psilopsyche molina* Navas and *P. kolbiana* Ulmer (Bergmann, 2002) in which the glands are absent in at least *P. molinai* (Chapter 2), these substances are probably not linked to the sternum V gland.

[b] Acetic acid and various –cosans also found in the male (Bergmann, 2002). As the sternum V glands are absent in the male (Chapter 2), these substances are unlikely to come from the sternum V gland.

TABLE 6-2. Significant correlations between the main types of methylated 3-ketones and the corresponding alcohols. 6-methyl-3-nonanol/one was not present in males. P-values were calculated using Pagel's 1994 Correlation test in Mesquite. F, female; M, male.

Compounds	4-methyl-3-heptanol/one	6-methyl-3-octanol/one	(4*S*,6*S*)-dimethyl-3-octanone	6-methyl-3-nonanol/one	(4*S*,6*S*)-dimethyl-3-nonanone
4-methyl-3-hexanol/one	M+F: p = 0.000 M: p = 0.006 F: p = 0.000	M+F: p = 0.004 F: p = 0.004	M+F: p = 0.004 F: p = 0.003	M+F: p = 0.001 F: p = 0.003	M+F: p = 0.001 F: p = 0.001
4-methyl-3-heptanol/one		M+F: p = 0.020 F: p = 0.019	M+F: p = 0.002 M: p = 0.005 F: p = 0.005	M+F: p = 0.007 F: p = 0.005	M+F: p = 0.006 M: p = 0.008 F: p = 0.010
6-methyl-3-octanol/one			M+F: p = 0.032 F: p = 0.020	M+F: p = 0.000 F: p = 0.000	M+F: p = 0.030 F: p = 0.023
(4*S*,6*S*)-dimethyl-3-octanone				F: p = 0.013	M+F: p = 0.000 M: p = 0.002 F: p = 0.000
6-methyl-3-nonanol/one					M+F: p = 0.049 F: p = 0.004

FIGURE 6-1. Phylogenetically correct regression of female gland reservoir size versus female body size. States of the terminal taxa are shown against the regression line, confidence intervals (CI) and prediction intervals (PI) generated by PDAP: PDTREE. Note the extremely small gland size in *Wormaldia arizonensis* (Philopotamidae).

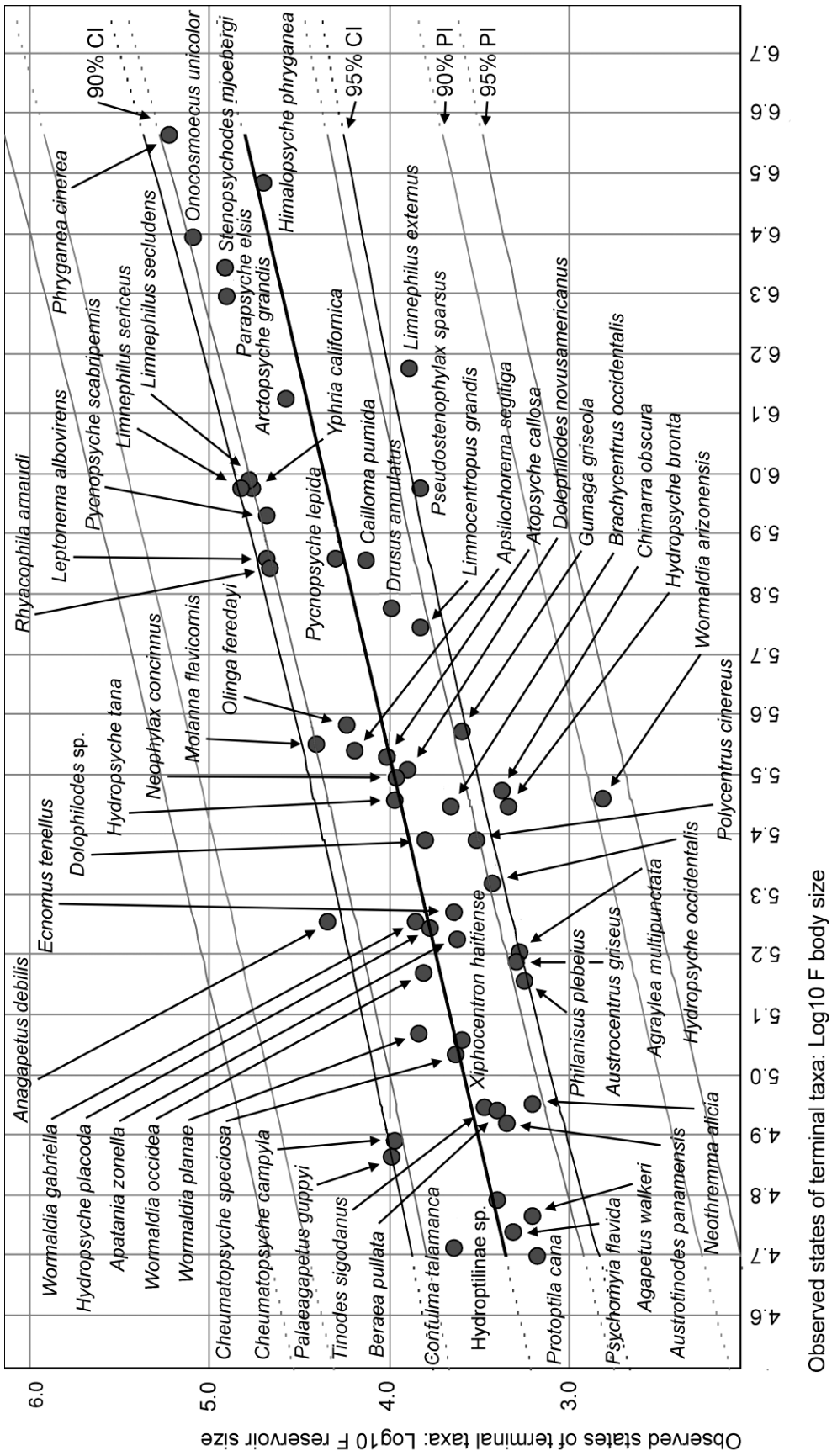

Observed states of terminal taxa: Log10 F reservoir size
Observed states of terminal taxa: Log10 F body size
90% CI
95% CI
90% PI
95% PI
Rhyacophila amaudi
Anagapetus debilis
Ecnomus tenellus
Dolophilodes sp.
Hydropsyche tana
Neophylax concinnus
Molanna flavicornis
Olinga feredayi
Pycnopsyche lepida
Yphria californica
Cailloma pumida
Drusus annulatus
Limnocentropus grandis
Apsilochorema segitiga
Atopsyche callosa
Dolophilodes novusamericanus
Gumaga griseola
Brachycentrus occidentalis
Chimarra obscura
Hydropsyche bronta
Wormaldia arizonensis
Polycentrus cinereus
Hydropsyche occidentalis
Agraylea multipunctata
Austrocentrus griseus
Philanisus plebeius
Xiphocentron haitiense
Hydroptilinae sp.
Protoptila cana
Psychomyia flavida
Agapetus walkeri
Austrotinodes panamensis
Neothremma alicia
Contulma talamanca
Beraea pullata
Tinodes sigodanus
Palaeagapetus guppyi
Cheumatopsyche campyla
Cheumatopsyche speciosa
Wormaldia planae
Wormaldia occidea
Apatania zonella
Hydropsyche placoda
Wormaldia gabriella
Onocosmoecus unicolor
Limnephilus secludens
Limnephilus sericeus
Pycnopsyche scabripennis
Leptonema albovirens
Phryganea cinerea
Himalopsyche phryganea
Arctopsyche grandis
Parapsyche elsis
Stenopsychodes mjoebergi
Pseudostenophylax sparsus
Limnephilus externus
6.0
5.0
4.0
3.0
6.7
6.6
6.5
6.4
6.3
6.2
6.1
6.0
5.9
5.8
5.7
5.6
5.5
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6

FIGURE 6-2. Phylogenetically correct regression of male gland reservoir size versus male body size. States of the terminal taxa are shown against the regression line, confidence intervals (CI) and prediction intervals (PI) generated by PDAP: PDTREE. Note the extremely large gland size in *Leptonema albovirens* (Hydropsychidae) and the relatively large gland sizes in *Hydropsyche* (Hydropsychidae) and *Pycnopsyche* (Limnephilidae). Also note the small relative gland size in *Limnephilus externus* (Limnephilidae).

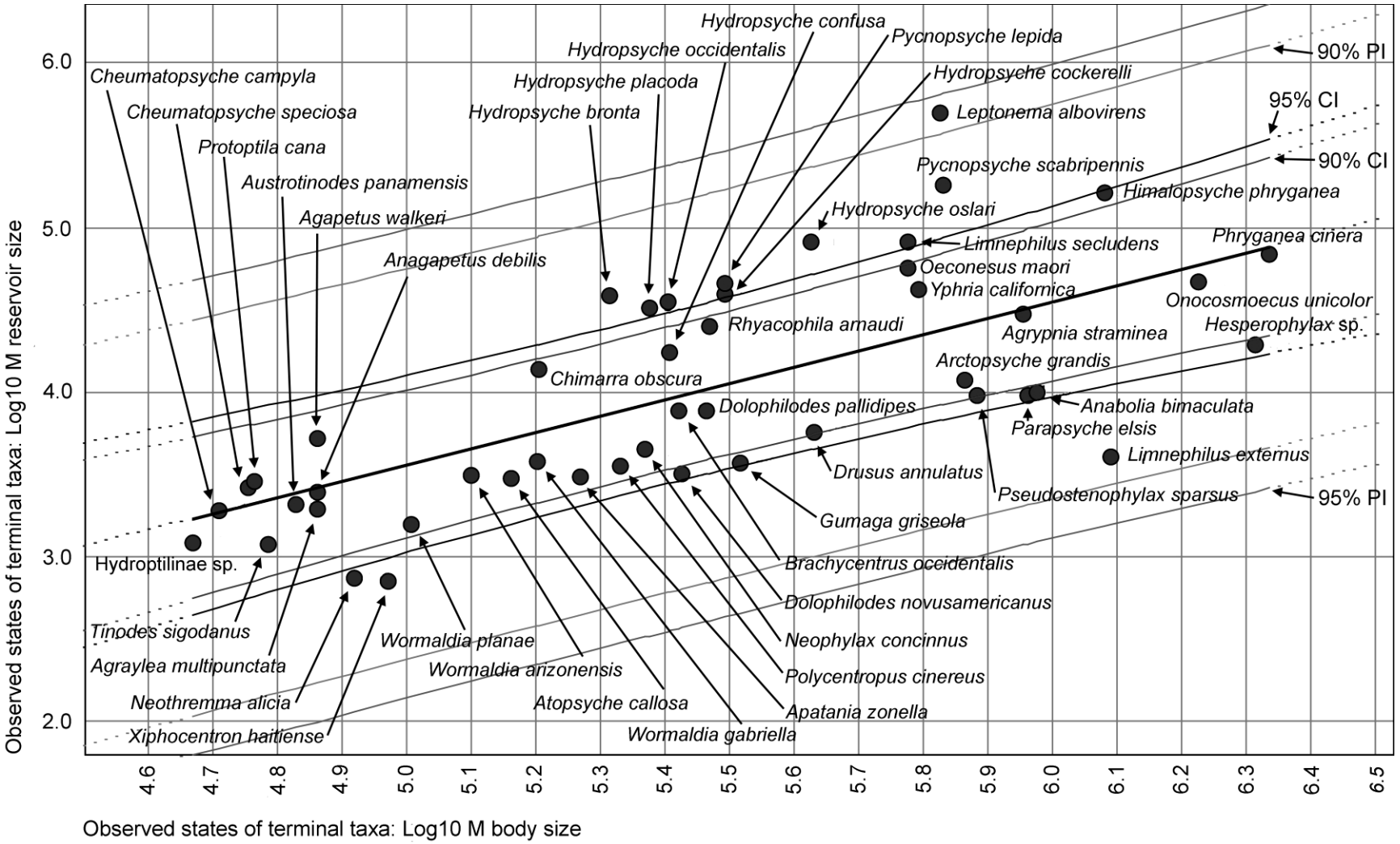

Observed states of terminal taxa: Log10 M reservoir size
Observed states of terminal taxa: Log10 M body size
6.0
5.0
4.0
3.0
2.0
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3
5.4
5.5
5.6
5.7
5.8
5.9
6.0
6.1
6.2
6.3
6.4
6.5
90% PI
95% CI
95% CI
90% PI
Cheumatopsyche campyla
Cheumatopsyche speciosa
Protoptila cana
Austrotinodes panamensis
Agapetus walkeri
Anagapetus debilis
Hydropsyche placoda
Hydropsyche bronta
Hydropsyche occidentalis
Hydropsyche confusa
Pycnopsyche lepida
Hydropsyche cockerelli
Leptonema albovirens
Pycnopsyche scabripennis
Himalopsyche phryganea
Hydropsyche oslari
Limnephilus secludens
Oeconesus maori
Yphria californica
Phryganea cinera
Onocosmoecus unicolor
Hesperophylax sp.
Agrypnia straminea
Rhyacophila arnaudi
Chimarra obscura
Arctopsyche grandis
Dolophilodes pallidipes
Anabolia bimaculata
Parapsyche elsis
Limnephilus externus
Drusus annulatus
Pseudostenophylax sparsus
Gumaga griseola
Hydroptilinae sp.
Brachycentrus occidentalis
Dolophilodes novusamericanus
Tinodes sigodanus
Wormaldia planae
Neophylax concinnus
Agraylea multipunctata
Wormaldia arizonensis
Polycentropus cinereus
Neothremma alicia
Atopsyche callosa
Apatania zonella
Xiphocentron haitiense
Wormaldia gabriella

FIGURE 6-3. Phylogenetically correct regression of residuals of male gland reservoir size regressed on male body size versus residuals of female gland reservoir size regressed on female body size. States of the terminal taxa are shown against the regression line, confidence intervals (CI) and prediction intervals (PI) generated by PDAP: PDTREE. Note the large gland sizes in males compared to conspecific females in *Leptonema albovirens*, *Hydropsyche* (both Hydropsychidae) and *Pycnopsyche* (Limnephilidae).

