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The male genital tract and aedeagal components of the Diptera with a discussion of their phylogenetic significance

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The male genital tract of Diptera is surveyed, based on whole dissections, with emphasis on nematocerous infraorders and especially the Culicomorpha. The genital tracts of all available nematocerous families are described and illustrated, including, for the first time, those of the families Thaumaleidae, Dixidae, Hesperinidae, and Pachyneuridae, and, as an outgroup, the mecopteran family Nannochoristidae (Mecoptera). On the basis of outgroup comparison, the Diptera + Mecoptera + Siphonaptera share the synapomorphies of accessory gland continuous with the vas deferens and U-shaped vasa deferentia. Further synapomorphies of the male genital tract provide evidence of the monophyly of the Mecoptera + Siphonaptera, Boreidae + Siphonaptera, and the Blephariceridae + Psychodidae + Trichoceridae + Anisopodidae + Brachycera. The Bibionomorpha, Culicomorpha, and Ptychopteromorpha share the synapomorphy of paired, two- to three-chambered accessory gland complexes, with secondary losses in Corethrellidae + Culicidae. It is concluded that there is no convincing evidence for the monophyly of the Chironomoidea. A sperm pump with an ejaculatory apodeme and the absence of a spermatophore are considered to be part of the ground plan of Diptera, but, because of uncertainty as to the identity of the basal lineage within Diptera and the homology of the sperm pump outside of Diptera, the polarity of these features cannot be presently interpreted. © 2007 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2007, **150**, 711–742.

ADDITIONAL KEYWORDS: Nematocera – male accessory gland – Mecoptera – phylogeny – seminal vesicle – Siphonaptera – spermatophore – Strepsiptera.

INTRODUCTION

The major groups of Diptera have a long history of phylogenetic study, especially in the nematocerous lineages or lower Diptera [see the review in Wood & Borkent (1989)]. The group is an excellent candidate for phylogenetic study because of the wealth of diverse features in each of its life stages, coupled with a remarkable fossil record. However, the component families within the major lineages and the relationships among the infraorders remain disputed (cf. Hennig, 1973; Wood & Borkent, 1989; Oosterbroek & Courtney, 1995; Michelsen, 1996; Krzemiński & Evenhuis, 2000; Saether, 2000). There remains a great need for detailed comparative morphological studies in the search for additional characters to test current phylogenies [as is well illustrated by Michelsen (1996)]. Here we report our investigation of a novel character system to partially address this need.

The internal male genital tract in Diptera is generally poorly known, with only a few detailed comparative studies (Loew, 1841; Dufour, 1851; Keuchenius, 1913), but none of these has addressed or searched for character states to interpret the phylogenetic relationships between families. In fact, no such approach is available for any insect order that we know of. Our study began with an examination of some Chironomoidea (*sensu* Wood & Borkent, 1989: Thaumaleidae, Simuliidae, Ceratopogonidae, and Chironomidae), in which the male genital tract is modified with the so-called accessory glands fused medially and each

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divided longitudinally into several chambers. The fused glands produce a preformed spermatophore containing two masses of sperm (Linley, 1981). In association with this internal modification are the presence of a membranous aedeagus and the absence of an ejaculatory apodeme and sperm pump (Wood, 1991). However, the condition in the Thaumaleidae, a member of the Chironomoidea, and several outgroup families was poorly or incompletely known, thus hampering further phylogenetic analyses. Our investigation of features of the Dixidae, a member of the Culicomorpha (to which the Chironomoidea belong), and families in other infraorders demanded a reappraisal of some character states.

This study grew from an investigation of the configuration of the genital tract in Thaumaleidae and its implications for our understanding of the phylogeny of the Culicomorpha (Sinclair, 1992a; Sinclair, Borkent & Wood, 1998). As we came to understand these structures, we studied additional taxa in our attempt to better understand the distribution of character states. As a result we describe and illustrate the male genital tract of all available families of nematocerous Diptera and some Brachycera, as well as pertinent outgroups of the Diptera, either from original dissections or from the literature. The dipteran families Thaumaleidae, Dixidae, Hesperinidae, and Pachyneuridae, and the mecopteran family Nannochoristidae are described here for the first time. The phylogenetic implications of the modifications of the male genital tract within these taxa are discussed below.

MATERIAL AND METHODS

The specimens examined for this study are listed in Table 1 and our analysis follows the classification and phylogenetic sequence of the nematocerous Diptera of Wood & Borkent (1989). Consequently, the families (except Hesperinidae), superfamilies, and infraorders recognized here are *sensu* Wood & Borkent (1989). The intention of this work is a morphological survey of the internal male genital tract and a search for phylogenetic informative characters. At this preliminary stage, we consider a detailed description of character states as an important component of determining which states are most indicative of phylogenetic relationships. An analysis using a total evidence approach was deemed beyond the scope of this project and such analyses are currently being conducted (see: http://www.inhs.uiuc.edu/cee/FLYTREE/). A character state

Table 1. Taxa examined in this study (arranged in alphabetical order by family)

Order/family	Taxon	Locality
Mecoptera		
Choristidae	*Chorista australis* Klug	NSW, Australia
Nannochoristidae	*Nannochorista maculipennis* Tillyard	Tasmania, Australia
Panorpidae	*Panorpa japonica* Thunberg	Honshu, Japan
Diptera		
Blephariceridae	*Philorus vividis* Kitakami	Kyushu, Japan
Bibionidae	*Bibio flavihalter* Hardy & Takahashi	Honshu, Japan
	Penthetria funebris Meigen	Bonn, Germany
	Plecia membranifera Hardy & Takahashi	Kyushu, Japan
Chironomidae	*Ablabesmyia monilis* (L.)	Kyushu, Japan
	Chironomus plumosus (L.)	London, UK
Dixidae	*Dixa rhathyme* Dyar & Shannon	British Columbia, Canada
	Dixa yamatoma Takahashi	Kyushu, Japan
	Dixella nicholsoni Tonnoir	Tasmania, Australia
	Dixella nova (Walker)	Ontario, Canada
Hesperinidae	*Hesperinus rohdendorfi* Krivosheina & Mamajev	Hokkaido, Japan
Pachyneuridae	*Pachyneura fasciata* Zetterstedt	Hokkaido, Japan
Ptychopteridae	*Ptychoptera contaminata* (L.)	Bonn, Germany
	Ptychoptera nr *japonica* Alexander	Kyushu, Japan
Rhagionidae	*Rhagio gracilis* (Johnson)	West Virginia, USA
Scatopsidae	*Scatopse chinensis* Cook	Lishan, Taiwan
Simuliidae	*Austrosimulium furiosum* group	Tasmania, Australia
Thaumaleidae	*Androprosopa americana* (Bezzi)	Ontario, Canada
	Androprosopa japonica (Okada)	Kyushu, Japan
	Austrothaumalea zentae Theischinger	NSW, Australia
Trichoceridae	*Trichocera* sp.	Honshu, Japan

matrix (Table 2) is included to aid the workers of these future analyses. The hand-generated Figure 9 uses the infraorders *sensu* Wood & Borkent (1989) as a basis for summarizing the distributions of the new characters described, otherwise all relationships are based on the characters presented.

Dissections were most easily performed on fresh material, although even specimens preserved in alcohol for 40 years could be dissected with limited success. Abdomens were removed and transferred to glycerol, where dissections were conducted in depression slides. Sclerites and pleural membranes were carefully peeled and removed, exposing the internal organs. To facilitate observation, dissections were conducted with a black background using reflected light. Digestive and reproductive tracts were identified and teased apart, starting from the anterior end of the abdomen. Details of the genital tract were observed and drawn using a compound microscope and a drawing tube.

Table 2. Character state matrix for the analysis. Inapplicable characters are indicated by '–' and missing data or scoring uncertainty are indicated by '?'

	1	2	3	4	5	6	7	8	9
Panorpidae	1	1	1	0	0	0	0	0	0
Boreidae	1	0	0	1	0	–	1	0	0
Nannochoristidae	1	1	1	0	?	–	1	0	0
Siphonaptera	1	0	1	0	0	0	0	0	0
Tipulidae	0/1	0	0	0/1	0	0	0	0	0
Hesperinidae	1	1	0	0	1	1	0	0	0
Pachyneuridae	1	1	0	0	1	1	0	0	0
Bibionidae	1	1	0	0	1	1	0	0	0
Blephariceridae	0	0	0	1	0	0	0	0	0
Trichoceridae	1?	0	0	1	0	0	0	0	0
Psychodidae	0	0/1	0	1	0	0	0	0	0
Anisopodidae	1?	1	0	1	0	0	0	0	0
Scatopsidae	0	0	0	1	0	0	0	0	0
Ptychopteridae	1	1	0	0	1	0	0	0	0
Thaumaleidae	1	1	0	0	1	–	1	1	1
Simuliidae	1	1	0	0	1	–	1	1	1
Chironomidae	1	1	0	0	1	–	1	1	1?
Ceratopogonidae	1	1	0	0	1	–	1	1	1
Dixidae	1	1	0	0	1	–	1	1	1
Corethrellidae	0	0	0	1	0	–	1	1	0
Culicidae	0	0	0	1	0	–	1	1	1
Rhagionidae	1	1	0	0	0	0	0	0	0
Tabanidae	0	1	0	1	0	0	0	0	0
Remaining Brachycera	0	0	0	1	0	0	0	0	0

Voucher specimens, either the remaining portion of the specimen or undissected males collected from the same swarm or seepage face (in the case of Thaumaleidae), are deposited in the Canadian National Collection of Insects, Ottawa. All material is stored separately in at least 75% ethanol. The terms for male genitalic structures follow those of Wood (1991) and Sinclair (2000). The nomenclature through the families and pertinent orders was compared and homologized. For example, the large, multichambered male genital gland of some nematocerous Diptera has been identified by various names, such as the accessory gland (Linley, 1981), ejaculatory duct (Wensler & Rempel, 1962) or seminal vesicle (Crosskey, 1990). In order to avoid confusion and to make comparison possible throughout orders, we use the term accessory gland for those glands with thickened walls and inner secretory cells. Enlarged ducts with no regard to cell wall structure are often labelled seminal vesicles, but we restrict seminal vesicles to only thin-walled inflated sacs or ducts where spermatozoa are stored. In several lineages the accessory gland is divided into several chambers and appears to perform different functions, including acting as a seminal vesicle and producing secretions. We therefore use the term accessory gland complex to distinguish it from simple accessory glands lacking internal divisions. Definitions of the major components of the male genital tract are defined in Table 3. The testes were in various states of

Table 3. Components of the male genital tract [definitions modified from Snodgrass (1935)]

Accessory gland	Thick-walled secretory gland, opening into the anterior portion of the ejaculatory duct and presumably providing secretions to facilitate the transfer of sperm to the female.
Ejaculatory duct (ductus ejaculatorius)	Median ectodermal exit tube.
Epididymis	Coiled or convoluted part of the vas deferens at the posterior part of the testis.
Seminal vesicle (vesicula seminalis)	Enlarged vas deferens serving as a spermatozoa reservoir.
Sperm pump	Muscular modified basal portion of the endophallus, containing an ejaculatory apodeme (Diptera).
Testis	Paired male reproductive glands, containing male germ cells that develop into spermatozoa.
Vas deferens	Duct leading from the testis to the ejaculatory duct.

maturity in the material examined and if internal divisions were visible this was stated and illustrated. In addition, pigment granules or fat bodies sometimes encased the organs, but their presence was also probably due to the relative maturity of the material.

COMPARATIVE SURVEY

DIPTERAN OUTGROUPS

In our survey of the structures of early lineages of extant Diptera, a review of dipteran outgroups is required in order to permit accurate polarity decisions. The Mecoptera and Siphonaptera are usually considered the outgroups of Diptera, and the three orders together comprise the Antliophora, named by Hennig (1981) for the shared presence of a sperm pump. Wood & Borkent (1989) tentatively listed it as a synapomorphy of all three orders, but noted the lack of comparative studies. For this reason, the presence of a sperm pump has been omitted from several reviews of the higher classification of the Insecta (e.g. Kristensen, 1981). Unfortunately this was not followed in recent phylogenetic studies of holometabolous insects, where the presence of a sperm pump in Strepsiptera was tentatively scored as homologous for the Antliophora and interpreted as evidence for a close relationship between Strepsiptera and the Antliophora (Whiting, 1998b). Recently, the sperm pump of all four orders was studied in detail by Hünefeld & Beutel (2005). They concluded that a functional and homologous pumping apparatus was not a ground plan character of the Antliophora. Consequently, the sperm pump of Diptera should be interpreted as a unique apomorphy. See under each order below for a detailed discussion of the sperm pump and a description of the male genital tract.

MECOPTERA

Description (Fig. 1A, B)

Testis: The general shape is similar in most genera, comprised of three to four longitudinal follicles fused medially into a single fusiform gland (Potter, 1938). The only known exception is *Boreus* Latreille, where two spherical lobes or two sets of five spherical lobes are fused medially (Potter, 1938; Cooper, 1940). The testis of *Nannochorista* Tillyard is also fusiform or spindle-shaped, similar to that of most other Mecoptera. Due to the age of the material at hand, no inner divisions could be detected.

Epididymis: Long, inflated, coiled tubes at the base of the testes are present in all genera, except *Boreus*

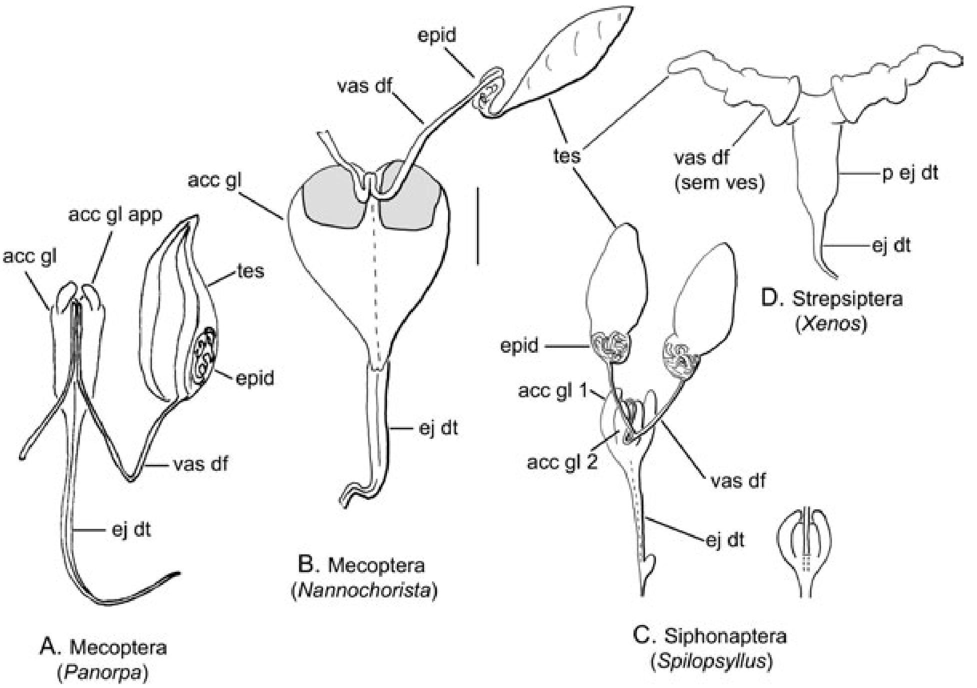


Figure 1. Male internal reproductive organs (dorsal view). A, *Panorpa* (Mecoptera), left testis removed [modified from Grell (1942)]. B, *Nannochorista* (Mecoptera), left testis removed, shaded areas mark discoloured regions (scale bar = 0.1 mm). C, *Spilopsyllus* (Siphonaptera), with ventral surface of accessory gland on right [modified from Mead-Briggs (1962)]. D, *Xenos* (Strepsiptera) [modified from Carcupino *et al*. (1995)]. acc gl, accessory gland; acc gl app, accessory gland appendix; ej dt, ejaculatory duct; epid, epididymis; p ej dt, proximal ejaculatory duct; sem ves, seminal vesicle; tes, testis; vas df, vas deferens.

(Potter, 1938). In *Boreus*, the testicular lobes empty into a thin-walled chamber (Cooper, 1940).

