Towards a phylogeny of the flesh flies (Diptera: Sarcophagidae): morphology and phylogenetic implications of the acrophallus in the subfamily Sarcophaginae

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The morphology of the acrophallus, the distal portion of the male phallus carrying the phallotreme, was studied in 72 exemplar species representing 56 genera and subgenera of the flesh fly subfamily Sarcophaginae. For 42 of those species, scanning electron microscopy was used to clarify the phallic morphology. Terms used to describe the male genitalia were updated based on new interpretations of homology. Male genitalic characters, combined with other morphological characters of adult males and females and of larvae, were used to construct a phylogeny. The monophyly of the subfamily was supported, and some generic-level sister-group relationships proposed in the literature, but without previous cladistic analyses, were also supported. The genus *Blaesoxipha* Loew, as currently recognized, was not monophyletic in our analysis. The genus *Helicobia* Coquillett is synonymized with *Sarcophaga* Meigen syn. nov. and treated as a subgenus of the latter. The *Sarcophaga* subgenera *Neobellieria* Blanchard and *Mehria* Enderlein were not monophyletic. Many of the clades in the analysis were supported primarily or exclusively by male genitalic character states, highlighting the importance of the male genitalia as a source of morphological characters for sarcophagine phylogeny.

INTRODUCTION

Although molecular characters are now widely used to reconstruct the phylogeny of Diptera and to test existing phylogenetic hypotheses, several recent studies have also used novel morphological characters with varying degrees of success (Yeates & Wiegmann, 2005), evidence that morphology as a source of information is far from exhausted in Diptera systematics. This is especially true of morphological character complexes that are difficult to study using conventional methods such as light microscopy, or structures for which homology is difficult to establish amongst taxa. Such character sets are a rich potential source of phylogenetic data that still remains to be explored.

The flesh fly subfamily Sarcophaginae is a well-supported monophyletic group that includes about 1800 described species worldwide, divided into 51 genera (Pape, 1992, 1996). Although flies in this subfamily vary greatly in size, they are externally uniform in appearance. In contrast, the male genitalia, especially the phallus, are highly distinctive at the species level and have long been used in species recognition (e.g. Pandellé, 1896; Aldrich, 1916; Rohdendorf, 1937; Roback, 1954; Pape, 1987; Dahlem & Downes, 1996; Povolný & Verves, 1997).

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The male genitalia also provide important characters for phylogenetic reconstruction (Roback, 1954; Sugiyama & Kano, 1984; Pape, 1994, 1998; Blackith, Blackith & Pape, 1997). However, because sarcophagine male genitalia are highly complex, genitalic characters used in generic diagnoses are often inconsistently defined or have obscure homologies.

Roback (1954) published a detailed morphological study of sarcophagine genitalia and introduced new terms for the phallic structures. He also defined generic relationships within the Sarcophaginae, based primarily on homologies amongst structures in the male genitalia. Although most of Roback's phylogenetic hypotheses have not been tested cladistically and some aspects of his classification were rejected by subsequent authors (Downes, 1955; Lopes, 1956; Pape, 1994), his work was a major contribution to sarcophagine morphology and many of his terms are still used (e.g. Dahlem & Downes, 1996; Mello-Patiu & Pape, 2000).

Pape (1987, 1994), Povolný & Verves (1997), Verves (2000), and Lehrer (2002b) reviewed and updated the morphology of sarcophagine male genitalia. Nevertheless, the homology of some structures remains ambiguous from genus to genus, which hinders their use in phylogenetic analyses. This is particularly true of the structures that make up the (functional) genital opening. In higher Diptera, the acrophallus is the part of the distiphallus (Fig. 2A) bearing the phallotreme (the sperm opening, Sinclair, 2000), which is a reliable landmark that can be considered homologous across the taxa. Because of the complexity and the high degree of sclerotization of the sarcophagine phallus, scanning electron microscopy (SEM) is the most feasible approach for studying its structure. However, other than a few studies of selected taxa (e.g. Leite & Lopes, 1989; Lopes & Leite, 1990, 1991; Chaiwong et al., 2007) SEM has not been used to homologize genitalic structures across a range of sarcophagine genera.