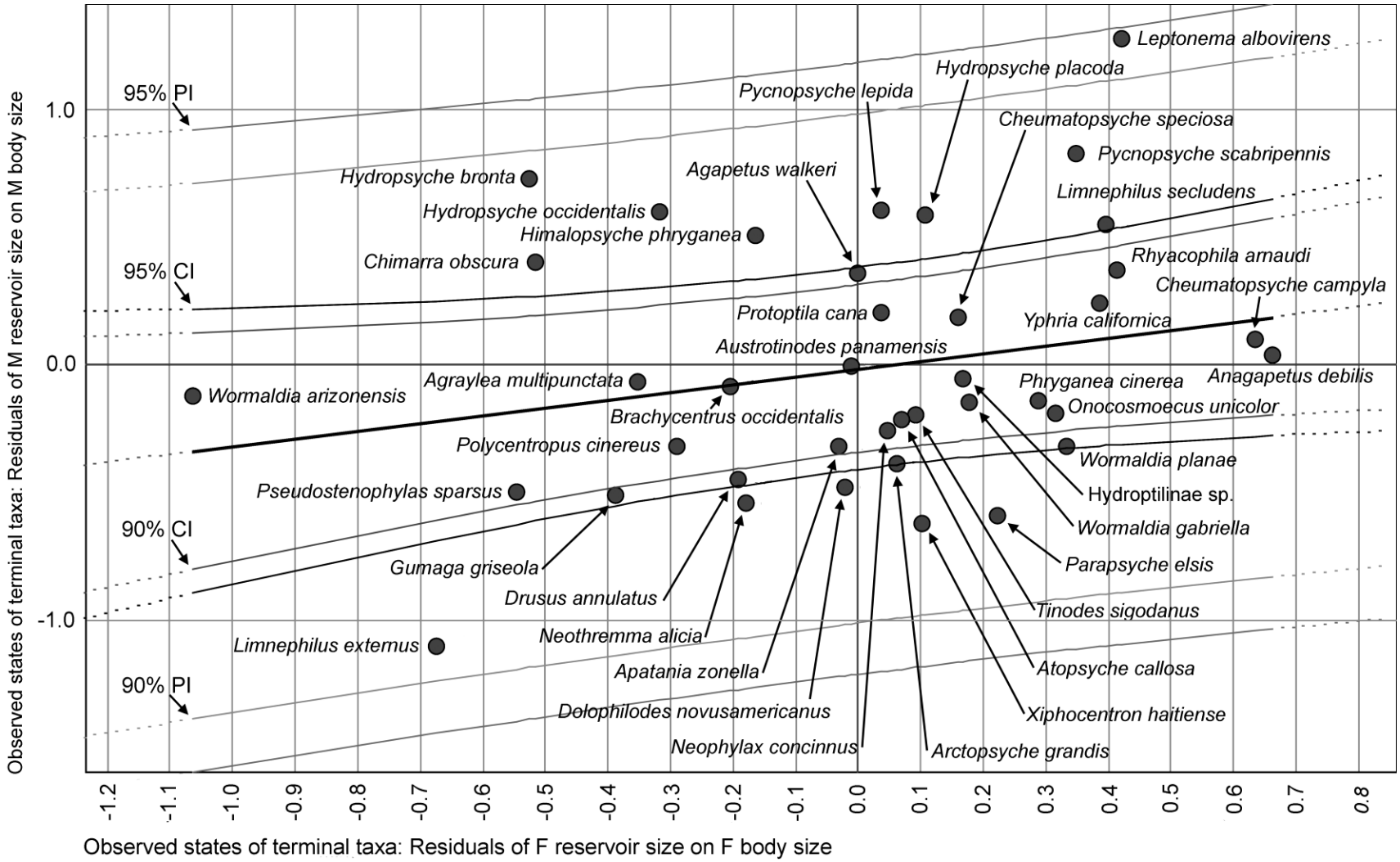

Leptonema albovirens
Hydropsyche placoda
Pycnopsyche lepida
Cheumatopsyche speciosa
Pycnopsyche scabripennis
Agapetus walkeri
Hydropsyche bronta
Limnephilus secludens
Hydropsyche occidentalis
Himalopsyche phryganea
Rhyacophila arnaudi
Chimarra obscura
Cheumatopsyche campyla
Protoptila cana
Yphria californica
Austrotinodes panamensis
Anagapetus debilis
Agraylea multipunctata
Phryganea cinerea
Wormaldia arizonensis
Onocosmoecus unicolor
Brachycentrus occidentalis
Polycentropus cinereus
Wormaldia planae
Hydroptilinae sp.
Pseudostenophylas sparsus
Wormaldia gabriella
Parapsyche elsis
Gumaga griseola
Drusus annulatus
Tinodes sigodanus
Neothremma alicia
Limnephilus externus
Atopsyche callosa
Apatania zonella
Xiphocentron haitiense
Dolophilodes novusamericanus
Neophylax concinnus
Arctopsyche grandis
95% PI
95% CI
90% CI
90% PI
1.0
0.0
-1.0
-1.2
-1.1
-1.0
-0.9
-0.8
-0.7
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Observed states of terminal taxa: Residuals of M reservoir size on M body size
Observed states of terminal taxa: Residuals of F reservoir size on F body size

FIGURE 6-4. Tree illustrating phylogenetic distribution of large and small gland reservoirs and amounts of secretory tissue. The scorings are based on residuals for taxa in which both gland size and secretory tissue was measurable. Score -2: residual value < -0.5. Score -1: residual value -0.5 - < -0.1. Score 0: residual value -0.1 - 0.1. Score 1: residual value > 0.1 - 0.5. Score 2: residual value > 0.5. The first number refers to gland reservoir size, the second number to the amount of secretory tissue.

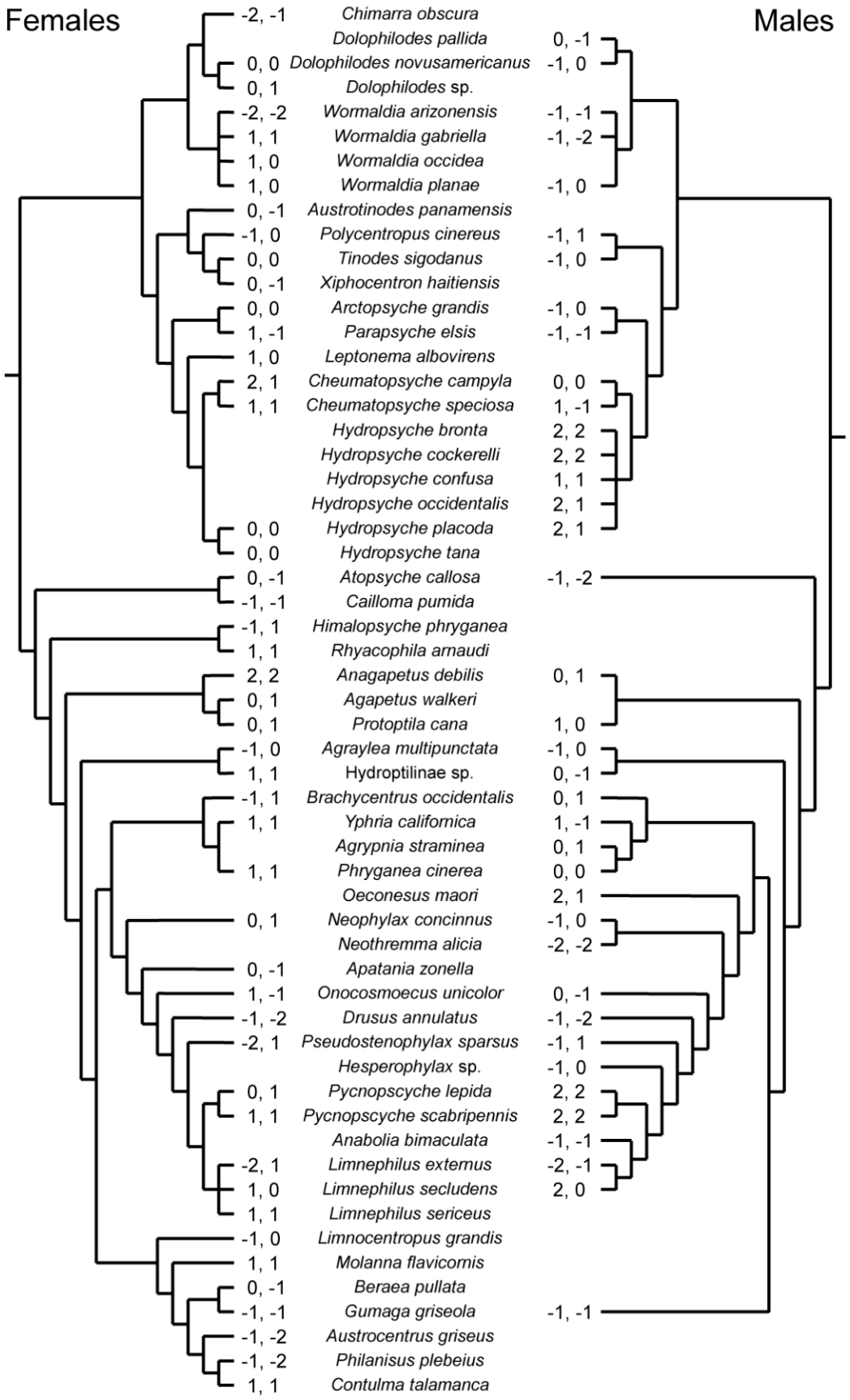
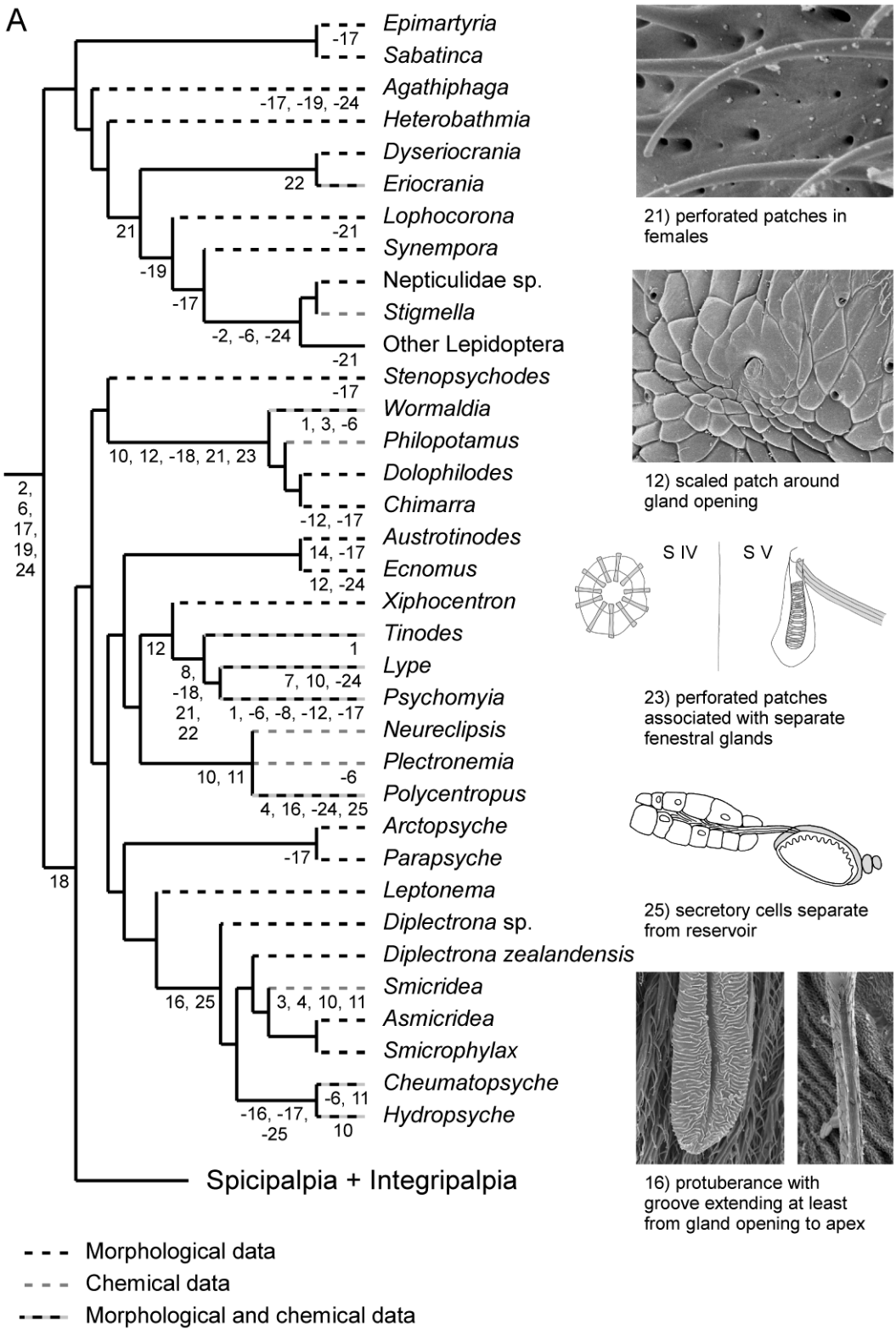