Vas deferens: In Mecoptera, each of the paired vasa deferentia extends posteriorly from the epididymis, bending back anteriorly before joining one another, then entering a pair of large glands. In Nannochoristidae, the two short vasa deferentia join medially just prior to entering the apex of a paired gland. In *Boreus*, the vasa deferentia curve slightly anteriorly before entering the most posterior of the three anterior lobes of the accessory gland (Cooper, 1940).

Accessory gland and seminal vesicle: In *Panorpa* (Fig. 1A), at the termination of each of the vasa deferentia is a large, often elongate, paired gland, closely approximated or fused medially (Potter, 1938; Grell, 1942; Matsuda, 1976). This gland is here referred to as the accessory gland, due to its thick walls and secretory cells (Grell, 1942). Spermatozoa are stored in the epididymis and no seminal vesicle is present. Often there is a pair of lobes or appendices of various lengths arising on the anterior end or laterally on this large gland, also of similar histology to the main gland (Grell, 1942). In Nannochoristidae, the accessory glands are joined medially, forming a heart-shaped gland. No internal divisions were visible (but the specimen was quite old), except for a flattened appendix anterodorsally (Fig. 1B, shaded areas). The accessory gland of *Boreus* differs from all other Mecoptera in comprising three closely positioned anterior lobes and a more distantly separated posterior lobe (Cooper, 1940).

Ejaculatory duct: Paired ducts arise from the posterior end of each of the accessory glands and join medially, but remain separate throughout their length and terminate in paired ejaculatory sacs. In Nannochoristidae, the slender, paired ducts are slightly shorter than the accessory gland. Prior to entering the ejaculatory sacs, the ducts are separated by the median depressor muscle of the piston (Grell, 1942; Hünefeld & Beutel, 2005). In Mecopteran studies (Potter, 1938; Grell, 1942; Hünefeld & Beutel, 2005) these ducts were labelled vasa deferentia, but we follow Matsuda (1976) in identifying ducts posterior to the accessory glands or exiting the seminal vesicle as ejaculatory ducts.

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme of Mecoptera functions as a piston, drawn in and out of the chamber (Willmann, 1981, 1987). The sperm pump (represented by the piston, not shown in Fig. 1A) is present in most families except Boreidae (Mickoleit, 1974) and Nannochoristidae (Willmann, 1981). In Boreidae, the reproductive tract is modified (Potter, 1938: fig. 30) and a preformed, two-chambered spermatophore is produced, but is not present in other Mecoptera (Mickoleit, 1974). The aedeagus lies in a genital cavity with a pair of lateral plates, which unite medially to form the sclerotized median piece (Matsuda, 1976). The opening of the sperm pump is narrow and lies on the distal end of the median piece.

Remarks: The accessory glands of Mecoptera have been termed seminal vesicles in the literature (e.g. Miyake, 1913; Potter, 1938; Grell, 1942; Matsuda, 1976), and sometimes only the small appendices have been referred to as accessory glands. We refer to the entire gland as the accessory gland due to its histology (i.e. the presence of large secretory cells) and due to the fact that no spermatozoa are stored in this gland, and thus cannot be termed the seminal vesicle (see Table 3). Mickoleit (1974) and Willman (1987) considered the spermatophore of Boreidae to be independently derived within Mecoptera and not a ground plan condition in this order.

Hünefeld & Beutel (2005) considered the sperm pump of Mecoptera to differ distinctly from the dipteran type. The genital segment, segment 9, is formed chiefly from the dorsomedially and ventromedially fused basal segments of the gonocoxites. The sperm pump is derived within this genital field (Hünefeld & Beutel, 2005). In contrast, the dipteran sperm pump is formed by a modified endophallus (Hünefeld & Beutel, 2005).

The male genital tract of *Nannochorista*, here described and illustrated for the first time (Fig. 1B), is similar to that of other Mecoptera illustrated in the survey by Potter (1938), although shorter and stouter. The paired accessory glands are not divided into separate chambers in Mecoptera (Grell, 1942).

The Nannochoristidae are often considered the most primitive lineage of the Mecoptera (Willman, 1987) or possibly the sister group to Siphonaptera + Diptera (Wood & Borkent, 1989). Recent molecular studies using multiple genes have assigned the Nannochoristidae as the sister group to Siphonaptera + Boreidae (Whiting, 2002). There is independent morphological evidence in the form of the structure of ovarioles (panoistic, initial stages of oogenesis completed postembryonically, multiple nucleoli in oocytes, etc.) that support this assemblage (see Simiczyjew, 2002; Grimaldi & Engel, 2005; Beutel & Pohl, 2006). Further analysis of the relationships of Mecoptera to other taxa is provided below in the Discussion.

SIPHONAPTERA

Description (Fig. 1C)

Testis: Each testis is spindle-shaped, not divided into follicles (Matsuda, 1976), but comprising bundles of spermatozoa (Mead-Briggs, 1962).

Epididymis: The outer tissue of the testis is enclosed posteriorly in a coiled tubular epididymis and probably functions as the seminal vesicle (Mead-Briggs, 1962).

Vas deferens: Each vas deferens leads posteriorly from the epididymis and the two are united at midlength in a common sheath. After uniting, the common duct arches anteriorly and loops around the median accessory glands, to which it is loosely attached (Mead-Briggs, 1962). This duct empties directly into the fused ejaculatory ducts.

Accessory gland and seminal vesicle: Paired accessory glands, sometimes comprising two pairs of lobes of unequal sizes, separately enter the ejaculatory duct prior to the ejaculatory bulb (see Fig. 1C, right). The glands are composed of columnar epithelial cells and have a secretory function (Günther, 1961; Mead-Briggs, 1962; Matsuda, 1976; Cheetham, 1988: fig. 84). No seminal vesicle has been identified.

Ejaculatory duct: The paired vasa deferentia become continuous with the paired, ejaculatory ducts, which unite at the level of the ejaculatory bulb (Mead-Briggs, 1962; Cheetham, 1988).

Ejaculatory apodeme, sperm pump, and aedeagus: There are two sperm pumping devices in Siphonaptera (Günther, 1961; Cheetham, 1988; Hünefeld & Beutel, 2005). The proximal pump is termed the ejaculatory bulb (sometimes the walls bear an ejaculatory sclerite) and is separated from the base of the endophallus (Günther, 1961: fig. 26; Mead-Briggs, 1962). Distally beyond the gonopore is the second pumping device, a musculated chamber (Günther, 1961; Cheetham, 1988; Hünefeld & Beutel, 2005). The aedeagus emerges as a narrow tube, bearing a pair of apical hooks or crochets (Matsuda, 1976). A pair of penis rods lies within the endophallus and at least one can be protracted from the aedeagus to enter the female bursa copulatrix (Matsuda, 1976).

Remarks: See the remarks under Mecoptera for a discussion of the phylogenetic position of the Siphonaptera. The vasa deferentia are looped anteriorly, as in many lower Diptera and Mecoptera. However, they do not enter the accessory glands anteriorly, but empty directly into the ejaculatory duct.

Hünefeld & Beutel (2005) also did not regard the sperm pump of Siphonaptera as being homologous with that of Mecoptera or Diptera. The proximal pump may include an ejaculatory sclerite, which is withdrawn anteriorly from the base of the endophallus.

STREPSIPTERA

Description (Fig. 1D)

Testis: Each testis consists of an anteriorly rounded, compact lobe (Hünefeld & Beutel, 2005) and with little external differentiation from the following vas deferens.

Epididymis: Not differentiated.

Vas deferens: These ducts are weakly distinguished externally from the testes, usually separated from each other (e.g. *Xenos* sp., *Mengenilla* sp.; Carcupino *et al.*, 1995; Hünefeld & Beutel, 2005) or they can be fused (e.g. *Corioxenos* sp.; Matsuda, 1976).

Accessory gland and seminal vesicle: No accessory glands are identified. The seminal vesicles are considered modified vasa deferentia and can be quite asymmetrical (Hünefeld & Beutel, 2005).

Ejaculatory duct: The duct is unpaired and its proximal portion functions as a sperm pump (Hünefeld & Beutel, 2005).

Ejaculatory apodeme, sperm pump, and aedeagus: The sperm pump is positioned at the anterior end of the ejaculatory duct (Matsuda, 1976) as opposed to the posterior end as in Mecoptera and Diptera. A sclerotized ejaculatory apodeme is absent and the pump is simply a muscular chamber, comprising an internal ring of circular muscle and an outer sheath of longitudinal muscle. The aedeagus is tubular or needle-like with a narrow hooked tip. No parameres are present. The spermatozoa are pumped through the narrow aedeagus and no spermatophore is probably formed (Hünefeld & Beutel, 2005).

Remarks: The reproductive tract is greatly reduced and modified in Strepsiptera. The seminal vesicle is in the same position as the epididymis in Mecoptera and is perhaps homologous. The sperm pump is clearly not homologous to that of Diptera, lacking sclerotized parts and derived from the proximal portion of the ejaculatory duct (Hünefeld & Beutel, 2005). The ejaculatory duct is normally encircled by circular muscle, but the presence of longitudinal muscle is possibly unique to Strepsiptera.

This order was included in the present outgroup survey because of the recent views concerning the relationships of Strepsiptera and recognition of the Halteria [e.g. Whiting & Wheeler, 1994; Whiting *et al.*, 1997; Whiting, 1998a, b, 2002, 2005; but see Rokas, Kathirithamby & Holland (1999) and Kukalová-Peck (1998) for evidence opposing this relationship]. Kristensen (1999) reviewed some of the morphological evidence for the Strepsiptera–Antliophora relationship and called into question most of the supporting characters either as regressive, problematical or possibly homoplastic. He concluded that the morphological evidence supporting the sister grouping Strepsiptera + Diptera was ambiguous. In fact, eight of the ten characters listed by Whiting (1998b) supporting Strepsiptera's inclusion in the Antliophora are absent in

Strepsiptera or inapplicable (characters 18–27; only 25 and 27 are present, with 26 a reversal). New information on the ground plan structure of the adult Strepsipteran mandible (character 18) shows that it is clearly chewing and not a dagger or blade-like structure as present in Antliophora (Grimaldi, Kathirithamby & Schawaroch, 2005; Pohl, Beutel & Kinzelbach, 2005). A further purported synapomorphy proposed by Whiting (1998b; character 25) is a ring-like segment 9 of the male abdomen. However, this feature is also present in the ground plans of male Trichoptera and Lepidoptera (Wood, 1991). The anterior position of the sperm pump and the absence of an inner ejaculatory apodeme make it unlikely that the pump (character 27) in Strepsiptera is homologous to that of the Antliophora, a conclusion also reached by Hünefeld & Beutel (2005). We agree with Kristensen (1999) that the proposed Strepsiptera/Antliophoran clade can be refuted on morphological grounds. The reduced nature of the strepsipteran male gonads, especially the absence of accessory glands, provides no additional evidence supporting such a relationship.

NEMATOCEROUS DIPTERA

TIPULOMORPHA

TIPULIDAE

Description (Fig. 2A)

Testis: Each testis is thin walled, small, rounded anteriorly and tapered posteriorly (Keuchenius, 1913).

Epididymis: Not differentiated.

Vas deferens: These ducts are slender and thin walled initially, increasingly inflated and merging together into a united tube with thicker walls. In *Tipula* L.,

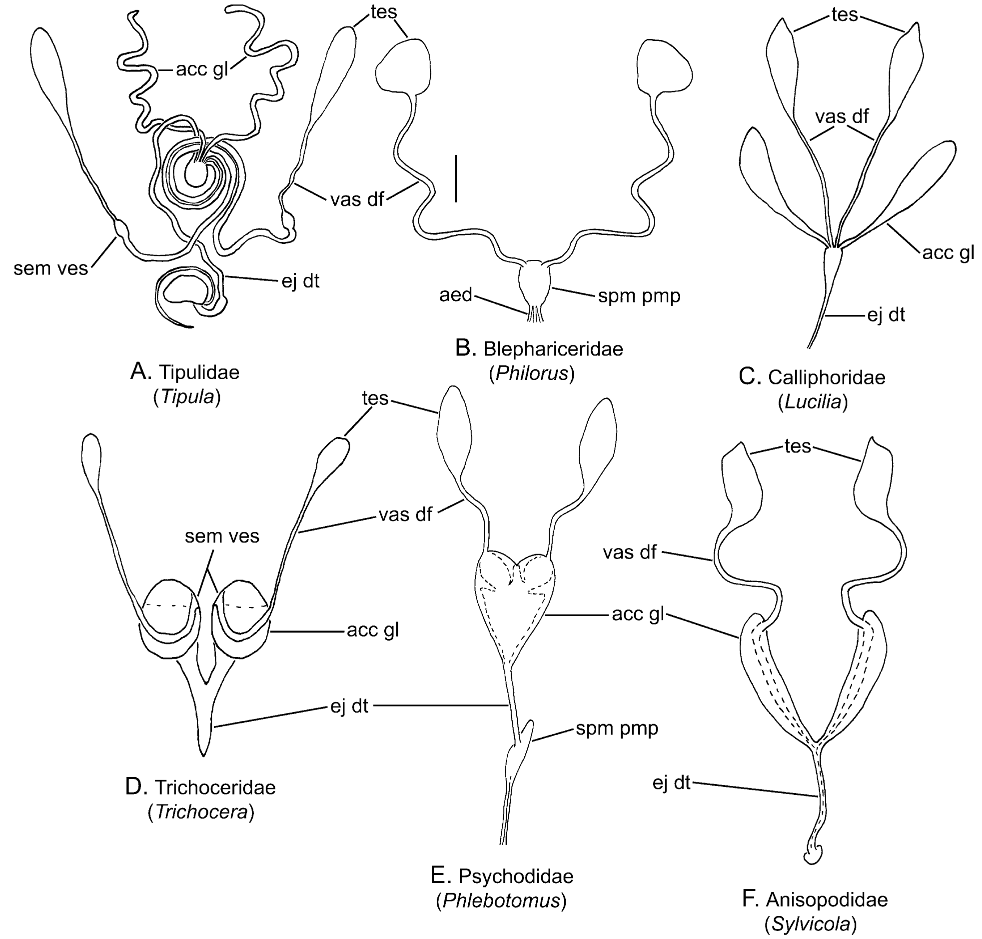


Figure 2. Male internal reproductive organs (dorsal view). A, *Tipula oleracea* L. (Tipulidae) [modified from Keuchenius (1913)]. B, *Philorus vividis* Kitakami (Blephariceridae) (accessory glands not observed) (scale bar = 0.1 mm). C, *Lucilia* sp. (Calliphoridae) [modified from Hori (1960)]. D, *Trichocera annulata* Meigen (Trichoceridae) [modified from Neumann (1958)]. E, *Phlebotomus* sp. (Psychodidae) [modified from Just (1973) and Perfil'ev (1968)]. F, *Sylvicola* sp. Anisopodidae [modified from Abul-Nasr (1950) and Dahl (1980)]. acc gl, accessory gland; aed, aedeagus; ej dt, ejaculatory duct; sem ves, seminal vesicle; spm pmp, sperm pump; tes, testis; vas df, vas deferens.

Dicranomyia Stephens and *Thaumastoptera* Mik there is a small bladder-like swelling prior to the fusion, filled with spermatozoa (Dufour, 1851; Keuchenius, 1913; Liang, 1925). The ducts remain medially separated until they empty into a sac-like swelling at the anterior apex of the ejaculatory duct (Neumann, 1958; Frommer, 1963). In *Chionea* Dalman the proximal half is very thin and at midlength turns sharply anteriorly and becomes greatly inflated, and probably functions as a seminal vesicle (Byers, 1983). In *Tipula*, the vasa deferentia extend posteriorly and appear to arch anteriorly prior to fusion, whereas in *Dicranomyia* and *Thaumastoptera* they are straight, extending posteriorly (Dufour, 1851; Keuchenius, 1913; Liang, 1925).

Accessory gland and seminal vesicle: The common vas deferens terminates in a ring or spherical-shaped, glandular-walled swelling of the ejaculatory duct (Keuchenius, 1913; Neumann, 1958; Byers, 1961). This has been termed the seminal vesicle due to its large lumen and presence of masses of spermatozoa (Byers, 1961, 1983). In contrast, Keuchenius (1913) never observed spermatozoa in this enclosure and considered it as having some sort of glandular function due to the thickened walls of this gland. The accessory glands also empty into this enlarged chamber. The accessory glands are often long, slender coiled tubes or greatly inflated, elongate sacs, each with a large lumen and walls of columnar cells (Byers, 1961, 1983; Frommer, 1963).