Because of the importance of the male genitalia in species recognition and especially because of the structural complexity of the male genitalia, particularly the acrophallus, in Sarcophaginae, it is reasonable to predict that these structures provide phylogenetically informative characters in determining generic relationships within the subfamily. There are several competing generic classifications within the Sarcophaginae (Downes, 1965; Lopes, 1969, 1982b; Verves, 1986; Shewell, 1987; Povolný & Verves, 1997), most of which were based on regional faunas and were not tested by cladistic analyses. Pape's (1996) classification included all described species of the family but his concept of large genera, sometimes with many subgenera, is not shared by some other researchers in Sarcophagidae (e.g.

Povolný & Verves, 1997; Peris, González-Mora & Mingo, 1998; Kano, Thinh & Kurahashi, 1999; Kano & Kurahashi, 2000; Verves, 2001; Lehrer, 2002a) although the various classifications are largely congruent in taxonomic content, if not the nomenclatural rank assigned to groups. To date, there have been few explicit phylogenetic analyses of sarcophagid genera or subgenera. Roback (1954) and Sugiyama & Kano (1984) both used genitalic, mainly phallic, morphology to reconstruct the phylogeny of the Sarcophaginae, but the former suffers from a noncladistic approach and the latter from a very narrow systematic sampling through a strict focus on the Old World fauna. Lopes (1990) studied the phylogeny of the New World genera with male proclinate orbital bristles. Pape (1994) tested the monophyly of *Blaesoxipha* Loew, but also investigated the phylogenetic relationships of ten sarcophagine genera based on a groundplan scoring approach (Yeates, 1995). Within the large genus *Sarcophaga* Meigen *s.l.* only the subgenus *Helicophagella* Enderlein has been revised using phylogenetic (and phenetic) analyses; Blackith *et al.* (1997) analysed genitalic and nongenitalic character sets combined as well as separately and found that male genitalic characters were more informative for phylogenetic relationships than external morphological characters.

Given that the male genitalia should provide an informative suite of characters for resolving phylogenetic relationships amongst sarcophagine genera and subgenera, and that explicit cladistic analyses are required to resolve the competing classifications within the subfamily, this study had two primary objectives. The first objective was to conduct a comprehensive study of the sarcophagine phallus, including SEM study, documenting and homologizing structures across genera and providing a consistent terminology reflecting homology. The second objective was to test our hypotheses of primary homology confirming synapomorphies in defining genera and subgenera by using genitalic and nongenitalic morphological characters of adult males and females as well as larvae, and constructing a hypothesis on phylogenetic relationships amongst selected genera and subgenera of Sarcophaginae.

MATERIAL AND METHODS

SOURCES OF MATERIAL AND SELECTION OF TAXA

The study was based on material housed in the Zoological Museum, University of Copenhagen, Denmark (ZMUC), the Canadian National Collection of Insects, Ottawa, ON, Canada (CNC), and the Lyman Entomological Museum, McGill University, Ste-Anne-de-Bellevue, QC, Canada (LEM). Given the large number

of taxa in the subfamily, an exemplar approach was used (Yeates, 1995).

Exemplar taxa were chosen to include representatives of major species-rich genera as well as selected smaller genera across the subfamily. Some of the smaller genera were selected because their status relative to other related genera was questionable. Availability of specimens was also taken into account. For most genera and subgenera, a single species (the type species where possible) was selected (Supporting Information Appendix S1). Multiple species of *Blaesoxipha, Helicobia* Coquillett, *Lepidodexia* Brauer & Bergenstamm, *Oxysarcodexia* Townsend, *Peckia* Robineau-Desvoidy, *Ravinia* Robineau-Desvoidy, *Sarcophaga*, and *Titanogrypa* Townsend were included because of high infrageneric variability of the genitalic structures.