Females
Males
-2, -1 Chimarra obscura
Dolophilodes pallida 0, -1
0, 0 Dolophilodes novusamericanus -1, 0
0, 1 Dolophilodes sp.
-2, -2 Wormaldia arizonensis -1, -1
1, 1 Wormaldia gabriella -1, -2
1, 0 Wormaldia occidea
1, 0 Wormaldia planae -1, 0
0, -1 Austrotinodes panamensis
-1, 0 Polycentropus cinereus -1, 1
0, 0 Tinodes sigodanus -1, 0
0, -1 Xiphocentron haitiensis
0, 0 Arctopsyche grandis -1, 0
1, -1 Parapsyche elsis -1, -1
1, 0 Leptonema albovirens
2, 1 Cheumatopsyche campyla 0, 0
1, 1 Cheumatopsyche speciosa 1, -1
Hydropsyche bronta 2, 2
Hydropsyche cockerelli 2, 2
Hydropsyche confusa 1, 1
Hydropsyche occidentalis 2, 1
0, 0 Hydropsyche placoda 2, 1
0, 0 Hydropsyche tana
0, -1 Atopsyche callosa -1, -2
-1, -1 Cailloma pumida
-1, 1 Himalopsyche phryganea
1, 1 Rhyacophila arnaudi
2, 2 Anagapetus debilis 0, 1
0, 1 Agapetus walkeri
0, 1 Protoptila cana 1, 0
-1, 0 Agraylea multipunctata -1, 0
1, 1 Hydroptilinae sp. 0, -1
-1, 1 Brachycentrus occidentalis 0, 1
1, 1 Yphria californica 1, -1
Agrypnia straminea 0, 1
1, 1 Phryganea cinerea 0, 0
Oeconesus maori 2, 1
0, 1 Neophylax concinnus -1, 0
Neothremma alicia -2, -2
0, -1 Apatania zonella
1, -1 Onocosmoecus unicolor 0, -1
-1, -2 Drusus annulatus -1, -2
-2, 1 Pseudostenophylax sparsus -1, 1
Hesperophylax sp. -1, 0
0, 1 Pycnopscyche lepida 2, 2
1, 1 Pycnopscyche scabripennis 2, 2
Anabolia bimaculata -1, -1
-2, 1 Limnephilus externus -2, -1
1, 0 Limnephilus secludens 2, 0
1, 1 Limnephilus sericeus
-1, 0 Limnocentropus grandis
1, 1 Molanna flavicornis
0, -1 Beraea pullata
-1, -1 Gumaga griseola -1, -1
-1, -2 Austrocentrus griseus
-1, -2 Philanisus plebeius
1, 1 Contulma talamanca

FIGURE 6-5. Tree illustrating mapping of selected characters with parsimony reconstruction of ancestral states, 6-5 A shows Annulipalpia, 6-5 B shows Spicipalpia + Integripalpia. The tree shows taxa down to genus level, except in the case of *Diplectrona* sp. and *Diplectrona zealandensis*, as the latter is more closely related to Smicrideinae than Diplectroninae according to Schefter (1996) and Geraci (2007). When data for more than one species in a genus were available, a concatenated taxon was used; in cases in which a trait is both absent and present in members of the same genus, the trait is shown as present. If two or more equally parsimonious reconstructions were presented for a single trait, we chose to minimize independent origins of the trait. The character numbers in the figure are only for the purpose of this figure. A minus in front of a character number indicate a loss. Characters: **1)** methyl-2-hexanol; **2)** heptan-2-ol/one; **3)** 3-hepten-2-one; **4)** octan-2-ol; **5)** 6-methyl-3-octanone; **6)** nonan-2-ol/one; **7)** 3-nonen-2-one; **8)** *Z*4-nonen-2-one; **9)** methylated 3-ketones and corresponding alcohols; **10)** organic acids; **11)** acetophenone and derivatives; **12)** scaly patch around gland opening; **13)** hexagonal cuticle around gland opening; **14)** bald area that extends from front edge of sternite and encompasses gland opening; **15)** internal cuticular ridge around gland opening; **16)** protuberance with groove that extends at least from gland opening to apex; **17)** enlarged evaporatory surface around gland opening; **18)** Trichoptera-type opening muscle; **19)** gland reservoir periform, ovoid or round; **20)** gland reservoir reniform; **21)** perforated patches in females; **22)** perforated patches associated with gland reservoir; **23)** perforated patches associated with separate fenestral glands; **24)** gland reservoir musculature; **25)** secretory cells separate from gland reservoir.



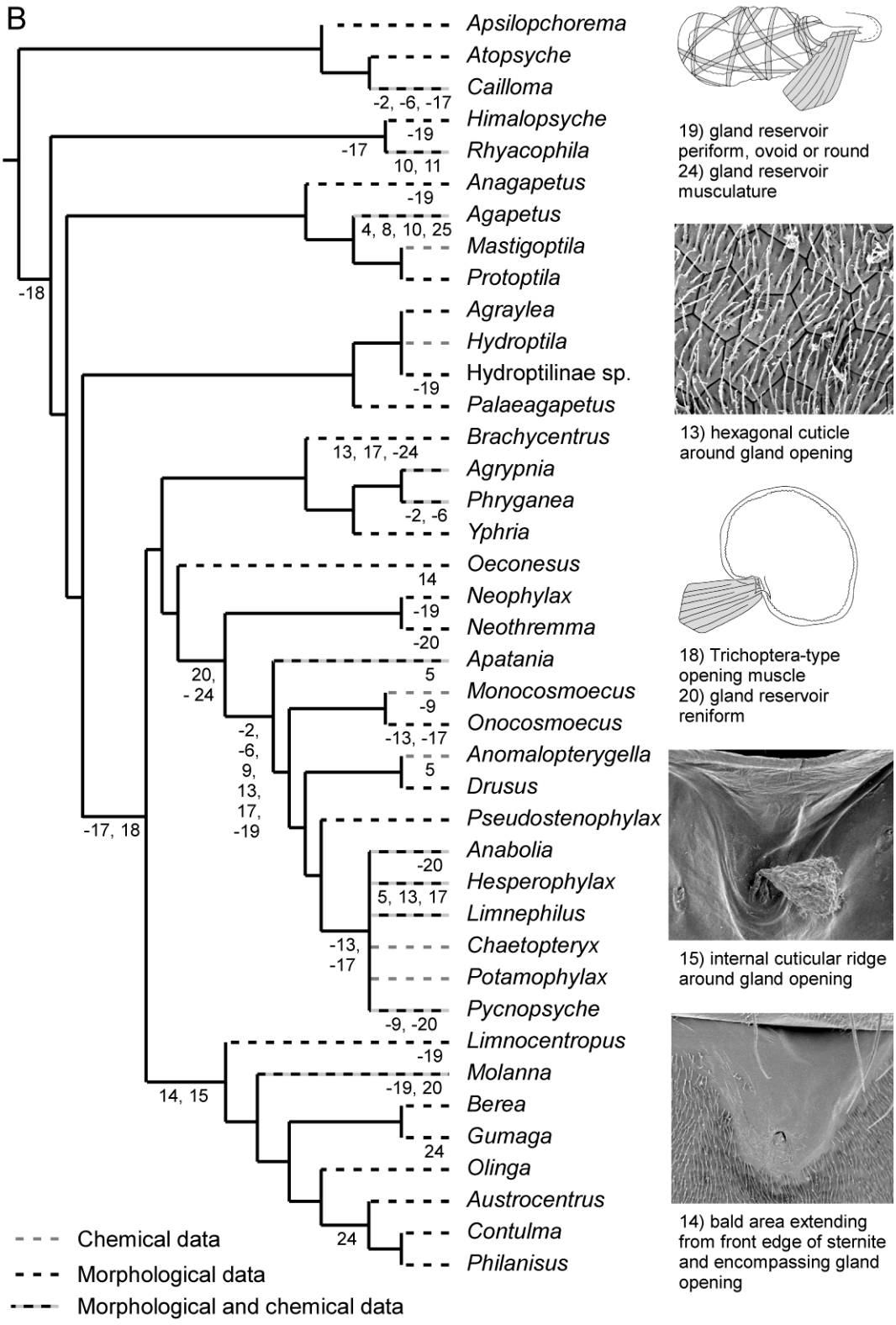
21) perforated patches in females

12) scaled patch around gland opening

23) perforated patches associated with separate fenestral glands

25) secretory cells separate from reservoir

16) protuberance with groove extending at least from gland opening to apex


B
Apsilopchorema
Atopsyche
Cailloma
-2, -6, -17
Himalopsyche
-19
-17
Rhyacophila
10, 11
Anagapetus
-19
Agapetus
4, 8, 10, 25
Mastigoptila
Protoptila
-18
Agraylea
Hydroptila
Hydroptilinae sp.
-19
Palaeagapetus
Brachycentrus
13, 17, -24
Agrypnia
Phryganea
-2, -6
Yphria
Oeconesus
14
Neophylax
-19
Neothremma
-20
Apatania
20,
- 24
5
Monocosmoecus
-9
Onocosmoecus
-13, -17
-2,
-6,
9,
13,
17,
-19
Anomalopterygella
5
Drusus
-17, 18
Pseudostenophylax
Anabolia
-20
Hesperophylax
5, 13, 17
Limnephilus
-13,
-17
Chaetopteryx
Potamophylax
Pycnopsyche
-9, -20
Limnocentropus
-19
Molanna
-19, 20
14, 15
Berea
Gumaga
24
Olinga
Austrocentrus
Contulma
24
Philanisus
Chemical data
Morphological data
Morphological and chemical data
19) gland reservoir periform, ovoid or round
24) gland reservoir musculature
13) hexagonal cuticle around gland opening
18) Trichoptera-type opening muscle
20) gland reservoir reniform
15) internal cuticular ridge around gland opening
14) bald area extending from front edge of sternite and encompassing gland opening

Chapter 7

Concluding remarks

The present work on the paired sternum V glands has answered many questions, but may have raised even more. It is my hope that my thesis work will function as a platform for further research on the sternum V glands.

MORPHOLOGY

Distribution of glands

The distribution of the gland is known for representatives of all major lineages of Trichoptera. However, there are still several family level groups in which the presence/absence is unknown for all taxa, especially in the Sericostomatoidea. Presence/absence has not been investigated in any representatives of Rossianidae, Barbarochthonidae, Hydrosalpingidae or Petrothrincidae, nor has it been investigated in representatives of the two 'sericostomatid' genera *Myotricha* and *Parasericostoma* that Holzenthal et al. (2007) found to be separate from the remaining (including *Sericostoma*) sericostomatid genera included in the analysis. Furthermore, presence/absence has not been investigated in any females of Plectrotarsidae, nor in any males of Phryganopsychidae or Limnocentropodidae.

In the single male specimen of *Philanisus plebeius* (Chathamiidae) available to me for study, I found indications that the sternum V gland was present, but could not conclusively confirm this. Ivanov and Melnitsky (2002) reported that the gland is absent in male Chathamiidae, but did not indicate which species had been studied. Further investigations of male representatives of Chathamiidae will hopefully clarify whether or not the glands are present.

Phylogenetic characters

My thesis work has identified several phylogenetically informative sternum V gland characters, which may contribute to future studies of Trichoptera phylogeny. It may be useful, for example, to determine the placement of the two subfamilies Pseudoneureclipsinae and Kambaitipsychinae in either Dipseudopsidae or Polycentropodidae. The representatives of Dipseudopsidae that I investigated have no sternum V glands, a very unusual condition in Annulipalpia, while the polycentropines included in both this and other studies (Nielsen, 1980; Ansteeg, 1989; Ivanov & Melnitsky, 1999, 2002) have sternum V glands with a well developed and distinctive cuticular protuberance. Absence of the sternum V gland in representatives of Pseudoneureclipsinae and Kambaitipsychinae would provide strong support for placement in Dipseudopsidae while presence of the sternum V gland and a polycentropine-like protuberance would provide strong support for placement in Polycentropodidae.

Ground plan of glands

Based on my studies of the sternum V gland in representatives of both Trichoptera and Lepidoptera I propose the following ground plan for the gland: The gland consists of a pair of invaginations from sternum V with slit-like gland openings. The gland duct is U-shaped just inside the opening. A pair of gland-opening muscles that originate on the anterior edge of sternum VI insert on the inside curve of the U. The saccular gland reservoir is pressed against the cuticle of sternum IV. In females, this cuticle is perforated and likely transparent; muscle fibres originating around the perforated cuticle insert on the gland reservoir.

Presently, this exact gland configuration is only known in representatives of Psychomyiidae (Trichoptera) and Eriocraniidae (Lepidoptera), but this gland configuration should have been present in stem groups of both orders. Extant taxa of interest that might possess the perforated patches on sternum IV are females of Agathiphagidae, Heterobathmiidae (other than *Heterobathmia pseuderiocrania*), Acanthopteroctetidae, Lophocoronidae, Mnesarchaeidae, Hepialoidea and Opostegidae (Lepidoptera) (Chapter 5).

FUNCTION

Trichoptera

I found two separate glandular structures in female philopotamids: regular sternum V glands and fenestral glands presumably derived from the sternum V gland (Chapter 5, e.g. figure 5-7). The relative sizes of the two structures in various philopotamids suggest a complementary function (Chapter 6). As previous studies of the gland secretions have not distinguished between the two structures, future studies would be enhanced by analysing the two structures separately.

Large gland size in males and production of male aggregation pheromones seems to be linked in *Hydropsyche* (Trichoptera: Hydropsychidae) (Chapter 6). If there is a connection, studies of pheromone use in *Leptonema albovirens* would be very interesting as males of this species had by far the largest glands found in this study, both in terms of relative and absolute size (Chapter 6).