Ejaculatory duct: A seminal duct, often quite long, extends from the enlarged chamber or seminal vesicle to the sperm pump.

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme of the sperm pump is normally connected to the base of the aedeagus by a stout membrane and rotates in a lever-like motion (Frommer, 1963; Wood, 1991; Sinclair, 2000). The aedeagus of Tipulomorpha is a slender, sclerotized tube, with one or three narrow openings (Frommer, 1963; Downes, 1968; Wood, 1991; Sinclair, 2000).

Remarks: Details of the male genital tract have been described and illustrated in a number of genera, including, for example, *Chionea*, *Dolichopeza* Curtis, and *Tipula* (Dufour, 1851; Keuchenius, 1913; Neumann, 1958; Byers, 1961, 1983; Frommer, 1963). The various ducts are usually long and slender, complexly intertwined and consequently not easily studied. Another aspect that differs from other nematocerous families is the central enlarged chamber or seminal vesicle, but the vasa deferentia are also expanded in these taxa and probably also function as seminal vesicles. In the latter, the configuration is similar to the generalized male insect condition as described by Snodgrass (1935: 568, fig. 292). The swelling of the duct where the accessory glands and vasa deferentia meet is also found in numerous Cyclorrhapha.

The vasa deferentia are uniquely fused near their midlength, but remain paired. This fusion is documented in *Tipula* and *Dolichopeza*, but does not occur in all Tipulidae (e.g. *Dicranomyia*). The fusion of the vasa deferentia also occurs independently in most Syrphidae.

The phylogenetic position of the Tipulomorpha remains disputed. Wood & Borkent (1989) assigned this infraorder as the sister group to the remaining Diptera, whereas Michelsen (1996) included it in the Polyneura (comprising several additional families) representing the sister to all remaining groups of Diptera. However in a ‘note added in proof’, the evidence supporting the monophyly of the Polyneura remains limited or conflicting because he studied a specimen of another species of Tipulidae, which conflicted with the main body of conclusions for this group. Hence, Tipulidae as a sister group of all remaining Diptera cannot be dismissed on the basis of Michelsen’s results.

Oosterbroek & Courtney (1995) assigned the Tipulidae as the sister group of the Trichoceridae and these together as the sister group to the Anisopodidae + Brachycera based on character states that were either of questionable homology (their characters 47 and 50 are dependent on the interpretation of the segmental homology of the terminal larval abdominal segment), reversals (i.e. characters 76 and 79 are wing venation features that are widespread in dipteran outgroups), or are poorly understood (e.g. only one-third of the ingroup was scored for characters 94 and 96, the remaining were left as unknown).

BIBIONOMORPHA

HESPERINIDAE

Description (Fig. 3A)

Testis: Each testis of *Hesperinus* Walker is oval and sack-like, partially collapsed in the material examined.

Epididymis: Not differentiated.

Vas deferens: These ducts are long, slender, and slightly twisted. The ducts extend posteriorly to the base of a large fused accessory gland complex. At their most posterior extension, the vasa deferentia are expanded into kidney-shaped chambers of unknown function (perhaps similar to the inflated sacs in Tipulidae). From these structures, the ducts join medially and arch anteriorly and are continuous with the apex of the accessory gland complex.

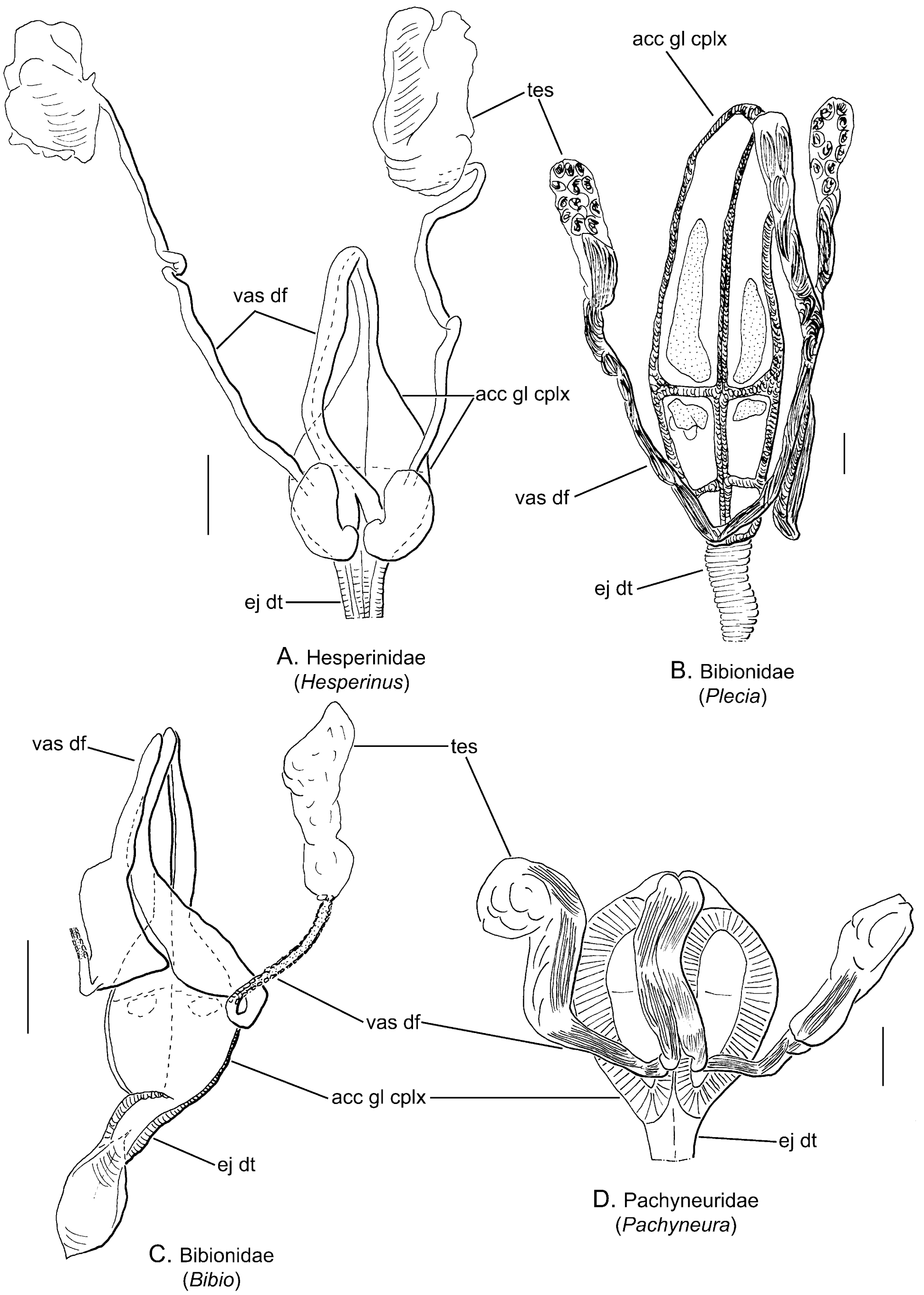


Figure 3. Male internal reproductive organs (dorsal view). A, *Hesperinus rohdendorfi* (Hesperinidae). B, *Plecia membranifera* (Bibionidae). C, *Bibio flavihalter* (Bibionidae), left vas deferens and testis removed (based on the specimen photographed in Fig. 4A). D, *Pachyneura fasciata* (Pachyneuridae). acc gl cplx, accessory gland complex; ej dt, ejaculatory duct; tes, testis; vas df, vas deferens. Scale bars = 0.1 mm.

Accessory gland and seminal vesicle: A large accessory gland complex is present with at least two distinct chambers visible. The gland remains divided medially throughout its length. No distinct seminal vesicle is present, although the large kidney-shaped expansions of the vasa deferentia may function as storage chambers.

Ejaculatory duct: At the posterior end of the accessory gland complex, a pair of medially joined ejaculatory ducts exits. They are rather thick walled and extend to the sperm pump.

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme is T-shaped in *Hesperinus*, with broad wing-like lateral arms (Sinclair, 2000: fig. 8). The apodeme extends posteriorly to near the apex of the aedeagus. The aedeagus is mostly sclerotized in *Hesperinus*, with a narrow apical phallotrema (or secondary gonopore at the apex of the aedeagus) (Sinclair, 2000: fig. 11).

Remarks: The male genital tract of *Hesperinus* is here described and illustrated for the first time. The broad lateral plates on the ejaculatory apodeme for the insertion of large muscles appear to be unique among the Bibionomorpha and similar to those observed in some Tipulidae (Wood, 1991: fig. 3a) and lower Brachycera (Sinclair, Cumming & Wood, 1994).

PACHYNEURIDAE

Description (Fig. 3D)

Testis: The testis is slender, sac-like, rounded anteriorly, and tapered posteriorly.

Epididymis: Not differentiated.

Vas deferens: These ducts extend posteriorly to the base of a large accessory gland complex and then arch and unite weakly medially (Fig. 3D). The vasa deferentia extend anteriorly as twin ducts closely applied to each other, before entering the anterior apex of the accessory gland complex. Layers of spermatozoa filling the entire ducts were observed in fresh specimens.

Accessory gland and seminal vesicle: A single large, rounded gland is present in *Pachyneura fasciata* Zetterstedt, referred to here as the accessory gland complex. The gland remains divided medially and at least two distinct chambers are visible on each side. No seminal vesicle is differentiated.

Ejaculatory duct: This duct remains paired upon exiting the accessory gland complex and is relatively short, slightly longer than one-half length of the accessory gland complex.

Ejaculatory apodeme, sperm pump, and aedeagus: The external genitalia of *Pachyneura fasciata* was illustrated in Wood (1991: fig. 7) with the various components shown in different colours. The ejaculatory apodeme is a small concave plate fused to the anterior end of the mostly sclerotized aedeagus and consequently the sperm pump is reduced. The apex of the aedeagus (phallotrema) is U-shaped and quite narrow.

Remarks: The male genital tract of *Pachyneura* Zetterstedt is here described and illustrated for the first time. The ejaculatory apodeme no longer functions as a piston in *Pachyneura*, but probably assists in drawing the spermatophore out and into the female genital chamber as in Bibionidae (see below).

BIBIONIDAE

Description (Figs 3B, C, 4A, B)

Testis: Each testis is an elongate, sac-like gland, sometimes encased in a dark fat body.

Epididymis: Not differentiated.

Vas deferens: The vasa deferentia are slender and delicate, extending posteriorly to near the base of the large accessory gland complex. In *Bibio* Geoffroy (Fig. 3C) and *Dilophus* Meigen (Blaschke-Berthold, 1994), the vasa deferentia arch anteriorly and become stout, greatly expanded ducts, which extend anteriorly and then posteriorly again before entering the anterior apex of the accessory gland complex. These expanded regions appear somewhat muscular and are only closely associated and not fused medially. In *Plecia* Wiedemann, these expanded regions are lacking and the vasa deferentia remain slender, although fused distally throughout (Fig. 3B). The vasa deferentia are somewhat enlarged anteriorly in *Penthetria* Meigen and the ducts are fused medially as they arch anteriorly and extend to the apex of the accessory gland complex without arching posteriorly, as in *Bibio*. The vasa deferentia of *Plecia membranifera* Hardy & Takahashi were observed to be completely full of spermatozoa throughout their length (Fig. 3B).

Accessory gland and seminal vesicle: The accessory gland complex in *Bibio* and *Penthetria* is distinctly oval-shaped, whereas the glands are very elongate and nearly parallel-sided in *Plecia*. At least two distinct chambers are visible in the glands of *Bibio flavihalter* Hardy & Takahashi (Fig. 4A, B) and *Penthetria funebris* Meigen. Three distinct chambers are visible in excellently preserved material of *Plecia membranifera* (Fig. 3B).

Ejaculatory duct: The ejaculatory ducts are stout and apparently muscular. The duct appears divided medially in *Penthetria* and perhaps at least anteriorly in *Bibio*. The ejaculatory duct in *Plecia* is clothed in a dark fat body and the inner duct was not visible.

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme is a concave plate, which cradles the endophallus and is moved by two pairs of opposing muscles in *Bibio* (Blaschke-Berthold, 1994; Sinclair, 2000: fig. 12). The ejaculatory apodeme slides in and out of the base of the aedeagus and compresses the sperm pump chamber (see 'Remarks – features of

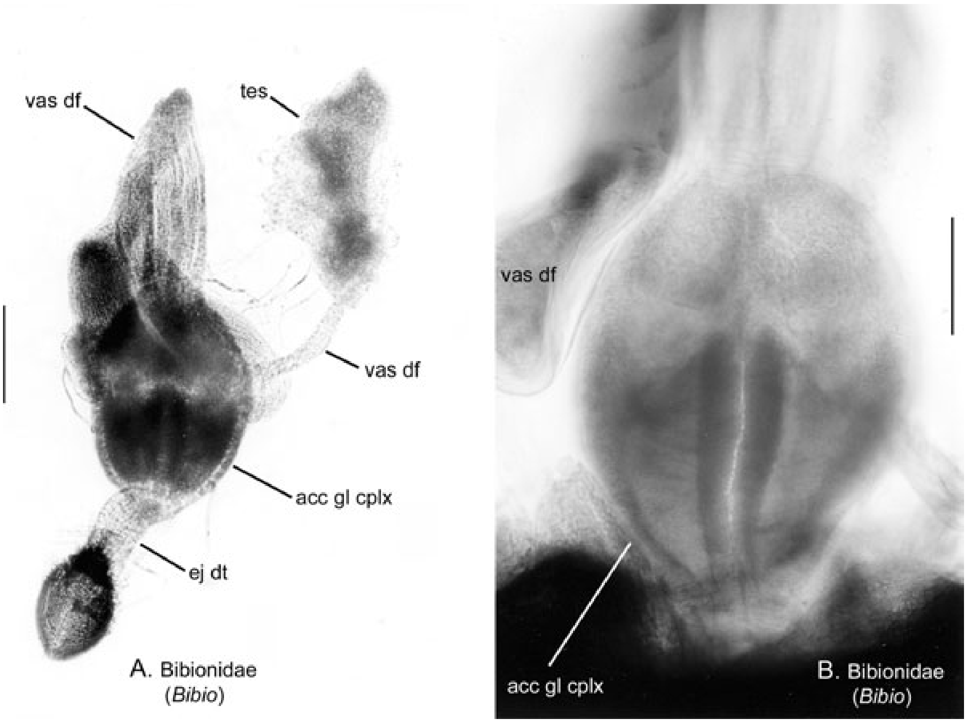


Figure 4. Male internal reproductive organs. A, *Bibio flavihalter* (Bibionidae) (dorsal view). B, *Bibio flavihalter* (Bibionidae) (dorsal view). acc gl cplx, accessory gland complex; ej dt, ejaculatory duct; tes, testis; vas df, vas deferens. Scale bars = 0.1 mm.

Bibionomorpha', below). The phallotrema is broad and flexible (Blaschke-Berthold, 1994).

Remarks: Our interpretation of the male gonads, especially of *Bibio*, differs from that of Blaschke-Berthold (1994). The very small delicate testes in *Bibio* were omitted from her illustrations (cf. Figs 3C, 4A and Blaschke-Berthold, 1994: fig. 72). In addition, we interpret the large medially fused gland as a complex fusion, identified as the accessory gland complex, whereas Blaschke-Berthold (1994) identified these glands as simply the seminal vesicle. The glands labelled 'akzessorische Drüse' (aD1) are possibly homologous to vesicular glands observed in Tipulidae (Byers, 1961; Frommer, 1963). Blaschke-Berthold (1994) made no observations on the internal divisions of the accessory gland complex (seminal vesicles *sensu* Blaschke-Berthold).