For the SEM study, 45 species, representing one genus of Miltogramminae, two genera of Paramacronychiinae, and 19 genera of Sarcophaginae were examined. Species selected for SEM study were those whose phallic morphology is difficult to study using light microscopy. Because of the species richness of the genus *Sarcophaga* a broad sampling of subgenera and species was not feasible. Instead, we restricted the sampling to nine species, representing eight subgenera and chosen from both the New World and Old World.

The cladistic analysis was based on 72 ingroup exemplar species (Supporting Information Appendix S1) representing 19 sarcophagine genera. The number of species used was the same as in the SEM study for most genera except *Lepidodexia* and *Sarcophaga*. Three additional species of *Lepidodexia* subgenus *Johnsonia* Coquillett were added because of the questionable limits of this genus (Pape, 1996). Because of the size of the genus *Sarcophaga* and the variation in phallic structure, 31 subgenera, as well as the unplaced species *Sarcophaga aldrichi* Parker, were included. Type species of subgenera were selected where possible (Supporting Information Appendix S1) and all biogeographical regions were represented. Two subgenera, *Neobellieria* Blanchard and *Mehria* Enderlein, were represented by multiple species (four and two, respectively) because their monophyly is the focus of a related study by the senior author.

To address intraspecific variability in males, at least three specimens of each species were examined except as follows: two specimens each of *Lepidodexia* (*Notochaeta*) *woodi* (Lopes), *Sarcophaga* (*Kramerea*) *schuetzei* Kramer, and *Sarcophaga* (*Phallanthisca*) *magensi* Kano; one specimen each of *Oxyvinia xanthophora* (Schiner) and *Sarcophaga* (*Sinonipponia*) *hervebazini* Ho. Females of 19 species were not available for examination (see characters 31–33, Supporting Information Appendix S1), and larval data were not available for 53 species (see characters 1–2, Supporting Information Appendix S1). Some larval character states used in the study were taken from the literature.

As Paramacronychiinae is the probable sister group of Sarcophaginae, and Miltogramminae is the probable sister group of these two (Pape, 1992, 1996), *Brachicoma devia* (Fallén), *Sarcophila* sp. and *Wohlfahrtia vigil* (Walker) (Paramacronychiinae), and *Macronychia aurata* (Coquillett) (Miltogramminae) were included as outgroups in the analysis.

PREPARATION AND EXAMINATION OF POSTABDOMENS AND LARVAE

The abdomen was removed from pinned male specimens, placed in hot 10% KOH for about 5 min, and transferred to glycerine, where the postabdominal structures, including sternite 5, were separated from the abdomen by cutting the membrane between sternite 4 and 5, and the epandrium was then separated from syntergosternite 7 + 8. If necessary, the postabdomen was returned to hot 10% KOH for about 2 min for further clearing. The epandrium and hypandrium were usually separated to facilitate examination of the phallus. Female terminalia were separated from the abdomen by cutting the membrane between segments 4 and 5 and were cleared in the same way as males.

All structures were rinsed twice in water, once in 70% ethanol, placed in 20% acetic acid for 5–8 min, and washed again in 70% ethanol. Once dried, the male abdomen (excluding genitalia) was glued into its original position on the pinned specimen. Genitalia were examined using a compound or a dissecting microscope and illustrated with the aid of a drawing tube, and subsequently stored in glycerine in a plastic microvial pinned below the source specimen.

Larvae were removed from the abdomen of pinned females when the latter were dissected, examined in glycerine and then stored in glycerine in a microvial pinned below the specimen.

PREPARATION OF PHALLIC STRUCTURES FOR SEM

Genitalia were dissected as described above except that the phallus was dissected in 70% ethanol instead of glycerine. The phallus was separated from the hypandrium, pregonite, and postgonite and given two successive changes of 70% ethanol, followed by two changes of 100% ethanol, and air-dried. Specimens of species with a more membranous phallus [*Macronychia aurata*, *Engelimyia inops* (Walker), *Helicobia surrubea* (Wulp)] were critical-point dried, to prevent the phallus from shrinking or collapsing. Each dried

phallus was glued to an aluminium stub with double-sided carbon adhesive tape and coated with 20 nm of platinum in a high resolution fine coater (Jeol JFC-2300HR). The specimens were examined using a Jeol-JSM-6335F Field emission SEM at the Zoological Museum of Copenhagen, except for specimens in Figures 6A–C, 8F, H, 10A–C, and 18C, F, G, which were gold-coated and examined with a Philips XL30 Environmental SEM at the Electron Microscope Unit of Agriculture and Agri-Food Canada, Ottawa, Ontario.