The chemical composition and/or the function of the sternum V gland secretions are known in a large number of trichopteran species. However, either function or composition of gland products is only known in representatives of two genera (Molannidae: *Molanna* and Sericostomatidae: *Gumaga*) in the infraorder Brevitentoria which contains nearly half of the trichopteran families. Functional studies of representatives of this group could potentially increase our understanding of the sternum V gland significantly.

Lepidoptera

Further studies of the function of the sternum V gland in representatives of Lepidoptera would improve our understanding of the ancestral function of the gland. While unlikely to improve our understanding of the ancestral gland function, clarifying the function of the highly derived gland in *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae) would provide a strong indication of whether there is a connection between derived form and derived function.

I found a glandular structure associated with the fenestra in female nepticulids (Chapter 3), and propose this as the source of the pheromones found

by Tóth et al. (1995). Löfstedt and Kozlov (1997) stated that identifying the pheromone-producing glands in nepticulids is critical for understanding the evolution of pheromone communication in Lepidoptera. Future research linking the pheromone production in female nepticulids to a specific body part would confirm or refute whether the sternum V gland is the source of the pheromones.

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Appendix 1

Supplemental information for Chapter 1

TRICHOPTERA IDENTIFIED TO SPECIES

Helicopsychidae

Helicopsyche borealis (Hagen)

Hydropsychidae

Arctopsychinae

Arctopsyche grandis (Banks)

Parapsyche elsis Milne

Hydropsychinae

Cheumatopsyche campyla Ross

Cheumatopsyche speciosa (Banks)

Hydropsyche bronta Ross

Hydropsyhe confusa (Walker)

Hydropsyche jewetti Denning, synonym of *H. cockerelli* Banks

Hydropsyche occidentalis Banks

Hydropsyche oslari Banks

Hydropsyche placoda Ross

Hydropsyche tana Ross

Hydroptilidae

Hydroptila waubesiana Betten

Zumatrichia notosa (Ross)

Lepidostomatidae

Lepidostoma cascadense (Milne)

Lepidostoma pluviale (Milne)

Leptoceridae

Leptocerinae

Ceraclea annulicornis (Stephens)

Ceraclea tarsipunctata (Vorhies)

Mystacides longicornis (Linnaeus)

Oecetis avara (Banks)

Oecetis cinerascens (Hagen)

Oecetis inconspicua (Walker)

Oecetis ochracea (Curtis)

Triaenodes reuteri McLachlan

Limnephilidae

Dicosmoecinae

Dicosmoecus atripes (Hagen)

Ecclisomyia conspersa Banks

Eocosmoecus frontalis (Banks)

Onocosmoecus unicolor (Banks)

Limnephilinae

Anabolia bimaculata (Walker)

Hydatophylax hesperus (Banks)

Limnephilus externus Hagen

Limnephilus frijole Ross

Limnephilus harrimani Banks

Limnephilus secludens Banks

Limnephilus sericeus (Say)

Molannidae

Molanna flavicornis Banks

Philopotamidae

Philopotaminae

Dolophilodes pallidipes Banks

Dolophilodes novusamericanus (Ling)

Wormaldia arizonensis (Ling)

Wormaldia gabriella (Banks)

Wormaldia occidea (Ross)

Chimarrinae

Chimarra aterrima Hagen

Phryganeidae

Phryganeinae

Agrypnia colorata Hagen

Agrypnia straminea Hagen

Phryganea cinerea Walker

Yphriinae

Yphria californica (Banks)

Polycentropodidae

Polycentropodinae

Polycentropus cinereus Hagen

Rhyacophilidae

Rhyacophila bifila Banks

Sericostomatidae

Gumaga griseola (McLachlan)

Appendix 2

Supplemental information for Chapter 2

DATA MATRIX AND TREE FOR MAPPING

The data matrix and the tree used for mapping of characters.

Characters

1) Presence/absence of gland: 0: absence; 1: presence (in either or both sexes)

2) Presence/absence of gland in females: 0: absence; 1: presence

3) Presence/absence of gland in males: 0: absence; 1: presence

4) Cuticular modifications with grooves demarcating borders between individual epidermal cells, binary: 0: absence; 1: presence

5) Cuticular modifications with grooves demarcating borders between individual epidermal cells, multistate: 0: absence; 1: scaly patch (e.g. Figures 2-2 B-E; 2-18 A); 2: polygons on protuberance (Figure 2-20); 3: hexagonal cuticle (e.g. Figure 2-23 A, B)

6) Bald area extending from front edge of sternite and encompassing gland opening: 0: absence; 1: presence (Figure 2-24 F-H)

7) Internal cuticular ridges extending from antecosta on both sides of gland opening and connecting in a smooth curve posterior to gland opening: 0: absence; 1: presence (Figures 2-15; 2-16; 2-26 G)

8) Presence/absence of protuberance: 0: absence; 1: presence (in either or both sexes), please refer to Figure 2-4 E for definition for protuberance versus bulge

9) Presence/absence of protuberance in females: 0: absence; 1: presence

10) Presence/absence of protuberance in males: 0: absence; 1: presence

11) Protuberance with groove extending at least from gland opening to apex with wavy cuticle along groove: 0: absence; 1: presence (Figure 2-18 E-H)

12) Protuberance with groove extending at least from gland opening to apex, protuberance covered with polygons separated by grooves: 0: absence; 1: presence (Figure 2-20)

13) Protuberance/bulge with setae: 0: absence; 1: presence (Figures 2-21 E, F; 2-22 A, C-E)

14) Short protuberance with very long setae: 0: absence; 1: presence (Figure 2-22 C)

15) Opening muscles: 0: absent while gland present (Figure 2-5 B, D, E); 1: present, originating on anterior edge of sternum VI (Figure 2-9 F); 2: present, originating mesad on cuticle of sternum V (e.g. Figure 2-13)

16) Gland reservoir shape: 0: none of the following (e.g. Figures 2-15 A; 2-26 F); 1: reniform (Figures 2-13 A; 2-26 C); 2: periform (e.g. Figures 2-2 A; 2-25 A); 3: ovoid or round (Figure 2-5 B); 4: elongate and compartmentalised (Figure 2-14 B)

17) Perforated patches on sternum IV in females (Figures 2-17 C, D; 2-18 C, D): 0: absent; 1: present and associated sternum V gland reservoir; 2: present and associated with separate fenestral glands (Figure 2-3 D)

18) Gland reservoir musculature: 0: absent (not observed) (e.g. Figure 2-13); 1: present (e.g. Figure 2-2)

19) Parallel gland reservoir musculature fibres: 0: absent; 1: present

20) Arrangement of secretory cells: 0: immediately adjacent to reservoir (e.g. Figure 2-8); 1: separate from reservoir (Figure 2-7)

Character matrix

Matrix with the characters listed above. Numbers in the matrix corresponds to character states as described above. The scoring ‘A’ equals 0/1, the scoring ‘B’ equals 0/2, the scoring ‘C’ equals 0/3, the scoring ‘D’ equals 1/2, and the scoring ‘E’ equals 2/3.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Stenopsychodes mjoebergi	1	1	?	0	0	0	0	1	1	?	0	0	0	0	2	2	0	1	0	?
Chimarra aterrima	1	1	1	0	0	0	0	0	0	0	0	0	0	0	?	?	2	?	–	?
Chimarra obscura	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	2	2	1	1	0
Dolophilodes sp.	1	1	?	1	1	0	0	0	0	?	0	0	0	0	?	2	2	1	0	0
Dolophilodes novusamericanus	1	1	1	1	1	0	0	1	0	1	0	0	0	0	1	2	2	1	0	0
Dolophilodes pallidipes	1	?	1	1	1	0	0	0	?	0	0	0	0	0	1	2	?	1	0	0
Philopotamus montanus	0	?	0	–	–	–	–	–	?	–	–	–	–	–	–	–	?	–	–	–
Wormaldia arizonensis	1	1	1	1	1	0	0	0	0	0	0	0	0	0	D	2	2	?	?	0
Wormaldia gabriella	1	1	1	1	1	0	0	1	0	1	0	0	0	0	1	3	2	1	0	0
Wormaldia planae	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	E	0	1	A	0
Wormaldia occidea	1	1	1	1	1	0	0	0	0	0	0	0	0	0	?	2	2	0	–	0
Austrotinodes panamensis	1	1	1	0	0	1	0	0	0	0	0	0	0	0	2	2	0	1	0	0
Ecnomus tenellus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	?	3	0	0	–	?
Dipseudopsis capensis	0	0	?	–	–	–	–	–	–	?	–	–	–	–	–	–	0	–	–	–
Phylocentropus placidus	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–	
Xiphocentron haitiense	1	1	1	1	1	0	0	0	0	0	0	0	0	0	2	2	0	1	0	0
Lype diversa	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	3	1	0	–	?
Psychomyia flavida	1	1	?	0	0	0	0	0	0	?	0	0	0	0	0	3	1	1	0	0
Tinodes sigodanus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0
Cyrnellus fraternus	1	1	?	0	0	0	0	1	1	?	1	0	0	0	?	?	0	?	–	?
Polycentropus cinereus	1	1	1	0	0	0	0	1	1	1	1	0	0	0	2	3	0	0	–	1
Arctopsyche grandis	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	2	2	0	1	0	0
Parapsyche elsis	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	2	2	0	1	0	0
Diplectrona sp.	1	1	1	1	2	0	0	1	1	1	0	1	0	0	2	3	0	1	0	1
Diplectrona zealandensis	1	?	1	1	2	0	0	1	?	1	0	1	0	0	2	2	?	1	0	1
Cheumatopsyche campyla	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	E	0	1	0	0
Cheumatopsyche speciosa	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	E	0	1	0	0
Hydropsyche bronta	1	1	1	0	0	0	0	1	0	1	0	0	0	0	2	E	0	0	–	0
Hydropsyche cockerelli	1	?	1	0	0	0	0	1	?	1	0	0	0	0	2	0	?	1	0	0
Hydropsyche confusa	1	?	1	0	0	0	0	1	?	1	0	0	0	0	2	2	?	1	0	0
Hydropsyche occidentalis	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	2	0	1	0	0
Hydropsyche oslari	1	1	1	0	0	0	0	0	1	0	1	0	0	0	2	0	0	1	0	0
Hydropsyche placoda	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	2	0	1	0	0
Hydropsyche tana	1	1	1	0	0	0	0	0	1	0	1	0	0	0	2	2	0	1	0	0
Leptonema albovirens	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	2	0	1	0	0
Asmicridea edwardsii	1	1	1	1	2	0	0	1	1	1	0	1	0	0	2	2	0	1	1	1
Smicrophylax sp.	1	1	?	1	2	0	0	1	1	?	0	1	0	0	2	2	0	1	1	1
Apsilochorema segitiga	1	1	?	0	0	0	0	0	0	?	0	0	0	0	2	3	0	1	1	?
Atopsyche callosa	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0	2	C	0	1	1	0
Cailloma pumida	1	1	0	0	0	0	0	0	0	0	–	0	0	0	0	?	2	0	1	1	0
Himalopsyche phryganea	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	C	0	1	0	0
Rhyacophila arnaudi	1	1	1	0	0	0	0	0	1	0	1	0	0	1	0	1	E	0	1	1	0
Agapetus walkeri	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	1	1	1
Anagapetus debilis	1	1	1	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	1	0	0
Protoptila cana	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	B	0	1	0	0
Hydroptilinae sp.	1	1	1	0	0	0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	0
Agraylea multipunctata	1	1	1	0	0	0	0	0	1	1	1	0	0	1	1	1	C	0	1	0	0
Palaeagapetus guppyi	1	1	1	0	0	0	0	0	1	1	1	0	0	1	0	1	3	0	1	0	?
Plectrotarsus tasmanicus	0	?	0	–	–	–	–	–	?	–	–	–	0	–	–	–	?	–	–	–
Pangullia faziana	0	?	0	–	–	–	–	–	?	–	–	–	0	–	–	–	?	–	–	–
Lepidostomatidae pluviale	0	0	0	–	–	–	–	–	–	–	–	–	0	–	–	–	0	–	–	–
Brachycentrus occidentalis	1	1	1	1	3	0	0	0	0	0	0	0	0	0	2	3	0	0	–	0
Agrypnia straminea	1	?	1	0	0	0	0	0	?	0	0	0	0	0	2	3	?	1	0	0
Phryganea cinerea	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	3	0	1	0	0
Yphria californica	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	C	0	1	0	0
Oeconesus maori	1	?	1	0	0	1	0	1	?	1	0	0	0	0	2	3	?	1	A	0
Goera calcarata	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Neophylax concinnus	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	–	0
Neothremma alicia	1	1	1	0	0	0	0	0	0	0	0	0	0	0	?	3	0	0	–	0
Apatania zonella	1	1	1	1	3	0	0	0	0	0	0	0	0	0	2	1	0	0	–	0
Onocosmoecus unicolor	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	–	0
Drusus annulatus	1	1	1	1	3	0	0	0	0	0	0	0	0	0	2	1	0	0	–	0
Anabolia bimaculata	1	?	1	0	0	0	0	0	?	0	0	0	0	0	2	0	?	0	–	0
Hesperophylax sp.	1	?	1	1	3	0	0	0	?	0	0	0	0	0	2	1	?	0	–	0
Limnephilus externus	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	–	0
Limnephilus harrimani	1	1	?	?	?	?	0	?	?	?	0	0	?	?	?	?	0	?	?	0
Limnephilus secludens	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	–	0
Limnephilus sericeus	1	1	?	0	0	0	0	0	0	?	0	0	0	0	2	1	0	0	–	0
Pycnopsyche lepida	1	1	1	0	0	0	0	1	1	1	0	0	0	0	2	4	0	0	–	0
Pycnopsyche scabripennis	1	1	1	0	0	0	0	1	0	1	0	0	0	0	2	4	0	0	–	0
Pseudostenophylax sparsus	1	1	1	1	3	0	0	0	0	0	0	0	0	0	2	1	0	0	–	0
Limnocentropus grandis	1	1	?	0	0	1	1	0	0	?	0	0	0	0	2	0	0	0	–	0
Trichovespula macrocera	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Psilopsyche molinai	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Atriplectides dubius	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Marilia flexuosa	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Molanna flavicornis	1	1	0	0	0	1	1	0	0	–	0	0	0	0	2	1	0	0	–	0
Anisocentropus bicoloratus	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Phylloicus aeneus	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Trianodes reuteri	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Helicopsyche borealis	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	0	–	–	–
Beraea pullata	1	1	?	0	0	1	1	0	0	?	0	0	0	0	?	3	0	0	–	0
Gumaga grisola	1	1	1	0	0	1	1	0	0	0	0	0	0	0	2	3	0	1	0	0
Caloca saeneva	0	?	0	–	–	–	–	–	?	–	–	–	–	–	–	–	?	–	–	–
Olinga feredayi	1	1	0	0	0	1	1	0	0	–	0	0	0	0	2	3	0	0	–	0
Austrocentrus griseus	1	1	0	0	0	1	1	0	0	–	0	0	0	0	2	3	0	1	0	0
Contulma talamanca	1	1	0	0	0	1	1	0	0	–	0	0	0	0	2	3	0	1	0	0
Philanisus plebeius	1	1	?	0	0	1	1	0	0	?	0	0	0	0	2	3	0	1	0	0

Tree

Tree used for mapping of characters and exploration of phylogenetic trends. See Materials and Methods for origin of tree.