Remarks – features of Bibionomorpha: There have been several studies made on the male genital tract of Bibionomorpha. Leppla, Carlysle & Guy (1975) presented detailed histological sections of the mechanics of spermatophore transfer in *Plecia nearctica* Hardy. The most comprehensive work is that of Blaschke-Berthold (1994), who detailed the internal tract and external genitalia of five families of Bibionomorpha, including the Sciaridae and Mycetophilidae *s.l.* In these two families, the anteriorly directed vasa deferentia are greatly enlarged, with thickened walls (Abul-Nasr, 1950; Blaschke-Berthold, 1994).

The male genital tract of this infraorder appears distinctly modified in comparison with the Tipulomorpha and very similar in basic configuration to Chironomoidea (+ Dixidae) and this is especially true of *Plecia membranifera.* However, the length or size of the various chambers distinctly differs. The accessory gland complex also differs from the accessory glands of Mecoptera in the presence of inner chambers and the absence of appendices. Undoubtedly, the configuration of the male genital tract in Bibionomorpha reflects the production of spermatophores, the existence of which is confirmed in the Bibionidae (Leppla *et al.*, 1975; Blaschke-Berthold, 1994) and Sciaridae (Eberhard, 2001).

The mechanics of spermatophore formation or transfer in Bibionomorpha would appear to differ from Culicomorpha because of the presence of the ejaculatory apodeme in the former. The ejaculatory apodeme no longer acts as a piston to compress the sperm pump, but is drawn in and out like a spatula, apparently assisting in delivering the spermatophore-forming material into the female genital chamber (Blaschke-Berthold, 1994; Sinclair, 2000). In contrast to the Culicomorpha, the spermatophore is actually never exchanged (at least in Bibionidae), but remains attached to the male genitalia and functions as a channel for the passage of spermatozoa (Leppla *et al.*, 1975; Blaschke-Berthold, 1994). The spermatophore fluids congeal within the female genital chamber, expanding to fill the chamber (Leppla *et al.*, 1975). In Sciaridae, the spermatophore has been observed attached to the female genitalia (Eberhard, 2001).

BLEPHARICEROMORPHA

BLEPHARICERIDAE

Description (Fig. 2B)

Testis: Each testis is oval and pale (*Philorus* Kellogg) or club-shaped, bent and twisted, and darkly pigmented (Zwick, 1977).

Epididymis: Not differentiated.

Vas deferens: These ducts are long and slender and extend directly to the sperm pump (*Philorus*) or are non-existent when the testis is rather elongate and empties directly into the sperm pump (Zwick, 1977).

Accessory gland and seminal vesicle: There are one to two pairs of pale glands, which empty separately at the base of the elongate testes (Zwick, 1977). The contents and function of these glands are unknown. No glands were observed in *Philorus*.

Ejaculatory duct: The ducts exiting the testis remain completely separate for their entire length and empty separately and directly into the sperm pump (Zwick, 1977; Sinclair, 2000: fig. 1). There appears to be no distinction between the vas deferens and the ejaculatory duct.

Ejaculatory apodeme, sperm pump, and aedeagus: The base of the aedeagus bears an enlarged ejaculatory apodeme, which compresses the sperm pump (Sinclair, 2000). The aedeagus in Blephariceridae is divided posteriorly into three narrow forks, with each narrow tube opening independently (Fig. 2B).

Remark: The male genital tract of this family was studied by Zwick (1977).

PSYCHODOMORPHA

TRICHOCERIDAE

Description (Figs 2D, 5A)

Testis: Each testis is oval to pyriform, with a thin epithelium continuous with the vasa deferentia.

Epididymis: Not differentiated.

Vas deferens: The vasa deferentia extend posteriorly to the base of the accessory glands, where they arch medially. Each duct abruptly terminates near the anteromedial margin of the accessory gland (Neumann, 1958; Dahl, 1980).

Accessory gland and seminal vesicle: The accessory glands are paired spherical structures, divided internally into two functionally different chambers (Neumann, 1958; 'Samenblase'). The chambers are lined with columnar cells, producing secretions that empty separately into the duct that separates the seminal vesicle and the ejaculatory duct. No spermatozoa have been observed within the chambers of the accessory gland (Neumann, 1958). The seminal vesicle is interpreted to occur at the apex of each of the vasa deferentia, adjacent to and fused with the accessory glands (Dahl, 1980). A narrowing of the duct separates the seminal vesicle from the anterior fork of the ejaculatory duct. The seminal vesicle and the accessory gland are enclosed in a continuous sheath of circular muscle (Neumann, 1958).

Ejaculatory duct: Anteriorly, the ejaculatory duct is paired for a short distance before fusing into a single duct. The circular muscle is very thin and does not function as a pump (Neumann, 1958).

Ejaculatory apodeme, sperm pump, and aedeagus: There is a small flexible ejaculatory apodeme at the base of a narrow sclerotized aedeagus (Neumann, 1958; Wood, 1991). The ejaculatory apodeme rotates, compressing the sperm pump and forcing the spermatozoa out through the narrow opening of the phallotrema.

Remarks: The configuration of the male genital tract of the Trichoceridae was thoroughly investigated by Neumann (1958). Our examination of material confirms his interpretation. The male genital duct of Trichoceridae is somewhat similar to Culicidae and Chaoboridae in basic configuration and no preformed spermatophore is produced. Matsuda (1976) assumed that one of the chambers in the accessory gland corresponded to the seminal vesicle in *Aedes* Meigen. This appears unlikely as spermatozoa are not stored in the accessory gland chambers of *Trichocera* Meigen.

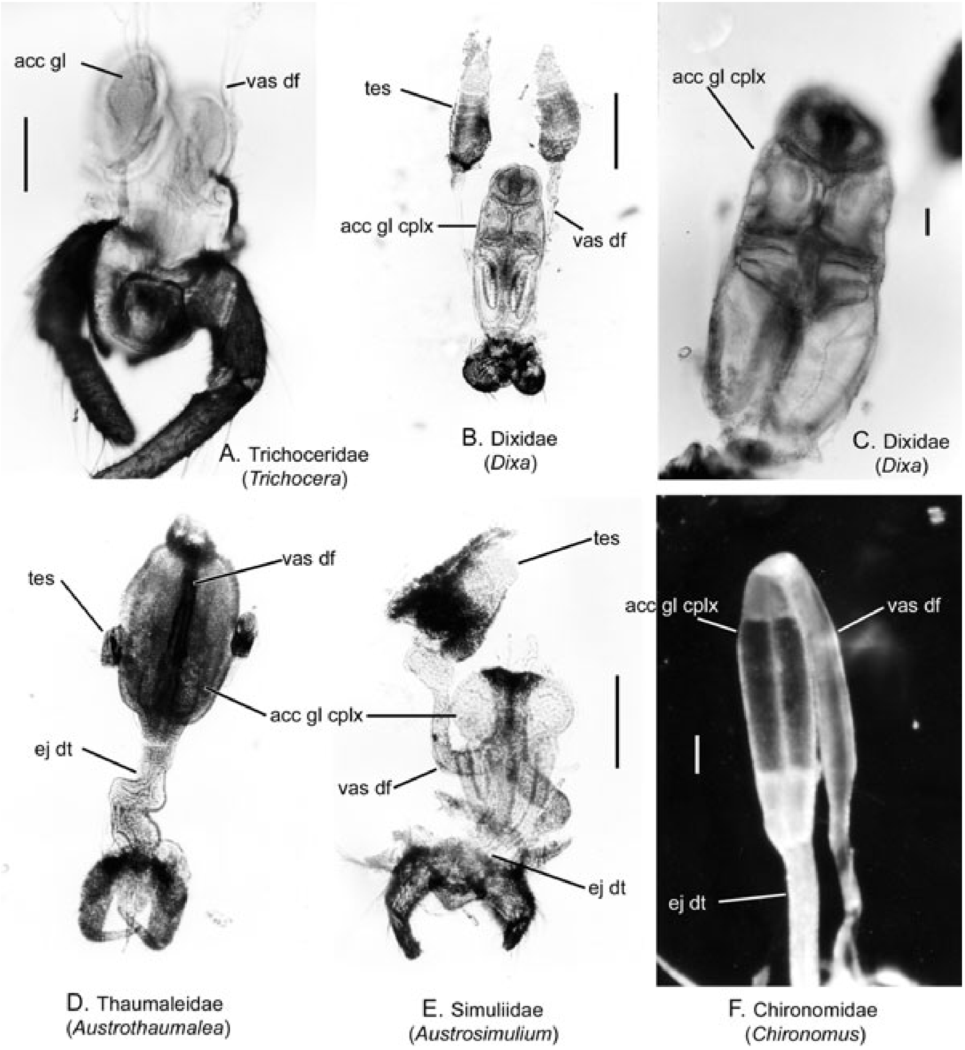
PSYCHODIDAE

Description (Fig. 2E)

Testis: The testes are oblong–oval or rounded, with tapered anterior ends. Spermatozoa are densely packed in the posterior chambers.

Epididymis: Not differentiated.

Vas deferens: A short, narrow duct enters the accessory gland separately in *Phlebotomus* Rondani & Berté (Davis, 1967; Perfil'ev, 1968; Just, 1973). In contrast, in other psychodids the entire reproductive tract



A. Trichoceridae (*Trichocera*)
B. Dixidae (*Dixa*)
C. Dixidae (*Dixa*)
D. Thaumaleidae (*Austrothaumalea*)
E. Simuliidae (*Austrosimulium*)
F. Chironomidae (*Chironomus*)

Figure 5. Male internal reproductive organs (dorsal view). A, *Trichocera* sp. (Trichoceridae). B, *Dixa yamatoma* (Dixidae). C, *Dixa yamatoma* (Dixidae). D, *Austrothaumalea zentae* (Thaumaleidae). E, *Austrosimulium furiosum* group (Simuliidae). F, *Chironomus plumosus* (Chironomidae). acc gl cplx, accessory gland complex; ej dt, ejaculatory duct; tes, testis; vas df, vas deferens. Scale bars = 0.1 mm.

is modified and the vasa deferentia are broad tubes little differentiated from the accessory glands (Friele, 1930; Matsuda, 1976).

Accessory gland and seminal vesicle: Only a single heart-shaped gland is present in *Phlebotomus* and is here referred to as the accessory gland, but its bilobed condition suggests fusion of paired glands. No seminal vesicle in *Phlebotomus* is present. The vasa deferentia enter each side of this gland and are enveloped, forming a pair of small inner chambers with broad gland cells and a narrow exit to the single large inner chamber of this gland (Just, 1973: fig. 7, Vsem; Jobling & Lewis, 1987). The cells within this larger chamber also have a secretory function. No seminal vesicle is identified in the modified genital tract of *Tinearia alternata* (Say) and *Satchelliella pulustris* (Meigen), although two pairs of accessory glands are present (Friele, 1930; Just, 1973).

Ejaculatory duct: A single narrow, tapered duct exits the accessory gland in *Phlebotomus* and enters the sperm pump (Just, 1973). The ejaculatory duct in *Satchelliella* Vaillant is broad and muscular, directly associated with the base of the aedeagus and lies immediately ventral to a long ejaculatory apodeme, which is articulated with the aedeagus. The inner lumen of the duct consists of finely folded epithelium, which aids in the flexibility and contraction of the duct.

Ejaculatory apodeme, sperm pump, and aedeagus: The single ejaculatory duct enters the sperm pump anteriorly. This pump bears a rod-like ejaculatory apodeme that compresses the chamber in a piston-like manner, ejecting the seminal fluid through long, thread-like paired ducts to the phallotrema in *Phlebotomus* (Perfil'ev, 1968; Just, 1973). The sperm pump is quite different in *Satchelliella* and sperm is probably forced out through the apex of the aedeagus by the combined action of the ejaculatory apodeme moving backwards and forwards and the muscular contractions of the ejaculatory duct.

Remarks: The narrow opening of the aedeagus and the presence of a sperm pump indicate that spermatophores are probably not produced in these genera of Psychodidae.

ANISOPODIDAE

Description (Fig. 2F)

Testis: Each testis is pear-shaped, with short anterior filaments, covered by brownish fat tissue (Abul-Nasr, 1950).

Epididymis: Not differentiated.

Vas deferens: These ducts are rather short, running posteriorly, clothed in brownish fat tissue, as found on the testis. The ducts consist of one thin layer throughout, except for a layer of glandular cells at the junction with the seminal vesicle (Abul-Nasr, 1950).

Accessory gland and seminal vesicle: Each vas deferens empties separately into the proximal end of a pair of elongate accessory glands (Abul-Nasr, 1950). These long glands remain separate and possess a layer of glandular cells that produce a secretion that surrounds the spermatozoan mass (Abul-Nasr, 1950). Each gland is coated with a thin layer of circular muscles and large amounts of spermatozoa are stored in this paired gland shortly after emergence of the adult (Abul-Nasr, 1950).

Ejaculatory duct: Each gland empties separately into the ejaculatory duct, which remains medially divided, entering the sperm pump closely appressed. This duct is long and convoluted, starting as a thickened tube, which gradually decreases in thickness posteriorly. Circular muscles coat the distal portion of the duct (Abul-Nasr, 1950).

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme is rod-shaped, and acts as a piston to compress the sperm pump, forcing the seminal fluid into the long, slender, coiled aedeagal tube (Abul-Nasr, 1950). The sperm pump is partially enclosed by the fused, concave, plate-like paramere.

Remarks: The male genital tract of Anisopodidae was illustrated by Abul-Nasr (1950) and Dahl (1980). The aedeagus is very long and thread-like and the sperm pump clearly functions to pump seminal fluid through this tube. The ejaculatory duct is a paired tube and no spermatophores are formed.

SCATOPSIDAE

Description

Testis: Each testis is oval-shaped (Loew, 1841).

Epididymis: Not differentiated.

Vas deferens: The ducts could not be traced in this study, but according to Loew (1841) a pair of rather long and convoluted ducts enters separately a small expanded region.

Accessory gland and seminal vesicle: A small oval expansion is present at the apex of the ejaculatory duct (Loew, 1841) and may correspond to a seminal vesicle. The paired vasa deferentia enter this vesicle separately. This structure could not be confirmed in our dissections.

A pair of long tubular glands, with posteriorly recurved tips, dominates the posterior region of the genital tract. These thick-walled accessory glands lie dorsal to the sperm pump and rectum, arch anteroventrally and taper to narrow ducts that enter the sperm pump ventrally. The recurved tip appears to taper to a short nipple-shaped appendix. These glands are rather stout and the narrow extension of the gland to the sperm pump is also thick walled, with a narrow inner canal.

Ejaculatory duct: This duct could not be traced in the present study, but according to Loew (1841) the duct is similar in size to the vas deferens and extends from the expanded anterior sac (seminal vesicle?) to the sperm pump.

Ejaculatory apodeme, sperm pump, and aedeagus: The sperm pump and associated ejaculatory apodeme are clearly separated from the base of the external genitalia. The outer wall of the sperm pump is sclerotized and remains intact after being cleared in lactic acid. The pump bears stout opposing muscle sets and clearly functions to pump sperm to the aedeagus. The pump is connected to the aedeagus by a very narrow, clear tube (see Cook, 1981: fig. 20.18).

Remarks: The reproductive tract of *Scatopse notata* (L.) was described by Loew (1841). Unfortunately, we were not able to trace the configuration of the vasa deferentia in the material available and consequently cannot confirm the observations of Loew (1841). The most striking difference in Scatopsidae from other families is the apparent direct connection of the accessory glands to the sperm pump. Further study of the scatopsid male reproductive tract is required in order to confirm and interpret these observations.

Given the presence of the narrow tube connecting the sperm pump and the aedeagus and the robust sperm pump, an aqueous solution containing spermatozoa is probably pumped through this system.

PTYCHOPTEROMORPHA

PTYCHOPTERIDAE

Description (Fig. 6D, E)

Testis: Each testis is long, slender, and spindle-shaped, often yellowish-orange in dissections. The testes of *Ptychoptera* nr *japonica* Alexander and *Ptychoptera contaminata* (L.) are positioned far posteriorly, lying near the accessory gland complex.

Epididymis: Not differentiated.

Vas deferens: In *Ptychoptera lacustris* Meigen (Fig. 6E), the vasa deferentia extend posteriorly to the level of the posterior chambers of the accessory gland complex, where they turn medially, fuse, and run anteriorly before arching abruptly into the accessory gland complex (Just, 1973). In *Ptychoptera contaminata*, the vasa deferentia appear little differentiated from the testes, extending first anteriorly dorsal to the testes. The vasa deferentia of *Ptychoptera* nr *japonica* (Fig. 6D) also run immediately anteriorly and empty into the apex of a large three-chambered accessory gland complex.