Usually, two or three phalli per species were examined. One phallus was kept intact and the others were dissected to make the details of the acrophallus more visible. In species for which only one male specimen was available, the coated phallus was first examined intact and then dissected on the stub before being recoated and re-examined.

MORPHOLOGY AND TERMINOLOGY

Terminology for adult structures, except the male genitalia, follows McAlpine (1981). Larval terminology follows Szpila & Pape (2005). The complex structure of the sarcophagine male genitalia has given rise to various interpretations of genital homology and, consequently, many workers on Sarcophagidae have defined their own terms or have modified those of earlier authors; these systems of terminology are not always consistent with terms applied to other families of Diptera. We follow the revised epandrial hypothesis summarized by Cumming, Sinclair & Wood (1995); Sinclair (2000) (Figs 1–3).

We define the acrophallus as the division of the distiphallus (Fig. 2A) bearing the phallotreme or sperm exit, which is sometimes clothed in small denticles (Sinclair, 2000). The sarcophagine
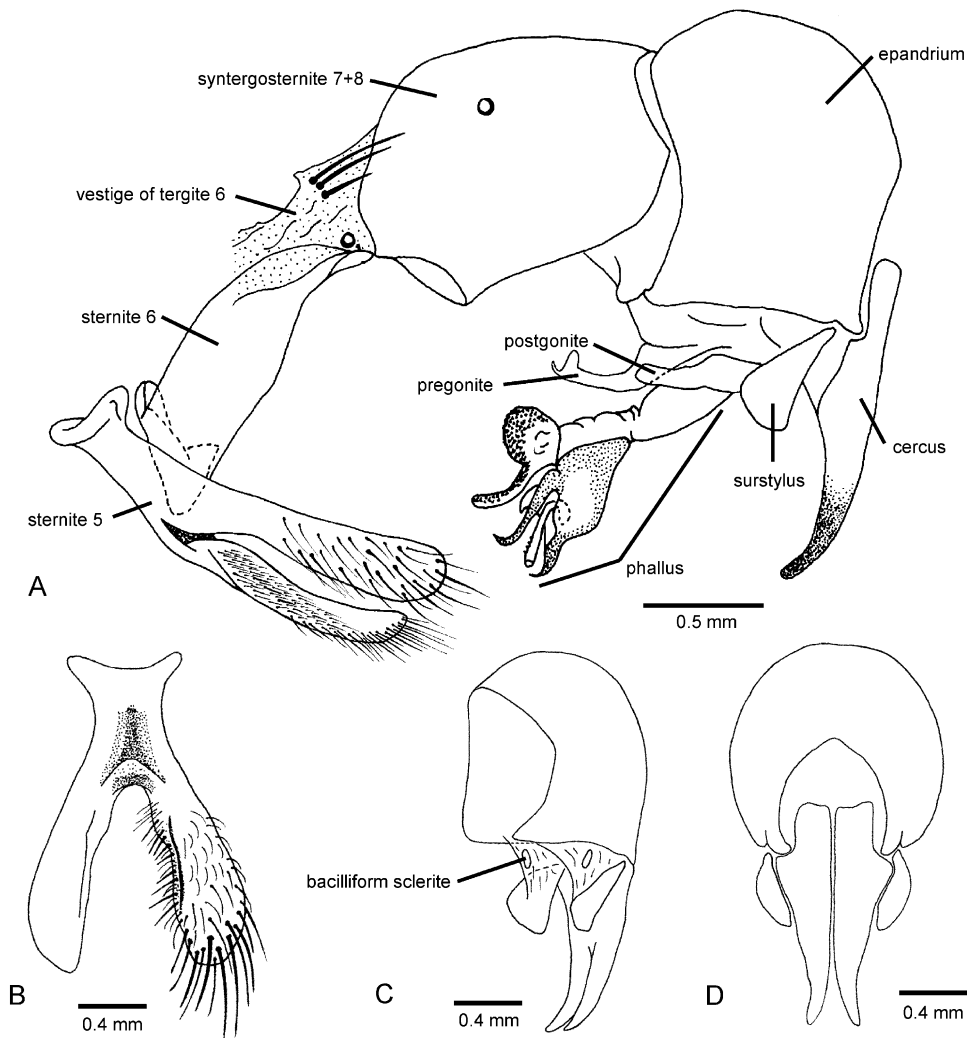