Stenopsychodes mjoebergi
Wormaldia arizonensis
Wormaldia gabriella
Wormaldia occidea
Wormaldia planae
Philopotamus montanus
Chimarra aterrima
Chimarra obscura
Dolophilodes sp.
Dolophilodes novusamericanus
Dolophilodes pallidipes
Austrotinodes panamensis
Ecnomus tenellus
Dipseudopsis capensis
Phylocentropus placidus
Cyrnellus fraternus
Polycentropus cinereus
Xiphocentron haitiense
Tinodes sigodanus
Lype diversa
Psychomyia flavida
Arctopsyche grandis
Parapsyche elsis
Leptonema albovirens
Diplectrona sp.
Diplectrona zealandensis
Asmicridea edwardsii
Smicrophylax sp.
Cheumatopsyche campyla
Cheumatopsyche speciosa
Hydropsyche bronta
Hydropsyche cockerelli
Hydropsyche confusa
Hydropsyche occidentalis
Hydropsyche oslari
Hydropsyche placoda
Hydropsyche tana
Apsilochorema segitiga
Atopsyche callosa
Cailloma pumida
Himalopsyche phryganea
Rhyacophila arnaudi
Anagapetus debilis
Agapetus walkeri
Protoptila cana
Palaeagapetus guppyi
Hydroptilinae sp.
Agraylea multipunctata
Plectrotarsus tasmanicus
Pangullia faziana
Lepidostoma pluviale
Brachycentrus occidentalis
Yphria californica
Agrypnia straminea
Phryganea cinerea
Oeconesus maori
Goera calcarata
Neophylax concinnus
Neothremma alicia
Apatania zonella
Onocosmoecus unicolor
Drusus annulatus
Pseudostenophylax sparsus
Hesperophylax sp.
Pycnopsyche lepida
Pycnopsyche scabripennis
Anabolia bimaculata
Limnephilus externus
Limnephilus harrimani
Limnephilus secludens
Limnephilus sericeus
Limnocentropus grandis
Trichovespula macrocera
Psilopsyche molinai
Atriplectides dubius
Marilia flexuosa
Molanna flavicornis
Anisocentropus bicoloratus
Phylloicus aeneus
Triaenodes reuteri
Helicopsyche borealis
Beraea pullata
Gumaga griseola
Caloca saeneva
Olinga feredayi
Austrocentrus griseus
Contulma talamanca
Philanisus plebeius

Appendix 3

Supplemental information for Chapter 3

BIOASSAYS WITH *EPIMARTYRIA AURICRINELLA*

Bioassays with *E. auricrinella* were carried out at Hopkin's Hole, a bog close to Ch. D'Eardley in Gatineau Park in Québec (Lat. 45.361 Long. -76.063) on June 12-15 2007.

During initial collection by sweep netting on June 11, Hopkins Hole was the best locality for *E. auricrinella* with 19 individuals caught. Based on this it was selected as the best locality for bioassay.

The bioassay was conducted using live traps with live animals as bait/pheromone source. The traps consisted of clear plastic tubes approximately 10 cm in diameter and 16 cm long with a funnel made from black window screen netting at either end. In each trap was a small metal cage (3x3x5 cm) covered with the same window screen netting. Inside each cage was a wet dental wick.

June 12: Nine traps were put out at Hopkin's Hole at noon. Four traps with three males in each, two traps with two females in each and three control traps. The traps were pulled and checked late in the afternoon, no animals had been caught in the traps and four males escaped.

June 13: Nine traps were put out at Hopkin's Hole at noon. Four traps with three males in each, two traps with two females in each and three control traps.

June 14: The traps were checked in the field at 4 pm. A fly had been caught in one trap, but no catch otherwise

June 15: The traps were pulled and checked at 4 pm. An additional fly had been caught, but no catch otherwise.

Thus no evidence was found to support that *E. auricrinella* employ long distance pheromones.

Appendix 4

Supplemental information for Chapter 6

Character matrices and trees used for mapping and correlations analyses.

CHEMICAL MATRIX AND TREE

Chemical matrix with both sexes included with each sex scored separately (except in cases in which scorings were identical for both sexes) and conspecific males and females treated as sister taxa. In all characters absence is scored as 0, presence is scored as 1.

Chemical characters

1) pentanol, 2) methyl-2-hexanol, 3) hexan-2-ol, 4) 4-methyl-3-hexanol/one, 5) heptan-2-ol, 6) heptan-2-one, 7) heptan-2-ol/one, 8) 4-methyl-3-heptanol, 9) 4-methyl-3-heptanone, 10) 4-methyl-3-heptanol/one, 11) 4-hepten-2-ol/one, 12) 3-hepten-2-one, 13) 6-methyl-5-hepten-2-ol, 14) hepten-2-ol/one / methyl-hepten-2-ol/one, 15) octan-2-ol, 16) octan-2-one, 17) octan-2-ol/one, 18) octan-3-ol/one, 19) 6-methyl-3-octanol, 20) 6-methyl-3-octanone, 21) 6-methyl-3-octanol/one, 22) (4S,6S)-dimethyl-3-octanone, 23) nonan-2-ol, 24) nonan-2-one, 25) nonan-2-ol/one, 26) 6-methyl-3-nonanol/one, 27) (4S,6S)-dimethyl-3-nonanone, 28) *Z*6-nonen-2-ol, 29) *Z*6-nonen-2-one, 30) *Z*6-nonen-2-ol/one, 31) *E*6-nonen-2-ol/one, 32) nonen/nonadien-2-ol/one, 33) *Z*4-nonen-2-one, 34) 3-nonen-2-one, 35) nonanal, 36) acetic acid, 37) propanoic/methylpropanoic acid, 38) butanoic/methylbutanoic acid, 39) hexanoic acid, 40) octanoic acid, 41) organic acids, 42) acetophenone, 43) acetophenone + derivatives, 44) decan-2-ol/one, 45) undecan-2-ol/one, 46) phenylethanol, 47) 1,8-cineol, 48) 3-hydroxy-2-butanon, 49) 7-episordidin, 50) 2,3-butandiol, 51) heptan-2-ol/one / nonan-2-ol/one, 52) methylated 3-ketones and corresponding alcohols

Chemical matrix

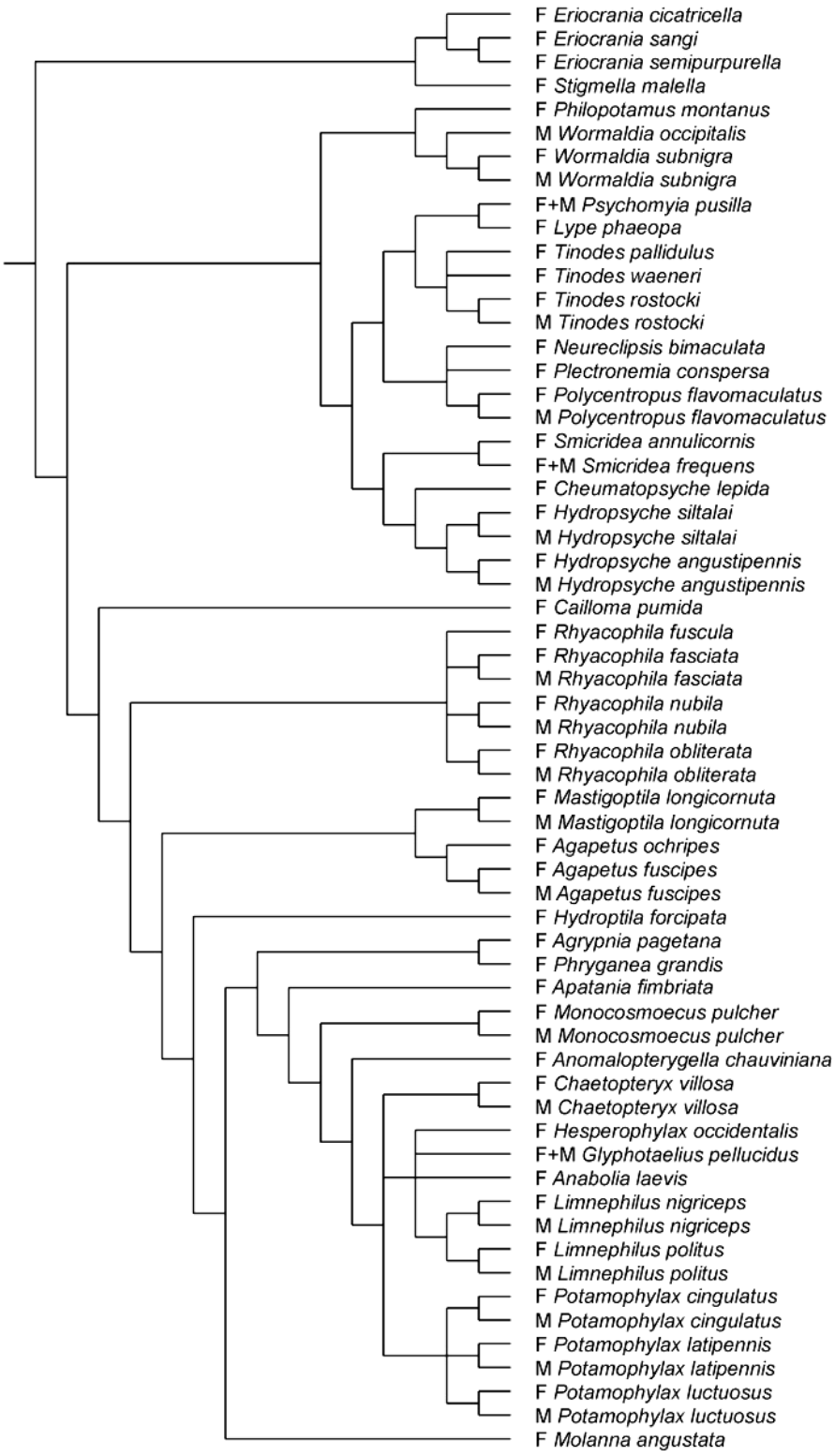
Matrix with the characters listed above (Chemical characters). Males and females are treated as separate taxa, except in cases in which scorings were identical for both sexes (see Materials and Methods). Absence is scored as 0 while presence is scored as 1, the scoring 'A' equals 0/1. F, female; M, male; F+M, female and male.