Accessory gland and seminal vesicle: In *Ptychoptera lacustris*, the accessory gland complex is divided into an elongate and slender anterior section connected to a rounded posterior gland by a short duct (Fig. 6E). The accessory gland complex of *Ptychoptera* nr *japonica* (Fig. 6D) is fused along the midline, forming a three-chambered gland. In *Ptychoptera contaminata*, the genital tract is folded over itself several times and what appear to be vasa deferentia could in fact be additional sections of the accessory gland complex lying beneath paired, rounded chambers. In this species, the vasa deferentia are quite elongate, extending anteriorly before strongly arching posteriorly as clear, thin-walled ducts that separately enter the paired posterior chambers.

Ejaculatory duct: The ducts are short, lined with thick muscles, and unite prior to entering the sperm pump (Just, 1973). The ejaculatory ducts of *Ptychoptera* nr *japonica* and *Ptychoptera contaminata* are bent sharply upon exiting the seminal vesicles and then arch to the sperm pump.

Ejaculatory apodeme, sperm pump, and aedeagus: Ptychoptera lacustris and *Ptychoptera contaminata* possess a large median ejaculatory apodeme and a pair of apodemes extended from the paramere, which compress the sperm pump [see explanation in Just (1973)]. In contrast, *Ptychoptera* nr *japonica* possesses a large sclerotized, spherical sperm sac and lacks an ejaculatory apodeme [similar to that of *Bittacomorpha clavipes* (Fab.); see Wood (1991: fig. 10)].

Remarks: The genital tract is quite variable in the genus *Ptychoptera*. The configuration of the vasa deferentia is similar to that observed in Bibionomorpha and Culicomorpha. The accessory gland complex comprises a number of chambers that are separated, connected by slender ducts or fused into a single gland.

CULICOMORPHA – CHIRONOMOIDEA

THAUMALEIDAE

Description (Figs 5D, 7A, B, 8A, B)

Testis: Each testis is rounded and oval, often thickly clothed with pigment granules (fat cells) and lying along the lateral margin of the accessory gland complex or lying free.

Epididymis: Not differentiated.

Vas deferens: The apical third of each of the ducts is enlarged in *Androprosopa japonica* (Okada), filled with spermatozoa and terminates near the anterior margin of the first chamber of the accessory gland complex (Fig. 7A). The vasa deferentia are fused medially only for a short distance, prior to entering the gland. In other species, the ducts unite along the midline, lying on the dorsal surface of the fused accessory gland complex (Fig. 7B). The ducts enter the gland complex either just posterior to the anterior margin at the base of small apical lobes (*Austrothaumalea zentae* Theischinger) or near the midpoint of the anterior section (*Androprosopa americana* (Bezzi)).

Accessory gland and seminal vesicle: The accessory gland complex is divided into a multichambered gland, comprising enlarged, elliptical sacs, united medially, and appearing to be divided into three to four pairs of chambers. In both *Androprosopa* species examined, the anterior-most chambers are much larger. In *Androprosopa japonica*, the middle and posterior pairs of chambers are oval and rather stout (Fig. 7A, the middle chamber is hidden by the curvature of the vasa deferentia). The opaque condition of the genital tract of the other two species prevented clear determination of the inner divisions of the gland. *Austrothaumalea zentae* was characterized by a small apical pair of chambers or swellings on the anterior end of the accessory gland complex (Figs 5D, 7B). No seminal vesicle was identified.

Ejaculatory duct: The modified accessory gland complex empties posteriorly into broad, paired ejaculatory ducts. The ejaculatory ducts fuse to form a single, wide duct just prior to the external genitalia. The duct

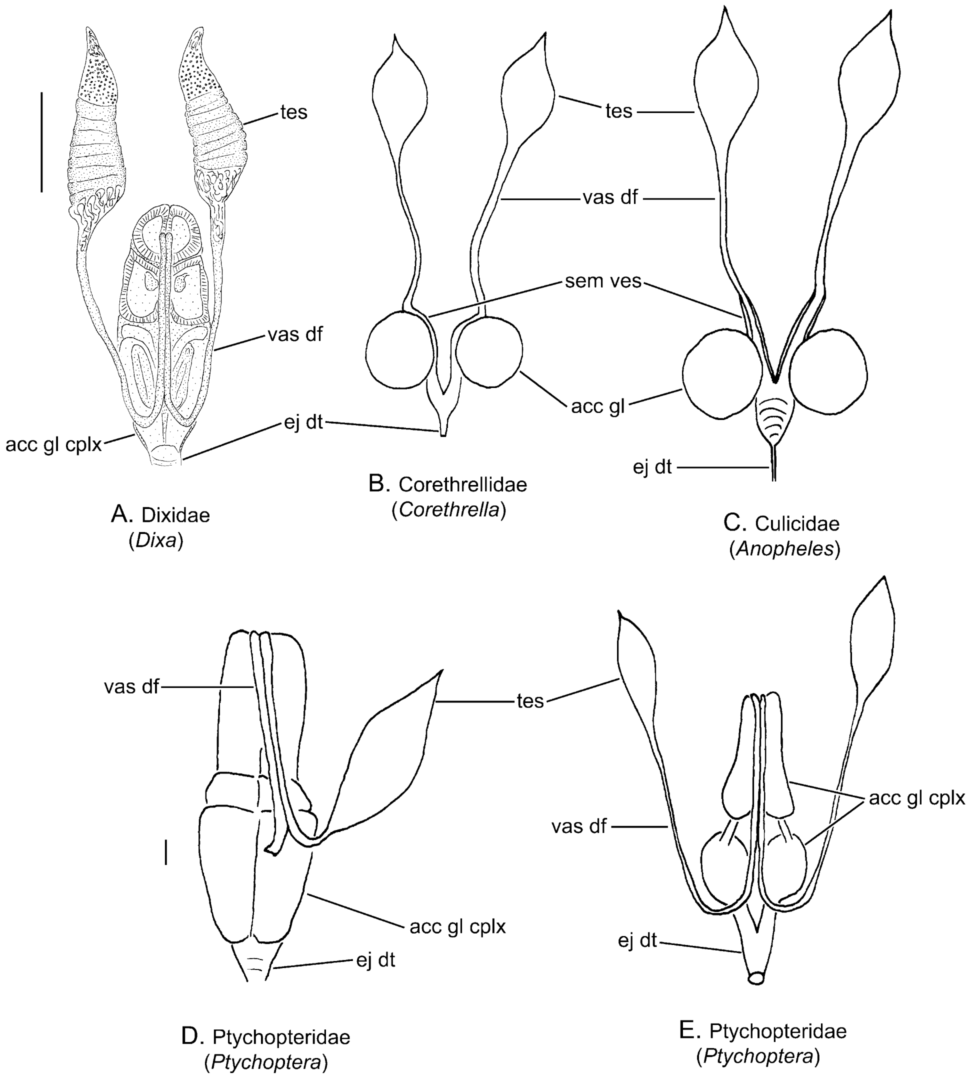


Figure 6. Male internal reproductive organs (dorsal view). A, *Dixa yamatoma* (Dixidae) (based on the specimen photographed in Fig. 5B). B, *Corethrella* sp. (Corethrellidae) [modified from McKeever (1985)]. C, *Anopheles quadrimaculatus* (Culicidae) [modified from Hodapp & Jones (1961)]. D, *Ptychoptera* nr *japonica* (Ptychopteridae) (scale bar = 0.1 mm). E, *Ptychoptera lacustris* (Ptychopteridae) [modified from Just (1973)]. acc gl, accessory gland; acc gl cplx, accessory gland complex; ej dt, ejaculatory duct; sem ves, seminal vesicle; tes, testis; vas df, vas deferens.

appears to be encircled by a layer of circular muscle and is characterized by a deep Z-like bend near the midlength.

Ejaculatory apodeme, sperm pump, and aedeagus: The aedeagus is represented by a flexible membranous tube (Fig. 8B), and the sperm pump and ejaculatory apodeme are absent. Dorsally, the aedeagus and flexible phallotrema are supported by the parameres, and ventrally by the ventral or gonocoxal plate (Fig. 8A, B). The parameres are normally paired structures, articulated with the gonocoxal apodemes. In Thaumaleidae, the ventral plate (= gonocoxal plate) is a simple, rectangular, thinly sclerotized plate broadly fused laterally to the gonocoxites (Sinclair, 2000: fig. 17). This plate lies nearly parallel to the gonocoxites, angled obliquely dorsally.

Remarks: The male genital tract of Thaumaleidae is here described and illustrated in detail for the first time. The configuration of the male tract was briefly described by Sinclair (1992a), but no figures were provided. In this study, three species were examined in detail and the configuration of each conforms to that of other Chironomoidea.

The homology of the anterior-most chamber in *Austrothaumalea zentae* remains uncertain. This small chamber is probably associated with the subapical

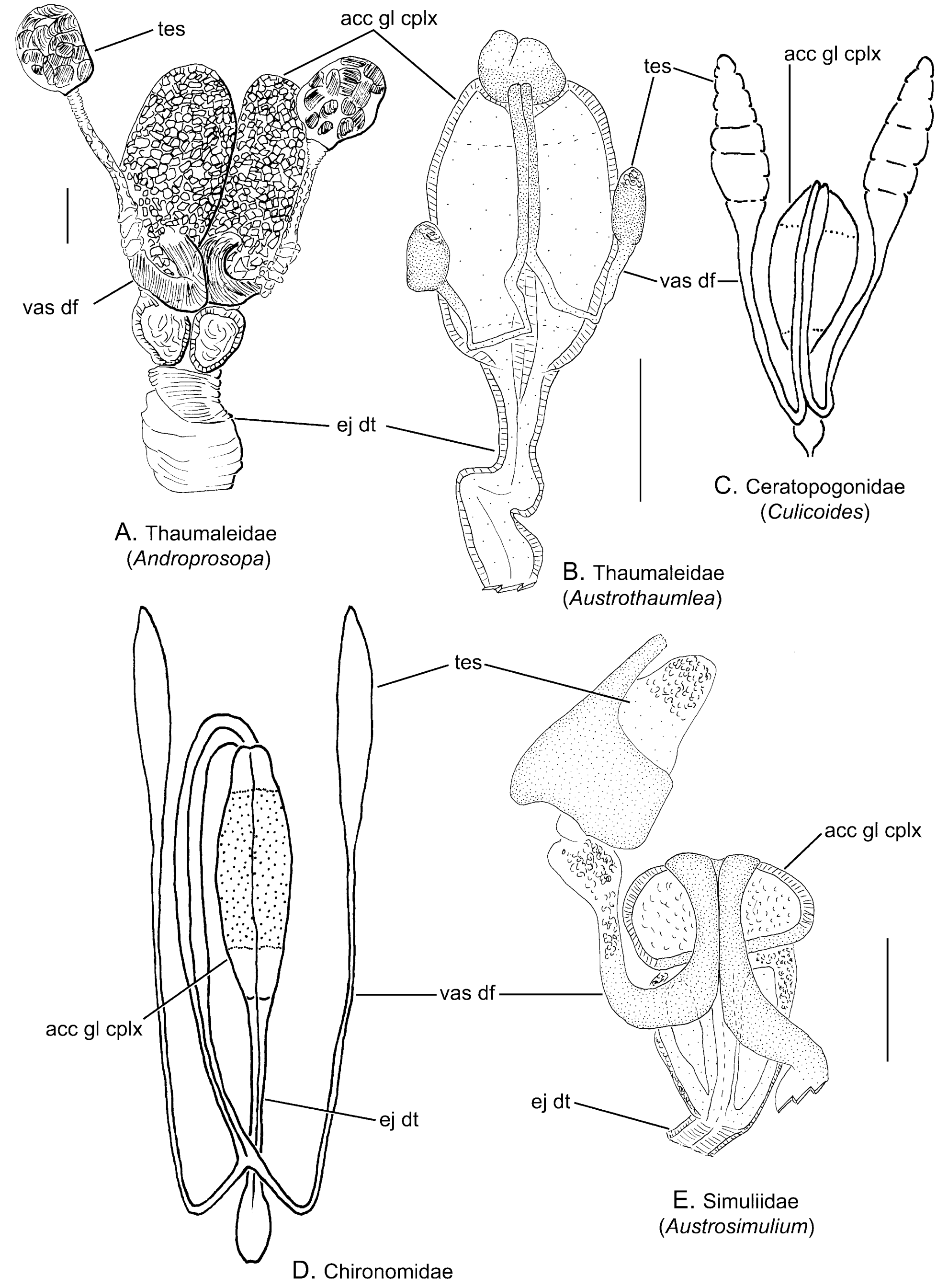


Figure 7. Male internal reproductive organs (dorsal view). A, *Androprosopa japonica* (Thaumaleidae). B, *Austrothaumalea zentae* (Thaumaleidae) (based on the specimen photographed in Fig. 5D). C, *Culicoides nubeculosus* (Meigen) (Ceratopogonidae) [modified from Pomerantzev (1932)]. D, *Chironomus plumosus* (L.) (Chironomidae) [modified from Wensler & Rempel (1962)]. E, *Austrosimulium furiosum* species group (Simuliidae) (based on the specimen photographed in Fig. 5E). acc gl cplx, accessory gland complex; ej dt, ejaculatory duct; tes, testis; vas df, vas deferens. Scale bars = 0.1 mm.

position of the vas deferens and may function as a seminal vesicle. The subapical termination of the vasa deferentia in Thaumaleidae is similar to that of the Dixidae, whereas in other Chironomoidea the ducts enter apically.

SIMULIIDAE

Description (Figs 5E, 7E)

Testis: Each testis is ovoid, pear-shaped or an elongate tapered organ, clothed in pigment granules or a fat

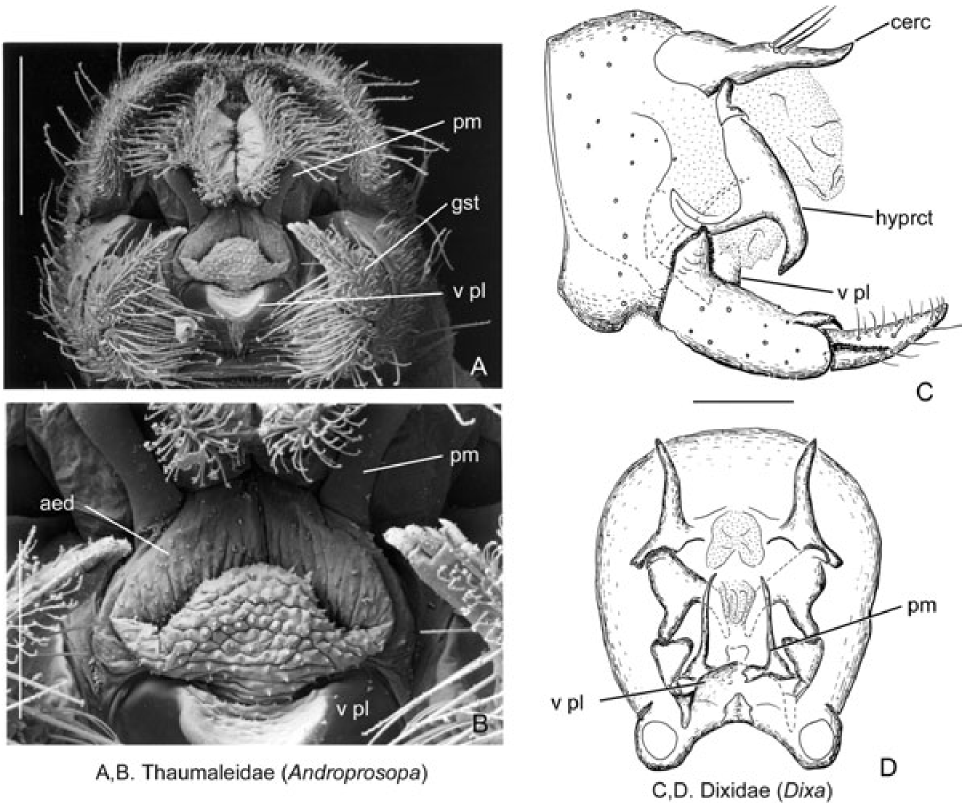


Figure 8. Male external terminalia. A, *Androprosopa americana* (Thaumaleidae) (posterior view). B, *Androprosopa americana* (Thaumaleidae) (posterior view). C, *Dixella nicholsoni* (Dixidae) (lateral view). D, *Dixella nicholsoni* (Dixidae) (posterior view; gonostyli removed). aed, aedeagus; cerc, cercus; gst, gonostylus; hyprct, hypoproct; pm, paramere; v pl, ventral plate. Scale bars = 0.1 mm, except in B scale bar = 0.05 mm.

body (Crosskey, 1990). The internal details are outlined by Raminani & Crupp (1978) and Rubtsov (1989).