Figure 1. *Sarcophaga* (*Neosarcophaga*) *occidentalis*: A, male postabdomen, left lateral; B, sternite 5, ventral; C, bacilliform sclerites, anterolateral; D, epandrium, cerci and surstyli, posterior.
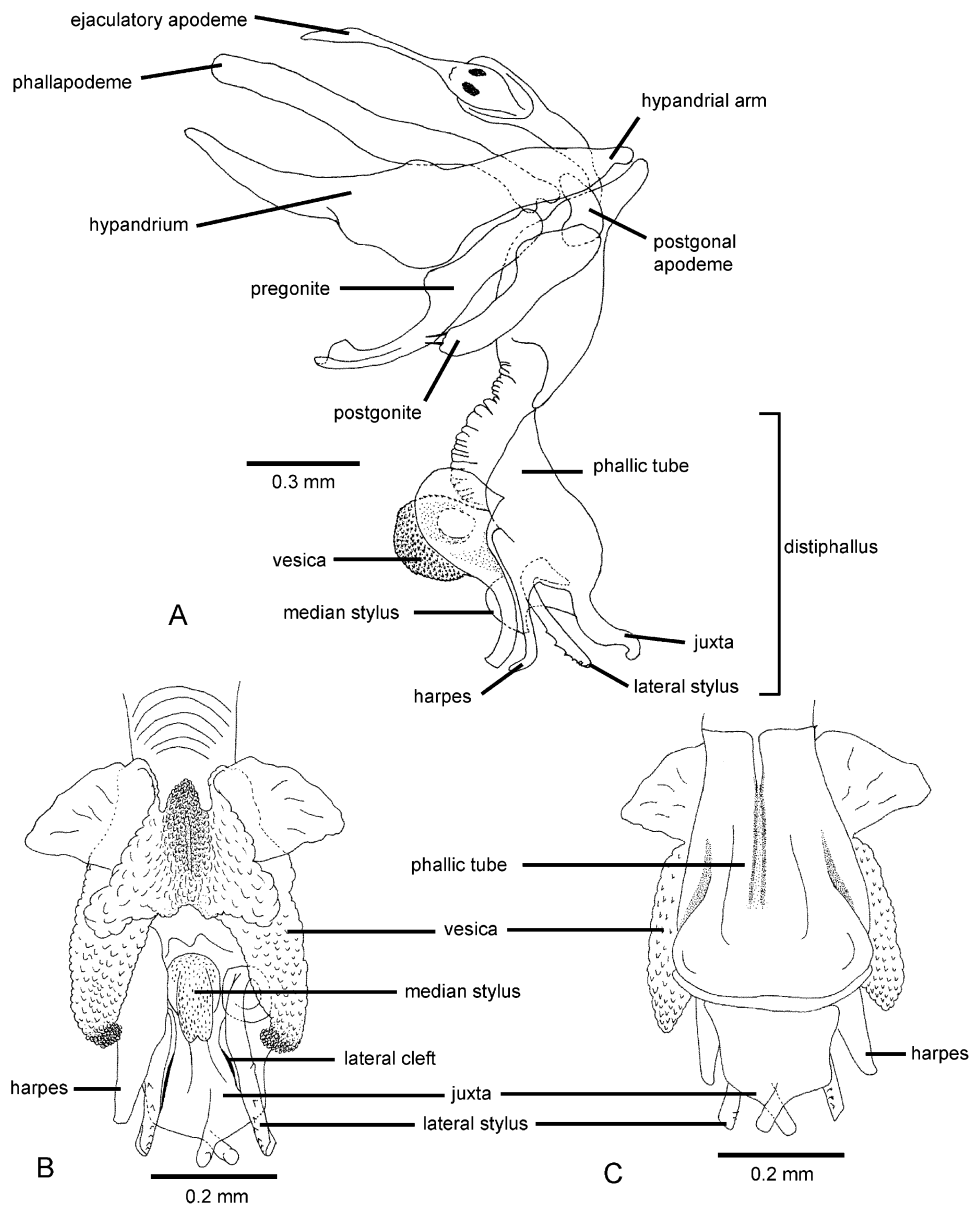
Figure 2. *Sarcophaga* (*Neosarcophaga*) *occidentalis*: A, phallus, left lateral; B, distiphallus, anterior, left harpes removed; C, distiphallus, posterior.