Characters	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
F *Eriocrania cicatricella*	0 0 0 0 1 0 1 0 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Eriocrania sangii*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 1 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Eriocrania semipurpurella*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
F *Stigmella malella*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
F *Philopotamus montanus*	0 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 1 1 0 0 1 1 1 1 1 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0
M *Wormaldia occipitalis*	0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 1 0 0 0 0 0 0 0 0 1 0
F *Wormaldia subnigra*	0 0 0 0 1 1 1 0 0 0 1 1 0 1 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0
M *Wormaldia subnigra*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0
F *Lype phaeopa*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 1 1 1 1 0 1 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0
F+M *Psychomyia pusilla*	0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Tinodes pallidulus*	0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Tinodes rostocki*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
M *Tinodes rostocki*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Tinodes waeneri*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Neureclipsis bimaculata*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 1 1 0 1 0 0 1 1 1 1 1 0 0 1 1 1 0 0 0 0 0 0 1 0
F *Plectronemia conspersa*	0 0 0 0 1 0 1 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 0 0 1 0 0 1 0 0 1 1 1 0 0 1 0 0 0 0 1 0
F *Polycentropus flavomaculatus*	0 0 0 0 1 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 1 0 1 1 1 0 0 0 0 0 1 0
M *Polycentropus flavomaculatus*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 1 0 1 0 0 0 0 0 0 1 0
F *Cheumatopsyche lepida*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 1 0
F *Hydropsyche angustipennis*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
M *Hydropsyche angustipennis*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 1 1 0 0 0 0 0 1 1 1 0 0 0 1 1 0 1 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 1 0
F *Hydropsyche siltalai*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 1 0 0 0 1 0 0 0 0 1 0
M *Hydropsyche siltalai*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 1 1 0 1 0 0 0 0 1 0 1 0 0 1 0 0 0 1 0 0 0 0 1 0
F *Smicridea annulicornis*	1 0 0 0 1 1 1 0 0 0 1 1 0 1 0 1 1 0 0 0 0 0 1 1 1 0 0 0 A A A 1 0 0 0 1 0 1 0 0 1 1 1 0 0 0 0 0 0 1 0
F+M *Smicridea frequens*	0 0 0 0 0 1 1 0 0 0 0 0 0 0 1 0 1 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1 0
F *Cailloma pumida*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
F *Rhyacophila fasciata*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
M *Rhyacophila fasciata*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 0 0 0 0 0 0 0
F *Rhyacophila fuscula*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Rhyacophila nubila*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
M *Rhyacophila nubila*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0
F *Rhyacophila obliterata*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
M *Rhyacophila obliterata*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0
F *Agapetus fuscipes*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 1 1 1 0 0 1 1 0 1 0 0 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0
M *Agapetus fuscipes*	0 0 0 0 1 0 1 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 1 1 1 0 0 1 1 1 0 1 0 0 0 1 1 0 0 0 1 0 0 1 1 0 0 0 0 0 1 0
F *Agapetus ochripes*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 1 1 0 0 0 1 1 0 1 0 0 0 1 0 0 0 0 1 0 0 0 1 0 0 0 0 0 1 0
F *Mastigoptila longicornuta*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
M *Mastigoptila longicornuta*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Hydroptila forcipata*	0 0 0 0 0 1 1 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Agrypnia pagetana*	0 0 0 0 1 0 1 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 1 0 1 0 0 1 1 1 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0
F *Phryganea grandis*	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0
F *Apatania fimbriata*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 1

F *Monocosmoecus pulcher*	0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 1 0 0
M *Monocosmoecus pulcher*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 1 0 0
F *Anomalopterygella chauviniana*	0 0 0 1 0 0 0 1 0 1 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
F *Anabolia laevis*	0 0 0 1 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 A A A A 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1
F *Chaetopteryx villosa*	0 0 0 1 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
M *Chaetopteryx villosa*	0 0 0 1 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
F+M *Glyphotaelius pellucidus*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
F *Hesperophylax occidentalis*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
F *Limnephilus nigriceps*	0 0 0 1 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1
M *Limnephilus nigriceps*	0 0 0 1 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
F *Limnephilus politus*	0 0 0 1 0 0 0 1 1 1 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
M *Limnephilus politus*	0 0 0 1 0 0 0 1 1 1 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 1
F *Potamophylax cingulatus*	0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1
M *Potamophylax cingulatus*	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0
F *Potamophylax latipennis*	0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1
M *Potamophylax latipennis*	0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1
F *Potamophylax luctuosus*	0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 1
M *Potamophylax luctuosus*	0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 0 1
F *Molanna angustata*	0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0

Chemical tree

Tree used for mapping of chemical characters and correlation analyses between chemical characters. Males and females are treated as separate taxa (except in cases in which scorings were identical for both sexes) with conspecific males and females treated as sister taxa. See Materials and Methods for origin of tree and treatment of males and females. F, female; M, male; F+M, female and male.


F Eriocrania cicatricella
F Eriocrania sangi
F Eriocrania semipurpurella
F Stigmella malella
F Philopotamus montanus
M Wormaldia occipitalis
F Wormaldia subnigra
M Wormaldia subnigra
F+M Psychomyia pusilla
F Lype phaeopa
F Tinodes pallidulus
F Tinodes waeneri
F Tinodes rostocki
M Tinodes rostocki
F Neureclipsis bimaculata
F Plectronemia conspersa
F Polycentropus flavomaculatus
M Polycentropus flavomaculatus
F Smicridea annulicornis
F+M Smicridea frequens
F Cheumatopsyche lepida
F Hydropsyche siltalai
M Hydropsyche siltalai
F Hydropsyche angustipennis
M Hydropsyche angustipennis
F Cailloma pumida
F Rhyacophila fuscula
F Rhyacophila fasciata
M Rhyacophila fasciata
F Rhyacophila nubila
M Rhyacophila nubila
F Rhyacophila obliterata
M Rhyacophila obliterata
F Mastigoptila longicornuta
M Mastigoptila longicornuta
F Agapetus ochripes
F Agapetus fuscipes
M Agapetus fuscipes
F Hydroptila forcipata
F Agrypnia pagetana
F Phryganea grandis
F Apatania fimbriata
F Monocosmoecus pulcher
M Monocosmoecus pulcher
F Anomalopterygella chauviniana
F Chaetopteryx villosa
M Chaetopteryx villosa
F Hesperophylax occidentalis
F+M Glyphotaelius pellucidus
F Anabolia laevis
F Limnephilus nigriceps
M Limnephilus nigriceps
F Limnephilus politus
M Limnephilus politus
F Potamophylax cingulatus
M Potamophylax cingulatus
F Potamophylax latipennis
M Potamophylax latipennis
F Potamophylax luctuosus
M Potamophylax luctuosus
F Molanna angustata

MORPHOLOGICAL MATRIX AND TREE

Morphological matrix with both sexes included with each sex scored separately (except in cases in which scorings were identical for both sexes) and conspecific males and females treated as sister taxa. The first character number is the number used in this matrix, the second number refers to the character numbers used in Chapter 2 (e.g. 2-4 corresponds to character 4 in Chapter 2). In all characters in the present chapter absence is scored as 0, presence is scored as 1, except in character 25. In some cases missing data (opening muscles, perforated patches on SIV, arrangement of secretory cells) was filled in based on expected character state as these characters were non-labile. Otherwise missing data was treated as absence of the character in question (e.g. presence/absence of reservoir musculature).

Morphological characters

Character 1 corresponds to character 2-4 (Cuticular modifications with grooves demarcating borders between individual epidermal cells, binary) in Chapter 2.
Characters 2-4 corresponds to character 2-5 (Cuticular modifications with grooves demarcating borders between individual epidermal cells, multistate) in Chapter 2. Character 2 corresponds to state 1 (scaly patch), character 3 to state 2 (polygons on protuberance) and character 4 to state 3 (hexagonal cuticle).
Character 5 corresponds to character 2-6 (Bald area extending from front edge of sternite and encompassing gland opening) in Chapter 2.
Character 6 corresponds to character 2-7 (Internal cuticular ridges extending from antecosta on both sides of gland opening and connecting in a smooth curve posterior to gland opening) in Chapter 2.
Character 7 corresponds to character 2-8 (Presence/absence of protuberance) in Chapter 2, but in this case scored specifically for each sex.
Character 8 corresponds to character 2-11 (Protuberance with groove extending at least from gland opening to apex with wavy cuticle along groove) in Chapter 2.
Character 9 corresponds to character 2-12 (Protuberance with groove extending at least from gland opening to apex, protuberance covered with polygons separated

by grooves) in Chapter 2.

Character 10 (Protuberance with groove extending at least from gland opening to apex) is a concatenation of characters 8 and 9.

Character 11 corresponds to character 2-13 (Protuberance/bulge with setae) in Chapter 2.

Character 12 corresponds to character 2-14 (Short protuberance with elongated setae) in Chapter 2.

Character 13 (enlarged evaporatory surface) concatenates characters 1, 8, 12 and add additional scorings.

Characters 14 and 15 correspond to character 2-15 (Opening muscles) in Chapter 2. Character 14 corresponds to state 1 ([Opening muscles] originating on anterior edge of sternum VI), character 15 to state 2 ([Opening muscles] originating mesad on cuticle of sternum V).

Characters 16-20 corresponds to character 2-16 (Gland reservoir shape) in Chapter 2. Character 16 corresponds to state 2 ([Gland reservoir] periform), character 17 to state 3 ([Gland reservoir] ovoid or round), character 19 to state 1 ([Gland reservoir] reniform), character 20 to state 4 ([Gland reservoir] compartmentalized). Character 18 is a concatenation of characters 16 and 17 (Gland reservoir periform, ovoid or round).

Characters 21-22 correspond to character 2-17 (Perforated patches on sternum IV in females) in Chapter 2. Character 21 corresponds to character 2-17 scored as presence/absence, character 22 corresponds to state 2 ([Perforated patches on sternum IV in females] present and associated with separate fenestral glands).

Character 23 corresponds to character 2-18 (Gland reservoir musculature) in Chapter 2.

Character 24 corresponds to character 2-19 (Parallel gland reservoir musculature fibres) in Chapter 2.

Character 25 corresponds to character 2-20 (Arrangement of secretory cells) in Chapter 2. 0: immediately adjacent to reservoir; 1: separate from reservoir.

Morphological matrix

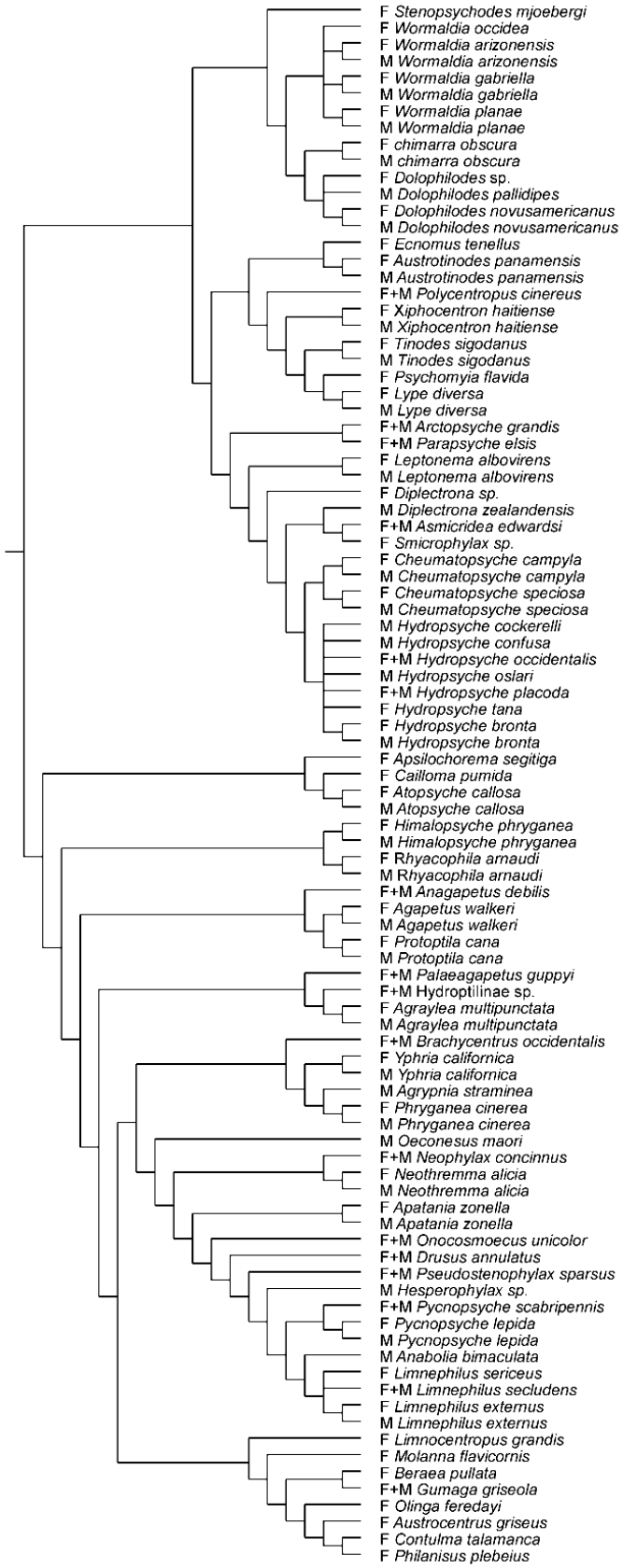
Matrix with the characters listed above (Morphological characters). Males and females are treated as separate taxa, except in cases in which scorings were identical for both sexes (see Materials and Methods). Absence is scored as 0 while presence is scored as 1. F, female; M, male; F+M, female and male.