Epididymis: Not differentiated.

Vas deferens: The vasa deferentia are short, a little longer than the testes. The ducts curve anteriorly to lie juxtaposed on the dorsal surface of the accessory gland complex. Each duct is rather broad, stout and enters the anterior chamber separately (Jobling & Lewis, 1987). Spermatozoa normally fill the distal portion of the vasa deferentia (Raminani & Crupp, 1978). The ducts are also often covered in pigmented fat cells.

Accessory gland and seminal vesicle: Each side of the accessory gland complex is two-chambered. The anterior chamber is oval and granular secretory material has been observed inside (Raminani & Crupp, 1978). The walls of this chamber are rich in glandular cells (Jobling & Lewis, 1987; Rubtsov, 1989: fig. 10A, fpsd). The second pair of chambers is elongate, approximately twice as long as the anterior chamber and more than three times as long as wide (Fig. 7E). Strong musculature encircles the anterior glands (Rubtsov, 1989: fig. 10A, epsd).

Ejaculatory duct: A pair of ducts exits the constricted end of the second pair of chambers, but remains divided until near the base of the aedeagus. The duct is rather stout, containing circular muscle.

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme and sperm pump are absent in Simuliidae. The aedeagus is mostly membranous, although a dorsal plate is occasionally present in the dorsal wall of the aedeagus. The median sclerite is viewed as a dorsal extension of the ventral plate rather than derived from the aedeagus (Adler, Currie & wood, 2004). The ventral plate (ventral aedeagal guide) articulates with the gonocoxite and serves to aid in opening the female genital chamber by lifting the anal lobes (Wood, 1978).

Remarks: In Simuliidae, two chambers within the accessory gland complex are present (Fig. 7E; Raminani & Crupp, 1978; Rubtsov, 1989; Crosskey, 1990), in contrast to the three chambers observed in other Chironomoidea.

Spermatophores have been confirmed in Simuliidae, with detailed descriptions of their shape and position during copulation (Davies, 1965; Wenk, 1965). A spermatophore in Simuliidae is an organized structure containing two packets of spermatozoa (Wood, 1978), which are partly separated by an internal ridge or groove (Davies, 1965). The presence of two separate packets is considered to have resulted from spermatozoa from each of the two halves of the accessory gland complex coming together within the ejaculatory duct (Wood, 1978).

CHIRONOMIDAE

Description (Figs 5F, 7D)

Testis: Each testis is a narrow, spindle-shaped gland (Abul-Nasr, 1950).

Epididymis: Not differentiated.

Vas deferens: The vasa deferentia are thread-like at the base of the testes, but expand when they abruptly turn and become juxtaposed. They lie to one side and do not lie on the dorsal surface of the accessory gland complex. The ducts are particularly enlarged near the anterior end, packed with spermatozoa. The ducts terminate at the anterior end of the accessory gland complex. The walls of the ducts are thin, often with an outer layer of fat cells (Wensler & Rempel, 1962: fig. 21).

Accessory gland and seminal vesicle: The accessory gland complex is fused into a slender, paired three-chambered complex (Wensler & Rempel, 1962: fig. 14; Abul-Nasr, 1950: fig. 9). Each section or chamber is separated by a plug (Wensler & Rempel, 1962). The anterior and posterior sections are short with large oval vacuoles in the epithelium, whereas the middle section is more than twice the length of either of the other chambers, with hexagonal epithelial vacuoles. The divisions of this glandular complex are readily differentiated in whole mounts. The first and third sections are pale compared with the dark middle chamber. The specific function of each section remains unknown in this family.

Ejaculatory duct: A small valve separates the accessory gland complex from the ejaculatory ducts. The paired ducts are bound by circular muscle and remain paired and enter the base of the aedeagus separately (Abul-Nasr, 1950). This duct is nonglandular throughout.

Ejaculatory apodeme, sperm pump, and aedeagus: As in all other Chironomoidea, the ejaculatory apodeme is absent and the aedeagus is membranous. The homology of the ventral plate remains unconfirmed in Chironomidae. Wood & Borkent (1982) considered the ventral sclerites fused between the gonocoxites of the genus *Buchonomyia* Fittkau as probable homologues, resembling the condition in thaumaleids. Dorsomedial lobes in several other genera are positioned where a ventral plate occurs in other Chironomoidea.

Remarks: The production of spermatophores has been verified in the Chironomidae, each characterized by two internal cavities (Nielsen, 1959).

CERATOPOGONIDAE

Description (Fig. 7C)

Testis: Each testis is narrow, spindle-shaped, tapered anteriorly, and organized into zones.

Epididymis: Not differentiated.

Vas deferens: The vasa deferentia are packed with mature spermatozoa, even at emergence (Linley, 1981). The ducts extend posteriorly to near segment 9, where they bend abruptly back, become fused to the midline of the accessory gland complex, and run juxtaposed to the anterior apex of the gland. The ducts enter their respective half of the gland through a sphincter (Linley, 1981).

Accessory gland and seminal vesicle: The accessory gland complex comprises three pairs of chambers in Ceratopogonidae, united along the midline. The most anterior chamber was referred to as the seminal vesicle by Linley (1981), and is lined with an inner layer of narrow secretory cells with spherical vacuoles containing secretions. A narrow aperture separates the seminal vesicle from the middle chamber, referred to as the glutinous gland (Linley, 1981). The secretory cells of this chamber produce a viscid material, which forms the envelope of the spermatophore. The third chamber is not easily observed in whole mounts and is not lined with glandular cells. The entire accessory gland complex is covered in a layer of circular muscle.

Ejaculatory duct: This duct comprises two sections. In the anterior half, the ducts remain separate, whereas the ducts unite near the base of the genitalia and in this section the duct is lined with columnar glandular cells (Linley, 1981). The ejaculatory duct is sheathed in a layer of circular muscles.

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme is absent and the true aedeagus is membranous. The ventral plate with its recurved tip, present in most Ceratopogonidae, is pressed into the opening of the dorsal wall of the common female spermathecal duct and may aid in stretching the opening (Linley, 1981). The ventral plate (termed aedeagus by authors in this family) is present throughout the family, including *Austroconops* Wirth

& Lee, an early lineage of Ceratopogonidae (Borkent, Wirth & Dyce, 1987; Borkent & Craig, 2004).

Remarks: The male genital tract of Ceratopogonidae was first described by Pomerantzev (1932) and the method of spermatophore formation in *Culicoides* Latreille was thoroughly described and excellently illustrated by Linley (1981). Two packages of sperm are prepared in the long, narrow, tapering glutinous gland (middle chamber) and are pushed into paired, secreted sheaths upon exiting, which fuse medially further down the ejaculatory duct. The spermatophore is then passed to the female during copulation. As observed by Linley (1981), spermatozoa remain in the vasa deferentia until ejaculation. In the present study, spermatozoa-packed vasa deferentia were observed in freshly collected, nonmated males of Bibionidae and Thaumaleidae (Figs 3B, D, 7A), suggesting a similar process of spermatophore production as is present in Ceratopogonidae.

Remarks – features of Chironomoidea. The accessory gland complex of Chironomoidea is similar in form among all four families. The elongate second chamber with its glandular cells is similar in Ceratopogonidae, Simuliidae (Fig. 5C; Rubtsov, 1989: fig. 10A, epsd), and Chironomidae (Fig. 5F). Only in Thaumaleidae do the vasa deferentia enter the accessory/seminal gland subapically (Fig. 7A).

The genital tract of the Chironomoidea is modified for the passage of a preformed spermatophore (Wood, 1978; Wood & Borkent, 1989), the existence of which has been confirmed in the families Simuliidae, Ceratopogonidae, and Chironomidae, and presumably also the Thaumaleidae, given the configuration of the internal genital tract (Sinclair, 1992a). The enlarged accessory gland complex is quite stout and generally easily identified in whole mount dissections, whereas the vasa deferentia and testes are often more fragile, the latter often distinctively clothed in pigment/fat granules.

CULICOMORPHA – CULICOIDEA

DIXIDAE

Description (Figs 5B, C, 6A, 8C, D)

Testis: Each testis is spindle-shaped and partially pigmented, tapered narrowly anteriorly, and nearly two-thirds as long as the accessory gland complex. In freshly collected swarming males, the internal zones of the testis are apparent, corresponding to the growth and maturation of the spermatozoa.

Epididymis: Not differentiated.

Vas deferens: The vasa deferentia are long and slender, extended to the posterior margin of the united accessory gland complex, where the ducts join medially and extend anteriorly as twin ducts closely abutting each other along the midline of the glands. The ducts enter the accessory gland complex prior to the anterior margin.

Accessory gland and seminal vesicle: In whole-mounted specimens of Dixidae, the accessory gland complex comprises three pairs of chambers, united along the midline. The anterior chamber is rounded, as observed in *Austrothaumalea*, with the vasa deferentia entering near the midlength of its corresponding side of this chamber. The middle chamber is nearly square in dorsoventral view. The third chamber appears cylindrical and narrow, with a small oval pocket at the anterior end, which appears narrowly separated from the remaining chamber. The anterior two chambers are thick walled.

Ejaculatory duct: The accessory gland complex empties posteriorly into short, paired ejaculatory ducts, which fuse medially just prior to the external genitalia, forming a large atrium. The ducts are strongly sinuous at the base of the accessory gland complex, curving sharply ventrally and then dorsally prior to entering the genitalia.

Ejaculatory apodeme, sperm pump, and aedeagus: The ejaculatory apodeme is absent and the ventral plate (= prosophallus) is present, lying between the gonocoxites (Fig. 8C, D). The aedeagus is membranous (see the discussion of its homology in 'Remarks'). The parameres are complex structures, often with long slender aedeagal guides.

Remarks: The Dixidae are considered to represent the sister group to the remaining Culicoidea, but despite its important phylogenetic position, the male genital tract has not been previously described and illustrated. Its configuration (based on four species) is extremely similar to that observed in the Chironomoidea and very unlike that of the remaining Culicoidea. Given the structure of the accessory gland complex, the formation of a preformed spermatophore is predicted in Dixidae.

As suggested by Belkin (1968), the 'aedeagus' of Dixidae (Fig. 8C, D) and Culicidae is probably a secondary subdivision of the paramere (= phallus *sensu* Belkin, 1968), and hence a sclerotized aedeagus is considered absent in all Culicomorpha (Sinclair, 2000). Downes (1968) viewed the slender 'aedeagal' filaments in southern Chilean Dixidae (Edwards, 1930: fig. 13) as representing a rigid-walled duct, but this was not observed in specimens examined in this study. These long filaments are probably homologous to the elongate structure labelled 'aedeagus' and ventral plate (= prosophallus) of *Dixella californica* (Johannsen) by Wood & Borkent (1982: fig. 9) and Wood (1991: fig. 11).

This long, filamentous structure in this species is not tubular, but merely a slender plate that possibly functions as an aedeagal guide. These slender processes are interpreted here as parameral in origin.

The ventral plate in Ceratopogonidae is used in the alignment of the spermatophore (Linley & Adams, 1971), where the tip of this plate ('aedeagus') is applied to the dorsal wall of the female spermathecal duct. The long filamentous paramere and the ventral plate (prosophallus) in some dixids could function in a similar manner, but also inserted into the correspondingly long female duct to ensure the passage of spermatozoa. The spermatophores of these species possibly possess a long neck, similar to that of *Culicoides melleus* (Coquillett) (see Linley & Adams, 1971: fig. 1) and the filamentous paramere and the ventral plate act as guides for correct insertion and positioning of the spermatophore.

CORETHRELLIDAE

Description (Fig. 6B)

Testis: Each testis is an elongate spindle-shaped organ, similar to that of other groups, with internal zones organized into the various stages of spermatozoan growth and maturation (McKeever, 1985).

Epididymis: Not differentiated.

Vas deferens: The vasa deferentia are a continuation of the outer walls of the testes, uniform and separate throughout their length, containing spermatozoa (McKeever, 1985).

Accessory gland and seminal vesicle: The vasa deferentia unite with the accessory glands, at which point the ducts may be termed seminal vesicles due to the presence of a densely packed mass of spermatozoa. However, these ducts are not enlarged at this point (McKeever, 1985). Each seminal vesicle terminates at a valve, at which point the duct continues and opens into the forked ejaculatory duct. The paired accessory glands are spherical and also open at the vasa deferentia–ejaculatory duct junction (McKeever, 1985). These glands are lined with tall inner glandular cells.

Ejaculatory duct: The ejaculatory duct begins as a pair of ducts each continuous with the vas deferens, uniting distally (McKeever, 1985). The duct is sheathed in circular muscle and is continuous posteriorly with the aedeagal sheath.

Ejaculatory apodeme, sperm pump, and aedeagus: There is no equivalent of the simuliid ventral plate in Corethrellidae (Wood & Borkent, 1982; McKeever, 1985). As in other families of Culicoidea, the ejaculatory apodeme is absent. The aedeagus is membranous, tapered to a narrow opening (McKeever, 1985). The only sclerotized structures that are readily observed are the fused parameres (penis valves) that form a V-shaped or tubular structure (Belkin, 1968; McKeever, 1985).

Remarks: The internal male genital tract was detailed by McKeever (1985). In contrast to the Culicidae, the seminal vesicles of *Corethrella* Coquillett (the only genus in the family) are not enlarged and although spermatozoa are found throughout the vasa deferentia, they form a densely packed mass in the seminal vesicle. The structure and arrangement of the accessory glands, seminal vesicles (weakly differentiated from vasa deferentia), ejaculatory duct, and parameres are similar in *Anopheles* Meigen (Lum, 1961a) and *Corethrella* (McKeever, 1985).

CHAOBORIDAE

Remarks: The internal arrangement of the male genital tract in Chaoboridae has not been investigated. Given the similarities of the male genital structures (membranous aedeagus, paramere, ventral plate absent) with Corethrellidae (Wood, 1991) and its known phylogenetic position as the sister group of the Culicidae, we predict that the genital tract is either very similar to those of Corethrellidae and Culicidae or show some further modifications.

CULICIDAE

Description (Fig. 6C)

Testis: Each testis is pyriform in shape, often partially clothed in a thick pigmented fat body. The testis is divided internally into a number of clearly defined zones (Hodapp & Jones, 1961).

Epididymis: Not differentiated.

Vas deferens: The ducts are initially thin walled, but distally a circular muscle sheath occurs (Hodapp & Jones, 1961). At the level of the accessory glands, the ducts of the vasa deferentia are enlarged and the seminal vesicle is differentiated (Jobling & Lewis, 1987).

Accessory gland and seminal vesicle: The accessory gland and seminal vesicle are separate glands. The seminal vesicles are either completely separated from each other or are fused through part or all of their length (Hodapp & Jones, 1961; Lum, 1961a; Jobling & Lewis, 1987). The chambers are united just prior to the entrance to the ejaculatory duct. The accessory glands are either spherical or pyriform glands separated throughout their lengths. The contents of both seminal vesicles and accessory glands empty separately into the ejaculatory duct (Hodapp & Jones, 1961). The accessory gland of *Aedes aegypti* (L.) is separated into an anterior and posterior zone on the basis of different secretory cells (Chen, 1984).

Ejaculatory duct: The ejaculatory duct is generally short, sheathed in circular muscle.

Ejaculatory apodeme, sperm pump, and aedeagus: The true aedeagus is membranous in Culicidae (see 'Remarks'). The ventral plate (= claspettes) is a complex structure in this family, with detailed arrangements of bristles and processes (Wood, 1991). The ejaculatory apodeme and sperm pump are absent in Culicidae.