acrophallus in its most widespread configuration comprises the median and lateral styli (Fig. 2B). To homologize the styli across the selected species, the phallotreme and the microserrations, present on the acrophallus in the ground-plan of Miltogramminae and Calliphoridae, were used as landmarks. In addition to some changes in interpretation of homology, the variation in the shape of the vesica, harpes, and juxta (see Fig. 2), and the difficulty in delimiting them in some species led us to modify Roback's (1954) original definitions; these changes are outlined in the Results.

CHARACTER MATRIX AND CLADISTIC ANALYSIS

Seventy-three characters (64 binary, nine multistate) were included in the analysis, including two larval characters, 27 external adult characters, 41 male genitalic characters, and three female genitalic characters (Supporting Information Appendix S2). The matrix was compiled using MESQUITE version 1.05 (Maddison & Maddison, 2004).

Phylogenetic analyses were performed using the program TNT version 1.0, 2005 (Goloboff, Farris & Nixon, 2003). Unweighted and implied weighting
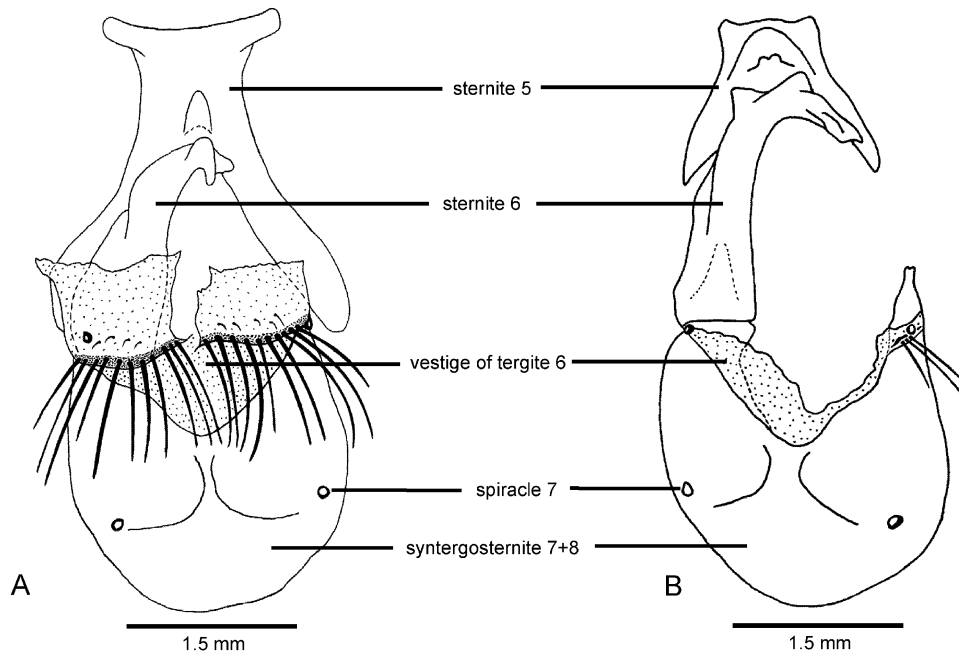
Figure 3. A, *Sarcophaga* (*s.s.*) *carnaria*. B, *Sarcophaga* (*Neobellieria*) *triplasia*. Syntergosternite 7 + 8, vestige of tergite 6, sternites 5 and 6, dorsal.