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
F *Stenopsychodes mjoebergi*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
F *Chimarra obscura*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	1	0
M *Chimarra obscura*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0
F *Dolophilodes* sp.	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	1	1	0	0
F *Dolophilodes novusamericanus*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	1	1	0	0
M *Dolophilodes novusamericanus*	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0
M *Dolophilodes pallidipes*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0
F *Wormaldia arizonensis*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0
M *Wormaldia arizonensis*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	0
F *Wormaldia gabriella*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	1	1	0	0
M *Wormaldia gabriella*	1	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0
F *Wormaldia planae*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0
M *Wormaldia planae*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	1	0
F *Wormaldia occidea*	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	1	0	0	0
F *Austrotinodes panamensis*	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0
M *Austrotinodes panamensis*	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
F *Ecnomus tenellus*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0
F *Xiphocentron haitiense*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0
M *Xiphocentron haitiense*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	0
F *Lype diversa*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0
M *Lype diversa*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
F *Psychomyia flavida*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0
F *Tinodes sigodanus*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0
M *Tinodes sigodanus*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0
F+M *Polycentropus cinereus*	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	1
F+M *Arctopsyche grandis*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0
F+M *Parapsyche elsis*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
F *Diplectrona* sp.	1	0	1	0	0	0	1	0	1	1	0	0	1	0	1	0	1	1	0	0	0	0	1	0	1
M *Diplectrona_zealandensis*	1	0	1	0	0	0	1	0	1	1	0	0	1	0	1	1	0	1	0	0	0	0	1	0	1
F *Cheumatopsyche campyla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0
M *Cheumatopsyche campyla*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0
F *Cheumatopsyche speciosa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0
M *Cheumatopsyche speciosa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0
F *Hydropsyche bronta*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0
M *Hydropsyche bronta*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
M *Hydropsyche cockerelli*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
M *Hydropsyche confusa*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
F+M *Hydropsyche occidentalis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
M *Hydropsyche oslari*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
F+M *Hydropsyche placoda*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
F *Hydropsyche tana*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
F *Leptonema albovirens*	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	0
M *Leptonema albovirens*	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0
F+M *Asmicridea edwardsi*	1	0	1	0	0	0	1	0	1	1	0	0	1	0	1	1	0	1	0	0	0	0	1	1	1
F *Smicrophylax* sp.	1	0	1	0	0	0	1	0	1	1	0	0	1	0	1	1	0	1	0	0	0	0	1	1	1
F *Apsilochorema segitiga*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	1	1	0
F *Atopsyche callosa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	1	1	0
M *Atopsyche callosa*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0
F *Cailloma pumida*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0
F *Himalopsyche phryganea*	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
M *Himalopsyche phryganea*	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0
F *Rhyacophila arnaudi*	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0
M *Rhyacophila arnaudi*	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0
F *Agapetus walkeri*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	1	1	1
M *Agapetus walkeri*	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	1	1
F+M *Anagapetus debilis*	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0
F *Protoptila cana*	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
M *Protoptila cana*	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	1	0	0
F+M Hydroptilinae sp.	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0
F *Agraylea multipunctata*	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0
M *Agraylea multipunctata*	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	1	1	0	0	0	0	1	0	0
F+M *Palaeagapetus guppyi*	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	1	0	0
F+M *Brachycentrus occidentalis*	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0
M *Agrypnia straminea*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0
F *Phryganea cinerea*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
M *Phryganea cinerea*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0
F *Yphria californica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
M *Yphria californica*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
M *Oeconesus maori*	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1	0
F+M *Neophylax concinnus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
F *Neothremma alicia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
M *Neothremma alicia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0
F *Apatania zonella*	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0
M *Apatania zonella*	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0
F+M *Onocosmoecus unicolor*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
F+M *Drusus annulatus*	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0
M *Anabolia bimaculata*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

M *Hesperophylax* sp.	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
F *Limnephilus externus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
M *Limnephilus externus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F+M *Limnephilus secludens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
F *Limnephilus sericeus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
F *Pycnopsyche lepida*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
M *Pycnopsyche lepida*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
F+M *Pycnopsyche scabripennis*	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
F+M *Pseudostenophylax sparsus*	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
F *Limnocentropus grandis*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F *Molanna flavicornis*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
F *Beraea pullata*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
F+M *Gumaga grisola*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0
F *Olinga feredayi*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0
F *Austrocentrus griseus*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0
F *Contulma talamanca*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0
F *Philanisus plebeius*	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0

Morphological tree

Tree used for correlation analyses between morphological characters. Males and females are treated as separate taxa (except in cases in which scorings were identical for both sexes) with conspecific males and females treated as sister taxa. See Materials and Methods for origin of tree and treatment of males and females. F, female; M, male; F+M, female and male.


F Stenopsychodes mjoebergi
F Wormaldia occidea
F Wormaldia arizonensis
M Wormaldia arizonensis
F Wormaldia gabriella
M Wormaldia gabriella
F Wormaldia planae
M Wormaldia planae
F chimarra obscura
M chimarra obscura
F Dolophilodes sp.
M Dolophilodes pallidipes
F Dolophilodes novusamericanus
M Dolophilodes novusamericanus
F Ecnomus tenellus
F Austrotinodes panamensis
M Austrotinodes panamensis
F+M Polycentropus cinereus
F Xiphocentron haitiense
M Xiphocentron haitiense
F Tinodes sigodanus
M Tinodes sigodanus
F Psychomyia flavida
F Lype diversa
M Lype diversa
F+M Arctopsyche grandis
F+M Parapsyche elsis
F Leptonema albovirens
M Leptonema albovirens
F Diplectrona sp.
M Diplectrona zealandensis
F+M Asmicridea edwardsi
F Smicrophylax sp.
F Cheumatopsyche campyla
M Cheumatopsyche campyla
F Cheumatopsyche speciosa
M Cheumatopsyche speciosa
M Hydropsyche cockerelli
M Hydropsyche confusa
F+M Hydropsyche occidentalis
M Hydropsyche oslari
F+M Hydropsyche placoda
F Hydropsyche tana
F Hydropsyche bronta
M Hydropsyche bronta
F Apsilochorema segitiga
F Cailloma pumida
F Atopsyche callosa
M Atopsyche callosa
F Himalopsyche phryganea
M Himalopsyche phryganea
F Rhyacophila arnaudi
M Rhyacophila arnaudi
F+M Anagapetus debilis
F Agapetus walkeri
M Agapetus walkeri
F Protoptila cana
M Protoptila cana
F+M Palaeagapetus guppyi
F+M Hydroptilinae sp.
F Agraylea multipunctata
M Agraylea multipunctata
F+M Brachycentrus occidentalis
F Yphria californica
M Yphria californica
M Agrypnia straminea
F Phryganea cinerea
M Phryganea cinerea
M Oeconesus maori
F+M Neophylax concinnus
F Neothremma alicia
M Neothremma alicia
F Apatania zonella
M Apatania zonella
F+M Onocosmoecus unicolor
F+M Drusus annulatus
F+M Pseudostenophylax sparsus
M Hesperophylax sp.
F+M Pycnopsyche scabripennis
F Pycnopsyche lepida
M Pycnopsyche lepida
M Anabolia bimaculata
F Limnephilus sericeus
F+M Limnephilus secludens
F Limnephilus externus
M Limnephilus externus
F Limnocentropus grandis
F Molanna flavicornis
F Beraea pullata
F+M Gumaga griseola
F Olinga feredayi
F Austrocentrus griseus
F Contulma talamanca
F Philanisus plebeius

COMBINED CHEMICAL AND MORPHOLOGICAL MATRIX AND TREE

Concatenated matrix (gland compounds and morphology) with both sexes included with each sex scored separately (except in cases in which scorings were identical for both sexes) and conspecific males and females treated as sister taxa. For the morphological characters the first character number is the number used in this matix, the second number refers to the character numbers used in Chapter 2 (e.g. 2-4 corresponds to character 4 in Chapter 2). In all characters absence is scored as 0, presence is scored as 1, except in character 76. In some cases morphological missing data (opening muscles, perforated patches on SIV, arrangement of secretory cells) was filled in based on expected character state as these characters were non-labile. Otherwise missing data was treated as absence of the character in question (e.g. presence/absence of reservoir musculature).

Chemical characters

1) pentanol, 2) methyl-2-hexanol, 3) hexan-2-ol, 4) 4-methyl-3-hexanol/one, 5) heptan-2-ol, 6) heptan-2-one, 7) heptan-2-ol/one, 8) 4-methyl-3-heptanol, 9) 4-methyl-3-heptanone, 10) 4-methyl-3-heptanol/one, 11) 4-hepten-2-ol/one, 12) 3-hepten-2-one, 13) 6-methyl-5-hepten-2-ol, 15) octan-2-one, 16) octan-2-ol/one, 17) octan-3-ol/one, 18) 6-methyl-3-octanol, 19) 6-methyl-3-octanone, 20) 6-methyl-3-octanol/one, 21) (4S,6S)-dimethyl-3-octanone, 22) nonan-2-ol, 23) nonan-2-one, 24) nonan-2-ol/one, 25) 6-methyl-3-nonanol/one, 26) (4S,6S)-dimethyl-3-nonanone, 27) *Z*6-nonen-2-ol, 28) *Z*6-nonen-2-one, 29) *Z*6-nonen-2-ol/one, 30) *E*6-nonen-2-ol/one, 31) nonen/nonadien-2-ol/one, 32) *Z*4-nonen-2-one, 33) 3-nonen-2-one, 34) nonanal, 35) acetic acid, 36) propanoic/methylpropanoic acid, 37) butanoic/methylbutanoic acid, 38) hexanoic acid, 39) octanoic acid, 40) organic acids, 41) acetophenone, 42) acetophenone + derivatives, 43) decan-2-ol/one, 44) undecan-2-ol/one, 45) phenylethanol, 46) 1,8-cineol, 47) 3-hydroxy-2-butanon, 48) 7-episordidin, 49) 2,3-butandiol, 50) heptan-2-ol/one / nonan-2-ol/one, 51) methylated 3-ketones and corresponding alcohols

Character 52: empty character denoting border between chemical and morphological characters

Morphological characters

Character 53 corresponds to character 2-4 (Cuticular modifications with grooves demarcating borders between individual epidermal cells, binary) in Chapter 2.

Character 54-56 corresponds to character 2-5 (Cuticular modifications with grooves demarcating borders between individual epidermal cells, multistate) in Chapter 2. Character 54 corresponds to state 1 (scaly patch), character 55 to state 2 (polygons on protuberance) and character 56 to state 3 (hexagonal cuticle).

Character 57 corresponds to character 2-6 (Bald area extending from front edge of sternite and encompassing gland opening) in Chapter 2.

Character 58 corresponds to character 2-7 (Internal cuticular ridges extending from antecosta on both sides of gland opening and connecting in a smooth curve posterior to gland opening) in Chapter 2.

Character 59 corresponds to character 2-8 (Presence/absence of protuberance) in Chapter 2, but in this case scored specifically for each sex.

Character 60 corresponds to character 2-11 (Protuberance with groove extending at least from gland opening to apex with wavy cuticle along groove) in Chapter 2.

Character 61 corresponds to character 2-12 (Protuberance with groove extending at least from gland opening to apex, protuberance covered with polygons separated by grooves) in Chapter 2.

Character 62 (Protuberance with groove extending at least from gland opening to apex) is a concatenation of characters 60 and 61

Character 63 corresponds to character 2-13 (Protuberance/bulge with setae) in Chapter 2.

Character 64 corresponds to character 2-14 (Short protuberance with elongated setae) in Chapter 2.

Characters 65 and 66 correspond to character 2-15 (Opening muscles) in Chapter 2. Character 65 corresponds to state 1 ([Opening muscles] originating on anterior edge of sternum VI), character 66 to state 2 ([Opening muscles] originating mesad

on cuticle of sternum V).

Characters 67-70 correspond to character 2-16 (Gland reservoir shape) in Chapter 2. Character 67 corresponds to state 2 ([Gland reservoir] periform), character 68 to state 3 ([Gland reservoir] ovoid or round), character 70 to state 1 ([Gland reservoir] reniform). Character 69 is a concatenation of characters 67 and 68 (Gland reservoir periform, ovoid or round).

Characters 71-73 corresponds to character 2-17 (Perforated patches on sternum IV in females) in Chapter 2. Character 71 corresponds to character 17 scored as presence/absence, character 72 corresponds to state 1 ([Perforated patches on sternum IV in females] present and associated sternum V gland reservoir), character 73 to state 2 ([Perforated patches on sternum IV in females] present and associated with separate fenestral glands).

Character 74 corresponds to character 2-18 (Gland reservoir musculature) in Chapter 2.

Character 75 corresponds to character 2-19 (Parallel gland reservoir musculature fibres) in Chapter 2.

Character 76 corresponds to character 2-20 (Arrangement of secretory cells) in Chapter 2. 0: immediately adjacent to reservoir; 1: separate from reservoir

Chemical part of combined matrix

Matrix with the chemical characters (listed abover) of the combined data set. The combined matrix was created by concatenating the chemical and morphological data (see Materials and Methods for details). Males and females are treated as separate taxa, except in cases in which scorings were identical for both sexes (see Materials and Methods). Absence is scored as 0 while presence is scored as 1, the scoring ‘A’ equals 0/1. F, female; M, male; F+M, female and male.

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
F *Eriocrania cicatricella*	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
F *Eriocrania semipurpurella*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
F Nepticulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
F *Wormaldia*	0	0	0	0	1	1	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	?
M *Wormaldia*	0	A	0	0	0	A	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	A	1	0	0	1	0	0	0	0	0	0	0	0	A	0	?
F *Lype*	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	?
F *Psychomyia*	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
F *Tinodes*	0	A	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	A	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	?	?
M *Tinodes*	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
F *Polycentropus*	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	1	0	?
M *Polycentropus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	?
F Smicrideinae	1	0	0	0	A	1	1	0	0	0	A	A	0	A	A	1	0	0	0	0	0	0	A	1	1	0	0	0	A	A	A	A	0	0	0	A	0	A	0	0	A	A	A	0	A	0	0	0	0	0	1	0	?
M Smicrideinae	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	?
F *Cheumatopsyche*	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	?
F *Hydropsyche*	0	0	0	0	0	A	1	1	0	0	0	0	0	0	0	0	A	A	0	0	0	0	0	A	1	1	0	0	0	0	0	0	0	0	0	0	A	0	A	0	0	A	0	0	0	A	0	0	0	0	1	0	?
M *Hydropsyche*	0	0	0	0	0	A	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	0	1	0	0	0	A	0	A	0	0	A	0	0	A	1	0	0	0	0	1	0	?
F *Cailloma pumida*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?
F *Rhyacophila*	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
M *Rhyacophila*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	?
F *Agapetus*	0	0	0	0	A	A	A	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	A	1	1	0	0	A	1	1	0	1	0	0	A	1	A	0	0	0	1	0	0	A	1	0	0	0	0	0	1	0	?
M *Agapetus*	0	0	0	0	A	A	A	0	0	0	0	0	0	0	A	A	0	0	0	0	0	0	A	1	1	0	0	A	1	1	0	1	0	0	A	1	0	0	0	0	1	0	0	0	A	0	0	0	0	0	1	0	?
F Protoptilinae	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
M Protoptilinae	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
F Hydroptilinae	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?
F *Phryganea*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	?
F *Apatania*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	?
F Onocosmoecinae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	?
M Onocosmoecinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	?
F Drusinae	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	?
F *Limnephilus*	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	A	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A	0	0	0	1	?
M *Limnephilus*	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	A	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A	0	A	0	1	?
F *Molanna*	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	?