Remarks: As suggested by Belkin (1968), the 'aedeagus' of Culicidae is probably a secondary subdivision of the paramere (phallus *sensu* Belkin, 1968), and hence a sclerotized true aedeagus is considered absent in all Culicomorpha (Sinclair, 2000). Consequently, the structures in red in Wood (1991: figs 11, 12 and 14, but not 15) represent articulated lobes of parameral origin. Nevertheless, this sclerite is trough-like and restricts the opening of the phallotrema.

The shape of accessory glands is variable among and within genera of the Culicidae (Hodapp & Jones, 1961; Lum, 1961a), ranging from spherical to tubular or pyriform. Secretions of the male accessory glands in some *Aedes* mosquitoes stimulate oviposition and induce monogamy (Chen, 1984).

Remarks – features of Culicoidea: Among the four families recognized in the Culicoidea, the shape and details of the male genital tract are very similar in both the Culicidae (Christophers, 1960; Hodapp & Jones, 1961; Lum, 1961a; Jobling & Lewis, 1987) and the Corethrellidae (McKeever, 1985).

A mating plug is produced in some Culicidae (Lum, 1961b; Giglioli & Mason, 1966), secreted from the accessory glands following seminal emission. It has been considered a vestige of the spermatophore rather than a specialized structure (Giglioli & Mason, 1966; Gerber, 1970). Wood (1978) did not consider the mating plug in Culicidae as homologous to the spermatophore of the Chironomoidea because it is deposited following deposition of the spermatozoa.

BRACHYCERA

TABANOMORPHA

Description

Testis: The testes are rounded to spindle-shaped (Loew, 1841; Dufour, 1851; Bonhag, 1951).

Epididymis: Not differentiated.

Vas deferens: In Rhagionidae, the ducts are long and slender and extend far posteriorly to near the posterior end of the accessory glands before arching sharply anteriorly to enter the anterior apex of the accessory glands (Loew, 1841; Dufour, 1851). The vasa deferentia run separately but parallel to one another above the accessory glands and fuse prior to entering. The ducts in Tabanidae are shorter and extend to only the anterior apex of the accessory glands before arching sharply anteriorly (Dufour, 1851) or enter the glands laterally (Bonhag, 1951).

Accessory gland and seminal vesicle: The accessory glands are elongate and tubular, medially separated in Rhagionidae emptying separately into the ejaculatory duct (Dufour, 1851). In contrast, the accessory gland in Tabanidae is somewhat heart-shaped, closely approximated or fused medially, tapered posteriorly and enters the ejaculatory duct separately (Dufour, 1851). No seminal vesicle is present.

Ejaculatory duct: A long and slender duct starts from the junction of the accessory glands and extends to the sperm pump.

Ejaculatory apodeme, sperm pump, and aedeagus: The Tabanomorpha are characterized by a sclerotized aedeagus with a narrow phallotrema and a sperm pump with a distinct ejaculatory apodeme and lateral ejaculatory processes (Sinclair *et al*., 1994). The apodeme compresses the sperm sac (or endophallus), which forces the sperm out through the phallotrema (Bonhag, 1951).

STRATIOMYOMORPHA, BOMBYLOIDEA, ASILOIDEA

Description

Testis: The testes are oval, spindle-shaped or tubular in shape (Loew, 1841; Dufour, 1851). In Asilidae they are long, filiform tubular organs, coiled in a spiral (Dufour, 1851; Owsley, 1946).

Epididymis: Not differentiated.

Vas deferens: The ducts enter separately at the apex of the ejaculatory duct.

Accessory gland and seminal vesicle: The accessory glands are simple and paired, usually long, and tube-like. The accessory glands together with the vasa deferentia enter at the anterior apex of the ejaculatory duct (Dufour, 1851). No seminal vesicle is present.

Ejaculatory duct: A long and slender duct starts from the junction of the accessory glands and vasa deferentia and extends to the sperm pump.

Ejaculatory apodeme, sperm pump, and aedeagus: The lower Brachycera are characterized by a sclerotized phallus with a narrow phallotrema and a sperm pump with a distinct ejaculatory apodeme and usually lateral ejaculatory processes (Sinclair *et al*., 1994).

EREMONEURA

Description (Fig. 2C)

Testis: There is a great variety of form in the testes, ranging from rounded or spindle-shaped, long and tubular to tight coils (Dufour, 1851; Keuchenius, 1913). In Glossinidae, the testis is a spirally coiled tube, enclosed by a thin epithelium (Roberts, 1972).

Epididymis: Not differentiated.

Vas deferens: The ducts enter the anterior end of the ejaculatory duct or enter into an inflated sac-like tube (Loew, 1841; Dufour, 1851), possibly terminating at a small sphincter (Kotrba, 1993b). The ducts unite after a short distance prior to entering the seminal vesicle or ejaculatory duct in many Syrphidae (Ovchinnikova, 1996) and Ephydridae (Loew, 1841). Keuchenius (1913) showed that the vasa deferentia are surrounded by a common sheath, but remain separate. The ducts exiting the testes run directly to the sperm pump, uniting just prior to entering the sperm pump in Sciadoceridae and Opetiidae (Brown, 1992; Cumming, Sinclair & Wood, 1995). These ducts are presumably a fusion of the vasa deferentia and ejaculatory ducts.

Accessory gland and seminal vesicle: The accessory glands are simple and paired, usually long, tube-like and thin walled (Sturtevant, 1926). Often the point of junction between the accessory gland and the vasa deferentia, at the beginning of the ejaculatory duct, is inflated and interpreted as the seminal vesicle. Keuchenius (1913) observed spermatozoa in the vesicle in Syrphidae. The accessory glands enter the sperm pump directly, together with the vasa deferentia (Cumming *et al.*, 1995). In Sciadoceridae, the ducts running between the testes and the sperm pump are enlarged proximally, with thickened glandular walls (Brown, 1992). In Lauxaniidae, the accessory glands are repeatedly branched, forming dense tangles (Sturtevant, 1926). The accessory gland is continuous with the vasa deferentia and ejaculatory duct in Muscidae and Scathophagidae (Hori, 1960). In these families, the vasa deferentia unite into a single duct with thick walls lined with glandular cells (Jobling & Lewis, 1987), referred to here as the accessory gland.

Ejaculatory duct: A long duct starts from the junction of the accessory glands and extends to the sperm pump.

Ejaculatory apodeme, sperm pump, and aedeagus: The Eremoneura are characterized by a sclerotized phallus with a narrow phallotrema and a sperm pump with a distinct ejaculatory apodeme (Cumming *et al.*, 1995). The apodeme compresses the sperm sac (or endophallus), which forces the sperm out through the phallotrema. The ejaculatory apodeme is absent in Glossinidae and/or reduced in the other families of the Hippoboscoidea (Pollock, 1973; Griffiths, 1976; McAlpine, 1989). Otherwise the ejaculatory apodeme is present in most eremoneuran families.

Remarks: In addition to the descriptions of Loew (1841), Dufour (1851) and Keuchenius (1913), the internal male genital tract is also described from the following families: Empididae (Smith, 1969: fig. 16), Dolichopodidae (Irwin, 1974), Opetiidae (Cumming *et al.*, 1995: fig. 16), Sciadoceridae (Brown, 1992), Syrphidae (Ovchinnikova, 1996), Platystomatidae (McAlpine & Kim, 1977), Diopsidae (Kotrba, 1993b), Lauxaniidae (Sturtevant, 1926), Drosophilidae (Chen, 1984), Chloropidae (Schwartz, 1965), Glossinidae (Roberts, 1972), Calliphoridae (Salzer, 1968), Tachinidae (Tschorsnig, 1985), and calyptrate muscoids (Hori, 1960).

Although male *Glossina* Wiedemann produce a spermatophore, the male genital tract appears no different from that of other nonspermatophore-forming Eremoneura (Roberts, 1972; Pollock, 1974). During mating, the secretions of the accessory glands pass to the female first, followed by the spermatozoa (Pollock, 1974). The spermatozoa form a single mass inside the spermatophore (Pollock, 1970, 1974). In Diopsidae, a spermatophore is constructed from secretions of the accessory gland in the female's vagina (Kotrba, 1993b, 1996). It forms a single chamber and the components are transferred by the sperm pump through a narrow opening of the phallus (Kotrba, 1993b, 1996).

Secretions of the male accessory gland in *Drosophila* Fallén reduce sexual receptivity of the mated female and also stimulate oviposition (Chen, 1984).

PHYLOGENETIC IMPLICATIONS

The morphological features and trends observed in the male reproductive system of the lower Diptera are discussed and summarized within a cladistic framework. In the following description of character states we give the plesiomorphic condition as 0 and the apomorphic condition as 1. The characters are traced on the cladogram (Fig. 9) based on our descriptions above.

1. Vasa deferentia configuration: (0) extended posteriorly, not arching anteriorly; (1) U-shaped, apically approximated.

The unique configuration of the vasa deferentia in Mecoptera, Siphonaptera, and many lineages of nematocerous Diptera and some lower Brachycera is quite striking and considered here evidence of a sister-group relationship between these three orders. This configuration of the vasa deferentia occurs in Siphonaptera (Fig. 1C) and Mecoptera (Fig. 1A, B), whereas in Diptera it is present in Bibionomorpha (Fig. 3A–D),

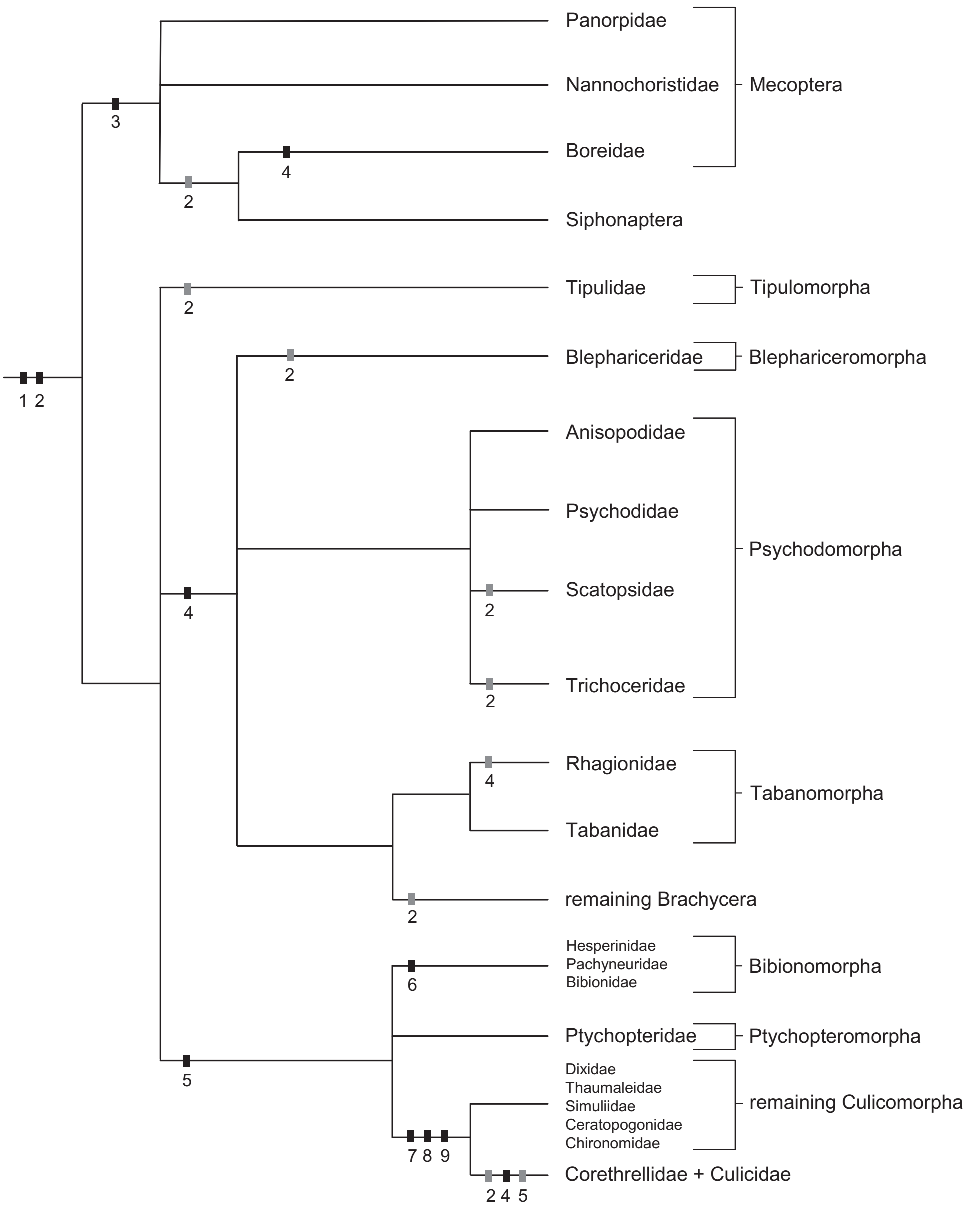


Figure 9. A possible phylogenetic arrangement of Diptera showing the distribution of internal male genitalic features. Black bars indicate the apomorphic condition and grey bars indicate reversals to the plesiomorphic condition. The numbers refer to characters listed below, which are discussed further in the text and scored in the data matrix (Table 2): 1, U-shaped vasa deferentia; 2, accessory gland continuous with vasa deferentia; 3, epididymus present; 4, apical fusion of vasa deferentia absent; 5, multichambered accessory gland; 6, sliding ejaculatory apodeme; 7, ejaculatory apodeme absent; 8, aedeagus membranous; 9, ventral plate present.

some Culicomorpha (Figs 6A, 7A–E), Ptychopteromorpha (Fig. 6D, E), Rhagionidae, and perhaps Trichoceridae (Fig. 2D), Anisopodidae (Fig. 2F), and Tipulidae (Fig. 2A) (although not apically approximated). We assume that secondary losses have occurred in other dipteran lineages, such as Tipulidae, Blephariceromorpha, Psychodomorpha, and most Brachycera. The configuration of the vasa deferentia in Trichoptera (Khalifa, 1949) and Lepidoptera (Kristensen, 2003) is not U-shaped or looped anteriorly.

2. Accessory gland: (0) separate or not continuous with vasa deferentia; (1) continuous with vasa deferentia.

The accessory glands are paired glands that empty into the anterior end of the ejaculatory duct (Snodgrass, 1935). The vasa deferentia do not empty into the anterior ends of the accessory glands in Trichoptera and Lepidoptera (Khalifa, 1949; Kristensen, 2003). In Mecoptera and most lower Diptera (including Tabanomorpha), the vasa deferentia and accessory glands are continuous, with the vasa deferentia emptying directly into the anterior end of the accessory glands (Figs 1A, B, 2E, F, 3, 6A, D, E, 7). Within some Diptera, the accessory glands are not in direct line with the vasa deferentia and are more similar to the basic Insecta condition as described by Snodgrass (1935) [i.e. Tipulidae (Fig. 2A), Trichoceridae (Fig. 2D), Blephariceridae (Fig. 2B), Corethrellidae (Fig. 6B), Culicidae (Fig. 6C), most Brachycera (Fig. 2C), except Tabanomorpha, Muscidae, and Scathophagidae; Loew, 1841; Dufour, 1851; Hori, 1960; Jobling & Lewis, 1987]. The lobes of the accessory glands in Siphonaptera (Fig. 1C) enter the ejaculatory duct separately and are not aligned with the vasa deferentia. The accessory glands also appear mostly separate from the vasa deferentia in Boreidae (Cooper, 1940).

3. Epididymis: (0) absent; (1) present.

The vasa deferentia are coiled at the base of the testes in Mecoptera (including Nannochoristidae) and Siphonaptera, forming the epididymis (Matsuda, 1976; Fig. 1A–C). Spermatozoa are stored in these ducts (Matsuda, 1976). This coiled structure is absent in Trichoptera, Lepidoptera, Diptera, and Strepsiptera, and is only sporadically present in other Insecta (e.g. Orthoptera). Its presence in the above two orders is a synapomorphy of Mecoptera and Siphonaptera. Coiled vasa deferentia are absent in Boreidae, although a small chamber is present at the anterior end of each of the ducts, which stores spermatozoa (Cooper, 1940).