analyses ($k = 3$) (Goloboff, 1993) were conducted under the parsimony criterion. Multistate characters were treated as non-additive. A heuristic search, using tree-bisection-reconnection (TBR) branch swapping, was conducted in both unweighted and weighted analyses. TBR branch swapping was conducted on 1000 random addition sequences, with ten trees saved per replicate (analyses using different numbers of trees did not provide more conclusive results). Branches were collapsed if the maximum branch length was zero.

The relative degree of support for individual nodes was assessed with bootstrap values calculated from 3000 bootstrap replicates. Relative Bremer support percentages (Goloboff & Farris, 2001) were also used to estimate support for groups recovered in the weighted analysis. Under weighting methods such as implied weighting (Goloboff, 1993), Bremer support may be difficult to compare (Bremer, 1994; Goloboff & Farris, 2001), but relative support is directly comparable (Goloboff & Farris, 2001). Relative support takes into account the amount of favourable/contradictory evidence and gives an idea of the residual number of synapomorphies supporting a branch (Simmons & Webb, 2006). We searched for suboptimal trees that were one step longer than the optimal trees, and a sample of 40 000 suboptimal trees (800 random addition replicates × 50 trees saved per replicate) was used to calculate relative Bremer support values.

RESULTS AND DISCUSSION

REVISED GENITALIC HOMOLOGIES

The SEM study of a broad sample of genera allowed us to reassess some of Roback's (1954) terminology and homology decisions in the male genitalia. In some cases, these revised interpretations provided additional support for monophyletic taxa within the ingroup.

Juxta

Roback (1954) defined the juxta as a ventral (= apical) appendage of the 'corpus' (see Phallic tube, below). However, in some taxa, there is no clear demarcation between the juxta and the phallic tube (e.g. Figs 12C, 21C). Thus, Roback referred to species with 'free' or 'fused' juxta, with intermediates between the two states. We treated the base of the median stylus as a landmark to delimit the site of origin of the juxta and we defined the juxta as any apical extension of the posterior side of the distiphallus arising from the base of the median stylus (Fig. 2). The major phylogenetic implication is that species of both Paramacronychiinae and Sarcophaginae in general have a juxta (character state 50 : 1), whereas Roback considered it absent in Paramacronychiinae. Absence of a juxta within these subfamilies is therefore to be considered as a homoplasious reversal.

Vesica

We define the vesica as a lobe-like structure protruding outwards from the anterior surface and originating close to the base of the distiphallus (e.g. Figs 2, 14D, 21D, 22D).

Harpes

Roback (1954) defined the harpes as a paired, 'anterodorsal' (= anterobasal) extension of the 'corpus' that usually arises at the 'anterodorsal' (= anterobasal) corners of the 'sclerous corpus' (see below) and runs ventromedially from it. We define the harpes as paired, sclerotized processes arising from the anterior margin of the phallic tube distal to the vesica (e.g. Figs 18A, 19A) and spreading ventromedially over the base of the lateral styli (e.g. Fig. 18C, F). As defined by Roback, harpes were present only in his *Sarcophaga* group (a subset of subgenera currently assigned to *Sarcophaga*) and, tentatively, in *Peckia*. In our definition, harpes are present only in *Lepidodexia* and *Sarcophaga*, although their homology is uncertain in some selected species of *Helicobia* (here treated as a subgenus of *Sarcophaga*, see below) and *Lepidodexia*.