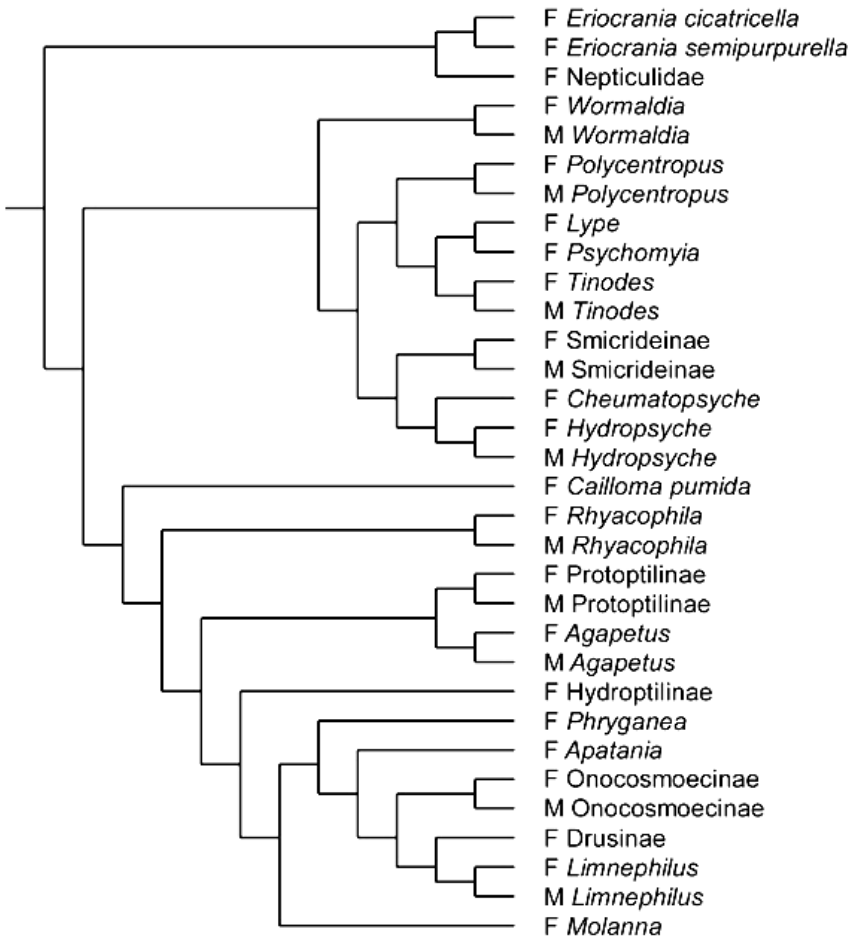
Morphological part of combined matrix

Matrix with the morphological characters (listed abover) of the combined data set. The combined matrix was created by concatenating the chemical and morphological data (see Materials and Methods for details). Males and females are treated as separate taxa, except in cases in which scorings were identical for both sexes (see Materials and Methods). Absence is scored as 0 while presence is scored as 1, the scoring 'A' equals 0/1. F, female; M, male; F+M, female and male.

	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7
Characters	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6
F *Eriocrania cicatricella*	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	1	0	1	0	0
F *Eriocrania semipurpurella*	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	1	1	0	1	0	0
F Nepticulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
F *Wormaldia*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1	0	0
M *Wormaldia*	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0
F *Lype*	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0
F *Psychomyia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0
F *Tinodes*	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0
M *Tinodes*	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
F *Polycentropus*	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	1
M *Polycentropus*	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	1
F Smicrideinae	1	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	1	0	0	0	0	1	1	1
M Smicrideinae	1	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	1	0	0	0	0	1	1	1
F *Cheumatopsyche*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	A	A	1	0	0	0	1	0	0
F *Hydropsyche*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
M *Hydropsyche*	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0
F *Cailloma pumida*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0
F *Rhyacophila*	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0
M *Rhyacophila*	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	1	1	0
F *Agapetus*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	1	1
M *Agapetus*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	1	1
F Protoptilinae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
M Protoptilinae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0
F Hydroptilinae	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0
F *Phryganea*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
F *Apatania*	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
F Onocosmoecinae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
M Onocosmoecinae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
F Drusinae	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
F *Limnephilus*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
M *Limnephilus*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
F *Molanna*	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0

Combined tree

Tree used for correlation analyses between chemical and morphological characters. The taxa in the tree are concatenated from the taxa in which chemical and morphological data were available (please refer to Materials and Methods for details). Males and females are treated as separate taxa (except in cases in which scorings were identical for both sexes) with conspecific males and females treated as sister taxa. See Materials and Methods for origin of tree and treatment of males and females. F, female; M, male; F+M, female and male.



Appendix 5

Supplemental information for Chapter 6

Raw data for gland size, size of secretory tissue and body size

Please refer to Materials and Methods for details about calculations. n.m. stands for not measurable, this was generally due to decomposition of the specimens.

Species	**Sex & structure**	**Gland**	**Gland**	**Body size**
		Reservoir (μm^2)	Secretory tissue (μm^2)	(Sternite lenght)^2 (μm^2)
Dolophilodes novusamericanus (Ling)	female, gland	8035.378	9754.575	321829.3
Dolophilodes novusamericanus	male, gland	3171.902	10382.35	267806.3
Dolophilodes sp.	female, gland	6312.028	11494.54	245025
Dolophilodes pallidipes Banks	male, gland	7773.761	10311.03	291600
Wormaldia arizonensis	female, gland	657.8614	1753.376	288691.3
Wormaldia arizonensis (Ling)	female, fenestra	19997.48	24639.19	288691.3
Wormaldia arizonensis	male, gland	3151.861	3243.22	126380.3
Wormaldia gabriella (Banks)	female, gland	7257.388	9501.263	179860.8
Wormaldia gabriella	male, gland	3824.771	2165.501	159680.2
Wormaldia occidea (Ross)	female, gland	6531.2	7134.08	147456
Wormaldia occidea	female, fenestra	4521.6	6405.6	147456
Wormaldia planae (Ross & King)	female, gland	6876.788	5830.321	116964
Wormaldia planae	male, gland	1565.549	5236.492	102080.3
Chimarra obscura (Walker)	female, gland	2375.316	4136.039	296480.3
Chimarra obscura	female, fenestra	3488.226	7076.116	296480.3
Chimarra obscura	male, gland	13604.08	n.m.	160400.3
Stenopsychodes mjobergi Ulmer	female, gland	82422.06	n.m.	2196324
Austrotinodes panamensis Flint	female, gland	2242.431	2363.941	82944
Austrotinodes panamensis	male, gland	2059.714	1229.184	67548.01
Ecnomus tenellus (Rambur)	female, gland	4372.74	n.m.	186624
Xiphocentron haitiense (Banks)	female, gland	3945.018	3156.014	113906.3
Xiphocentron haitiense	male, gland	717.1446	n.m.	93636
Tinodes sigodanus (Ross & Merkley)	female, gland	3010.671	4650.968	88209
Tinodes sigodanus	male, gland	1195.963	3662.637	61256.25
Psychomyia flavida Hagen	female, gland	2071.27	n.m.	54756
Polycentropus cinereus Hagen	female, gland	3340.96	8591.04	246016

Polycentropus cinereus	male, gland	3579.6	17521.2	214276.4
Arctopsyche grandis (Banks)	female, gland	38021	39650.48	1332639
Arctopsyche grandis	male, gland	11915.51	30790.22	732051.4
Parapsyche elsis Milne	female, gland	80117.1	23192.63	1971216
Parapsyche elsis	male, gland	9537.75	20347.2	917572.4
Cheumatopsyche campyla Ross	female, gland	9301.936	7424.938	77841
Cheumatopsyche campyla	male, gland	1928.274	4103.368	51438.24
Cheumatopsyche speciosa (Banks)	female, gland	4273.077	8907.434	107912.3
Cheumatopsyche speciosa	male, gland	2649.391	2333.789	56882.25
Hydropsyche bronta Ross	female, gland	2204.688	n.m.	279946.8
Hydropsyche bronta	male, gland	38684.8	38056.8	206752.1
Hydropsyche confusa (Walker)	male, gland	17305.29	18261.61	255934.8
Hydropsyche occidentalis Banks	female, gland	2677.258	n.m.	207936
Hydropsyche occidentalis	male, gland	35607.6	37833.08	254016
Hydropsyche placoda Ross	female, gland	6021.343	8230.552	175142.3
Hydropsyche placoda	male, gland	32180.6	31588.35	237851.3
Hydropsyche cockerelli Banks	male, gland	39582.58	51667.83	311364
Hydropsyche oslari Banks	male, gland	81269.9	n.m.	423801
Hydropsyche tana Ross	female, gland	9447.279	1453.428	286760.3
Leptonema albovirens (Walker)	female, gland	48324.6	24843.93	720122
Leptonema albovirens	male, gland	500545.6	n.m.	669778.6
Apsilochorema segitiga Weaver & Huisman	female, gland	15626.65	n.m.	345508.8
Atopsyche callosa Navas	female, gland	10326.2	5758.258	336748.1
Atopsyche callosa	male, gland	2975.464	1171.515	145618.6
Cailloma pumida Ross	female, gland	13571.58	9319.018	719443.2
Himalopsyche phryganea (Ross)	female, gland	49881.39	94890.63	3040838
Himalopsyche phryganea	male, gland	162670.6	n.m.	1206043
Rhyacophila arnaudi Denning	female, gland	45901.81	33897.68	694388.9
Rhyacophila arnaudi	male, gland	24904.97	n.m.	294957.6
Agapetus walkeri (Betten & Mosely)	female, gland	1627.289	4044.87	58177.44
Agapetus walkeri	male, gland	5208.883	n.m.	72900
Anagapetus debilis (Ross)	female, gland	22158.48	31725.05	179860.8
Anagapetus debilis	male, gland	2472.185	11282.52	72900
Protoptila cana Flint	female, gland	1535.052	5562.73	49818.24
Protoptila cana	male, gland	2847.721	4749.478	58177.44
Hydroptilinae sp.	female, gland	2547.922	4867.455	61702.56
Hydroptilinae sp.	male, gland	1225.746	3397.778	46656
Agraylea multipunctata Curtis	female, gland	1915.274	5926.546	159680.2
Agraylea multipunctata	male, gland	1943.527	4289.782	72900
Palaeagapetus guppyi Schmid	female, gland	9827.698	n.m.	72900
Brachycentrus occidentalis Banks	female, gland	4595.908	12627.67	278995.2
Brachycentrus occidentalis	male, gland	7706.424	18404.54	263579.6
Agrypnia straminea Hagen	male, gland	29757.78	47704.01	899652.3
Phryganea cinerea Walker	female, gland	169473.8	114345.2	3669906
Phryganea cinerea	male, gland	69349.84	57178.45	2171497
Yphria californica (Banks)	female, gland	57976.48	36927.91	947507.6
Yphria californica	male, gland	42322.18	15392.66	621890
Oeconesus maori McLachlan	male, gland	56341.4	43207.28	598766.4

Neophylax concinnus	female, gland	9115.546	25515.39	311364
Neophylax concinnus McLachlan	male, gland	4517.078	10865.4	233869
Neothremma alicia Dodds & Hisaw	female, gland	1626.567	n.m.	89281.44
Neothremma alicia	male, gland	745.3104	1484.843	82944
Apatania zonella (Zetterstedt)	female, gland	4224.87	3590.778	168428.2
Apatania zonella	male, gland	3060.856	n.m.	186192.3
Onocosmoecus unicolor (Banks)	female, gland	123917.6	24550.12	2470555
Onocosmoecus unicolor	male, gland	47042.73	20530.32	1684544
Drusus annulatus (Stephens)	female, gland	9827.698	4181.35	598766.4
Drusus annulatus	male, gland	5697.216	3479.371	428632.1
Anabolia bimaculata (Walker)	male, gland	9970.128	24640.46	947507.6
Hesperophylax sp.	male, gland	19472.27	54998.48	2059799
Limnephilus externus Hagen	female, gland	7833.672	46218.66	1495729
Limnephilus externus	male, gland	4069.44	25484.87	1233654
Limnephilus secludens Banks	female, gland	60827.77	30944.31	971998.8
Limnephilus secludens	male, gland	82452.9	23951.24	598766.4
Limnephilus sericeus (Say)	female, gland	67227.79	40323.17	947507.6
Pycnopsyche lepida (Hagen)	female, gland	19940.26	46473	720122
Pycnopsyche lepida	male, gland	45613.52	55677.47	311364
Pycnopsyche scabripennis (Rambur)	female, gland	47959.26	63821.21	852852.3
Pycnopsyche scabripennis	male, gland	179943.1	97874.93	678481.7
Pseudostenophylax sparsus (Banks)	female, gland	6765.444	35556.73	947507.6
Pseudostenophylax sparsus	male, gland	9614.052	65385.73	763177
Limnocentropus grandis Banks	female, gland	6836.659	17030.61	553536
Molanna flavicornis Banks	female, gland	25342.44	23053.38	354263
Beraea pullata (Curtis)	female, gland	2531.744	2252.108	87143.04
Gumaga griseola (McLachlan)	female, gland	3986.544	7564.323	372222
Gumaga griseola	male, gland	3705.231	11359.86	328214.4
Olinga feredayi (McLachlan)	female, gland	17457.9	n.m.	381306.3
Austrocentrus griseus Schmid	female, gland	1990.211	1921.397	153977.8
Contulma talamanca Holzenthal & Flint	female, gland	4385.198	7268.51	51438.24
Philanisus plebeius Walker	female, gland	1784.729	1686.164	142884