4. Apical fusion of vasa deferentia: (0) present; (1) absent.

The vasa deferentia are apically fused in the ground plan of Diptera, Mecoptera, and Siphonaptera (see character 1). The ducts are not fused apically in some Tipulidae, in all Blephariceridae, Psychodidae, Trichoceridae, Anisopodidae, Scatopsidae, Corethrellidae, and Culicidae, and in early lineages of Brachycera. The separated condition of the vasa deferentia has probably evolved at least twice in Diptera, once as shown in Figure 9 and at least once within the Tipulidae. Among the Brachycera, Rhagionidae and Syrphidae have reverted to the plesiomorphic fused condition.

5. Accessory gland: (0) undivided internally; (1) divided into two to three chambers.

The enlarged accessory glands found in many Mecoptera are undivided internally and their various lobes or appendices do not form a continuous path. The additional lobes appear as discoloured regions on the accessory gland of Nannochoristidae (Fig. 1B) and it is uncertain whether this is homologous to the appendices in other Mecoptera or if they are actually internal subdivisions homologous to those of Diptera (see below). The paired lobes of the accessory glands in Siphonaptera are possibly homologous to those found in Mecoptera. The presence of these accessory gland lobes could be regarded as a synapomorphy of the Mecoptera and Siphonaptera, with perhaps secondary reduction in Nannochoristidae or a synapomorphy of all three orders with reduction in Nannochoristidae and loss in Diptera.

The multichambered accessory glands in Bibionomorpha, Ptychopteridae, and most Culicomorpha are unique within Diptera, with each chamber presumably performing a specific function towards the formation of spermatophores. The function and specialization of each chamber has only been studied in detail in the Ceratopogonidae (Linley, 1981). As in character 2, the separation of the accessory glands from the vasa deferentia in Corethrellidae and Culicidae is viewed as having evolved in their common ancestor independently from the remaining Culicomorpha.

6. Ejaculatory apodeme: (0) compressing the sperm chamber or pump; (1) slides in and out, not compressing the pump.

Following the interpretation of Sinclair (2000), the ejaculatory apodeme aids in the delivery of the spermatophore-forming material to the female vagina in Bibionomorpha.

7. Ejaculatory apodeme: (0) present; (1) absent.

The ejaculatory apodeme is absent in all Culicomorpha, and this appears to be correlated to the passage and presentation of a preformed spermatophore (Wood & Borkent, 1989; Wood, 1991), although spermatophores (as packages of spermatozoa) are not produced in Corethrellidae, Chaoboridae or Culicidae. However, as stated under 'Remarks – features of Culicoidea', a mating plug is secreted from the accessory glands in some Culicidae and it has been considered a vestige of the spermatophore rather than a specialized structure (Giglioli & Mason, 1966; Downes, 1968; Gerber, 1970). Given the phylogenetic relationships discussed here we would agree with the former interpretation.

The absence of the ejaculatory apodeme has traditionally been considered as evidence of spermatophore production in Chironomoidea (e.g. Downes, 1968), but this is certainly not true in other lineages; for example, Bibionomorpha (see character 6) and Diopsidae (Kotrba, 1996). Given the scattered distribution within Diptera, differing delivery methods, and the number of inner chambers, it appears unlikely that spermatophores in all these groups are homologous.

8. Aedeagus: (0) sclerotized; (1) membranous.

The aedeagus is membranous in Culicomorpha, presumably allowing the passage of a preformed spermatophore, although spermatophores containing spermatozoa are not produced in Corethrellidae, Chaoboridae, and Culicidae (but see character 7).

9. Ventral plate or aedeagal guide: (0) absent; (1) present.

The homology of this structure within the Culicomorpha was initially clearly discussed by Wood & Borkent (1982) and discussed further by Wood (1991) and Sinclair (1992a, 2000). We consider the ventral plate to be lost in Corethrellidae and Chaoboridae.

DISCUSSION

This study provides additional evidence for the monophyly of Mecoptera + Siphonaptera + Diptera on the basis of characters 1 and 2 described above. The presence of the epididymis (character 3) is considered a synapomorphy of Mecoptera and Siphonaptera. The presence of accessory gland lobes is possibly also synapomorphic for these two orders. Alternatively, the epididymis and accessory gland lobes could be regarded as secondarily lost in Diptera, with Nannochoristidae and Diptera regarded as sister groups. The latter grouping is supported by the synapomorphies of the sensory pit on the third maxillary palpal segment (adult), the elongate laciniae (adult; also in Siphonaptera), the loss of the galea (adult; also in Siphonaptera), and the loss of the larval eye lens (which is probably correlated with becoming aquatic, the probable plesiotypic condition in Diptera). The latter cannot be scored for Siphonaptera because all known larvae lack eyes. It seems clear that further studies are needed to resolve these conflicting character states.

The apomorphic state of character 4 (loss of the apical fusion of the vasa deferentia) provides fresh evidence that the Psychodomorpha (*sensu* Wood & Borkent, 1989; here including Psychodidae, Trichoceridae, Anisopodidae) are a natural group, with the recognition that the Brachycera are closely related to the Psychodomorpha, as proposed by Woodley (1989) and Sinclair (1992b). The suggestion that Blephariceromorpha (here represented by Blephariceridae) are related to this assemblage has been previously proposed by Courtney (1991) based on four larval synapomorphies.

This analysis and study have also shown that the peculiar genital tract, which Wood & Borkent (1989: character 83) thought was a good indicator of the monophyly of the Chironomoidea (see also Sinclair, 1992a), is far more widespread. Hence, it is no longer pertinent as an indicator of phylogenetic relationships within the Culicomorpha. In addition, a reversal to a separated condition of the accessory gland from the vas deferens provides additional evidence of the monophyly of the Corethrellidae and Culicidae. The latter group probably includes the Chaoboridae, but the male genital tract of this family is unknown.

The modification of the accessory glands into a multichambered complex supports the monophyly of the clade, Bibionomorpha + Ptychopteromorpha + Culicomorpha. The clade is a new phylogenetic arrangement that has not been previously proposed [see Wood & Borkent (1989), Oosterbroek & Courtney (1995), and Yeates & Wiegmann (1999, 2005) for reviews of lower Diptera classification]. We are not, however, proposing that the classification of the lower Diptera should be immediately altered. The characters proposed here are mostly new and demand further analysis and assessment and we consider our results to be tentative.

CHIRONOMOIDEA MONOPHYLY

Support for the monophyly of the Chironomoidea (*sensu* Wood & Borkent, 1989) has been based on the following morphological characters.

Prothoracic larval prolegs present (Wood & Borkent, 1989)

This was originally proposed as a synapomorphy of this lineage due to its presence in Thaumaleidae, Simuliidae, Chironomidae, and early lineages of Ceratopogonidae (Forcipomyiinae and first-instar *Culicoides*) (Wood & Borkent, 1989). More recently, prolegs have been observed in the first-instar larva of *Corethrella appendiculata* Grabham by Borkent & McKeever (1990). They suggested that the presence of the prothoracic prolegs was probably a synapomorphy of the entire Culicomorpha. Additional first instars of all lineages of the Culicomorpha should be examined.

Presence of procerci (Wood & Borkent, 1989)

Cylindrical processes bearing setae are present only in Chironomidae and Thaumaleidae, and assumed to be secondarily lost in Simuliidae and Ceratopogonidae (Wood & Borkent, 1989). Oosterbroek & Courtney (1995) doubted the value and homology of this character. This character appears weak and does not confidently support the monophyly of the Chironomoidea. The homology of the posterior segments of nematocerous Diptera requires a careful morphological study to determine homologous character states. The paired dorsal setae in *Austroconops* are possibly homologous to the procerci of Chironomidae (Borkent & Craig, 2004: fig. 2C–G), but this remains speculative at this point.

Last abdominal segment bearing crochet-tipped posterior prolegs (Oosterbroek & Courtney, 1995)

Oosterbroek & Courtney (1995) considered the presence of crotcheted posterior prolegs as a synapomorphy of the Chironomoidea, but scored the 'hooked spines' in Corethrellidae and Chaoboridae as questionably homologous. We disagree with this interpretation and consider that the details of the 'hooked spines' of Corethrellidae and Chaoboridae are almost certainly homologous to those of Chironomoidea. They are in paired groups and their detailed structure is virtually identical.

The presence of anal prolegs is quite widespread and possibly subject to homoplasy among aquatic families. Within the nematocerous Diptera, posterior prolegs are otherwise present in Tanyderidae and Nymphomyiidae and similar structures are also known in Nannochoristidae (Oosterbroek & Courtney, 1995). Consequently, posterior prolegs may be a ground plan condition of Diptera, but the homologies of this character require careful interpretation. Wood & Borkent (1989) interpreted the presence of posterior prolegs as plesiomorphic for the Chironomoidea. Similar hooked spines are known in the Siphonaptera.

Preformed spermatophore (Wood & Borkent, 1989)

This character was originally proposed as a synapomorphy of Chironomoidea, exclusive of Thaumaleidae (Wood & Borkent, 1989). Sinclair (1992a) and the present study suggest that spermatophores are also probably produced in Thaumaleidae on the basis of the form of the male genital tract. The same evidence from this study also suggests that a preformed spermatophore is produced in Dixidae. Thus, it is highly probable that spermatophore production and the modified genital tract are ground plan features of the Culicomorpha, secondarily lost in Corethrellidae, Chaoboridae, and Culicidae (see 'Culicoidea monophyly').

Given our phylogenetic inferences, spermatophore production and the modification of the accessory gland into a multichambered complex are now viewed as synapomorphies for the Bibionomorpha + Ptychopteromorpha + Culicomorpha clade. Although no material of Tanyderidae was available, we predict that their male genital tract may be similarly modified.

It can be concluded from the above discussion of characters that there appears to be little or no support for the monophyly of the Chironomoidea, as already stated by Borkent & McKeever (1990). Evidence from the current survey does not definitely refute chironomoid monophyly, but simply moves previous synapomorphies (e.g. spermatophore production) down to the base of the Culicomorpha or deeper. Molecular evidence also has not supported the monophyly of the Chironomoidea. Pawlowski *et al*. (1996) tested the phylogeny of the Culicomorpha using partial 28S RNA gene sequences. Their results did not support the traditional subdivision of the Culicomorpha into two superfamilies, the position of Dixidae proved variable and Chironomidae appeared as a possible sister group to the remaining Culicomorpha. In another analysis, Miller, Crabtree & Savage (1997) tested the phylogeny of the Culicomorpha using 18S and 5.8S ribosomal DNA. The monophyletic clade Corethrellidae + Chaoboridae + Culicidae was resolved with the Chironomidae as its sister group. The Chironomoidea did not form a monophyletic group and the phylogenetic relationships of Ceratopogonidae, Simuliidae, and Dixidae were unresolved. Unfortunately a representative of the Thaumaleidae was not included in their study. In both of these studies, the taxon sampling was very limited and there was weak support for most internal nodes (Yeates & Wiegmann, 1999).

CULICOIDEA MONOPHYLY

In all recent morphological phylogenetic studies, the Culicoidea is a well-supported monophyletic group (Wood & Borkent, 1989; Oosterbroek & Courtney, 1995; Saether, 2000). Its monophyly is supported by two unique synapomorphies: large, stout, one-segmented larval antenna, and labiohypopharynx connected to the paraclypeal phragma (Wood & Borkent, 1989; Oosterbroek & Courtney, 1995). The discovery of a modified male genital tract in Dixidae does not contradict the above two synapomorphies, but suggests that the presence of a multichambered accessory gland complex is a ground plan feature of the Culicomorpha, lost secondarily in Culicoidea, exclusive of Dixidae.

In molecular analyses, the monophyly of Corethrellidae + Chaoboridae + Culicidae is strongly supported, but the position of Dixidae is variable (Pawlowski *et al*., 1996; Miller *et al*., 1997).

CULICOMORPHA MONOPHYLY

Saether (2000) suggested that the family Nymphomyiidae was, either alone or together with the Thaumaleidae, the sister group of the remaining Culicomorpha. The style of his presentation, which presented merely a list of character states, a matrix, and concluding cladograms, has made it very difficult to clearly identify the characters used to support these conclusions. We have tentatively determined the following characters from Saether (2000) to be possible synapomorphies for the assignment of Nymphomyiidae: character 4 (reduced number of male flagellomeres); 12 (posterior veins of wing reduced); 14(?) (radial vein with two sectors); 19(?) (gonocoxite with lobes). None of these characters appears very convincing, all are homoplastic, and in our opinion offer little support for the inclusion of Nymphomyiidae in the Culicomorpha.

Specimens of Nymphomyiidae were made available for this study, but only a long longitudinal mass could be observed anterior to the aedeagus. The methods used in this study were not adequate for interpreting such minute specimens and serial sectioning of appropriately preserved material is required. The details of the aedeagus remain uncertain, although the aedeagus appears to be tube-like and weakly sclerotized (Courtney, 1994). It is unlikely that there is a need for a spermatophore (or spermathecae, etc.), as the males and females of at least some species, and perhaps all, oviposit and die *in copula* (Courtney, 1994).

CONCLUSIONS

The complex character state consisting of a sperm pump and its associated ejaculatory apodeme, found in Mecoptera, Siphonaptera, and Diptera, was originally presented by Hennig (1981) as a synapomorphy that formed the basic supporting evidence for grouping these three orders into the Antliophora. Although the sperm pump was recently considered to be nonhomologous among the three orders (Hünefeld & Beutel, 2005), we regard the sperm pump and associated apodeme as part of the ground plan of the Diptera. This conclusion (plesiomorphic within the order) is based on the broad distribution of the sperm pump within the Diptera, including most early lineages (Wood, 1991).

Aside from some families of Culicomorpha, spermatophores have also been confirmed in the Bibionomorpha and the following families of cyclorrhaphan flies: Diopsidae, Sepsidae, and Glossinidae, and perhaps the Phoridae (Kotrba, 1996; Eberhard & Huber, 1998). The form and production of spermatophores differ among several of these groups (e.g. one vs. two inner chambers; or simply a solid mass of sperm), and the genital tract appears correspondingly modified only in the lower Diptera. It is proposed that spermatophores containing two sperm masses in at least a partially divided inner chamber has evolved in the Bibionomorpha + Culicomorpha. Spermatophores in Glossinidae (Pollock, 1970), Diopsidae (Kotrba, 1996), and Sepsidae (Eberhard & Huber, 1998) have, based on their distant phylogenetic position, each probably evolved independently. In these latter three families of Brachycera, a single sperm mass is deposited into the solidified spermatophore, the material of which is secreted by the accessory gland and moulded on to the inner surface of the vagina (Kotrba, 1993a, 1996). Gerber (1970) and Pollock (1972) proposed an alternative scenario, where the presence of spermatophores is proposed as a ground plan feature of Diptera. If so, there would have been a very large number of independent losses of this feature throughout much of the order and we consider that very unlikely, In addition, the different construction of the cyclorrhaphan spermatophore suggests that they are not homologous to that of the nematocerous families. Consequently, we conclude that the evolution of spermatophores in Diptera has occurred independently more than once, based on the mechanism and timing of their formation, and whether they are preformed, or are produced during or after copulation.

The unique multichambered accessory gland complex in Bibionomorpha + Ptychopteromorpha + Culicomorpha requires further study. Histological sectioning to determine and compare cell types of each chamber would be of great interest and assist in homologizing these specialized regions. This would probably lead to further conclusions about phylogenetic relationships among the families and infraorders.

The re-evaluation of the spermatophore-forming multichambered accessory gland complex further weakens cladistic support for the superfamily Chironomoidea. There remains a great need to examine further characters within the Culicomorpha to discover how these families are related. There is a great deal of striking variation in the configuration of the genital tract of nematocerous Diptera, for example within Tipulidae (length and configuration of the vasa deferentia), Thaumaleidae (differences in the size of chambers in the accessory gland complex), and Ptychopteridae, which suggests that the male genital tract will be useful for interpreting intrafamilial relationships.

Our survey of the male genital tract did not include all families. It is especially important that the male genital tracts of Axymyiidae, Nymphomyiidae, and Tanyderidae be examined and illustrated.

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