Phallic tube

This is the structure that Roback (1954) called the 'corpus'. He defined the corpus as the tubular basal portion of the phallus and further divided it into the 'sclerous corpus' and 'membranous corpus'. Povolný & Verves (1997) considered the corpus *sensu* Roback equivalent to the distiphallus. Roback (1954) also recognized a structure that he referred to as a phallic tube; however, that structure is an 'anteroventral' (= anterodistal) prolongation of the membranous corpus present in a small number of Sarcophaginae. It is not homologous with the phallic tube as we define it: the tube-shaped part of the distiphallus surrounding the sperm duct and supporting processes such as the harpes and the vesica (e.g. Figs 8A, 11A, 18A, 19A).

Median stylus

This structure includes the capitis and the median process of Roback (1954). Pape (1987) was the first to use the term 'median stylus' for this structure. Roback defined the capitis as a cap or helmet-like structure on the median process of his *Sarcophaga* group and *Helicobia*. However, this structure is simply the distal part of the median stylus and needs no special term. The apex of the median stylus is bifurcate in *Sarcophaga* (e.g. Figs 8D, F, 9C, 18D, F, H) and is sometimes bulbous (e.g. Fig. 18C, E, G). Roback's 'median process' is the keel-shaped base of the median style. In *Sarcophaga* this rather large, plate-like structure merges into the base of the juxta.

Hillae

Roback (1954) proposed this term for specific structures found in the genus *Ravinia*. The hillae are paired, tube-like (sometimes hatchet-like) structures protruding outwards from the anterior surface of the distiphallus proximally to the lateral and median styli and distally to the vesica (Fig. 14). The hillae can be grooved (Fig. 15E) or not (Fig. 15F), with (Fig. 14A, B, E, F, H) or without (Fig. 14C, D, G) a membranous bladder. The hillae have been considered derivatives of lateral styli (Verves, 2000) or the lateral styli themselves (Pape, 1994). However, our observations did not support these hypotheses, in that the hillae do not take part in the formation of the acrophallus proper, i.e. the lateral plus median styli (Fig. 15C). Also, what we consider as lateral styli are present, although flattened (Figs 15G, 16A–F), in some of the species of *Ravinia* studied, although apparently absent (Fig. 16G–H) in others.

Tree topology and branch support

The unweighted analysis yielded 100 trees of 379 steps each. The strict consensus tree was poorly resolved and bootstrap and Bremer support values were generally low.

The analysis using implied weighting resulted in 120 most parsimonious trees of 396 steps each (Fig. 27A, B). The most parsimonious trees differed only in arrangements of some nodes within the genus *Sarcophaga*. Because of this, the strict consensus tree (Figs 25, 26) was more resolved than that from the unweighted analysis. Bootstrap and relative Bremer support values (calculated from 34 205 suboptimal trees) were generally low in the weighted analysis (Figs 27, 28).

The low bootstrap values and relative Bremer support in many nodes of both analyses were an indication of the amount of homoplasy in the character matrix. Relatively few monophyletic groups were supported by uniquely derived character states. We do not consider this a weakness in the data matrix arising from the choice of characters or exemplar taxa. Sarcophagidae, like other families of higher Diptera, are subject to extensive homoplasy in morphological characters (e.g. Couri & Pont, 2000; O'Hara, 2002); this makes it difficult to obtain high branch support based on measures like bootstrapping or Bremer support. Nevertheless, the weighted trees were well resolved, even though they were based primarily on homoplasious character states.

GENERIC MONOPHYLY AND SUPRAGENERIC RELATIONSHIPS

Subfamily limits

Because the monophyly of the Sarcophaginae has been well demonstrated previously (Pape, 1996), testing it was not a primary objective of this study. Thus, known autapomorphies for the subfamily were not necessarily included in the matrix. Despite this, monophyly of the Sarcophaginae was supported by multiple character states (Fig. 27A). Previous studies (Pape, 1992; Wells, Pape & Sperling, 2001) placed Paramacronychiinae as the sister group of Sarcophaginae; this relationship was supported by two uniquely derived character states in our analysis: the possession of a juxta (50 : 1) and an anteriorly displaced acrophallus (54 : 1) (Figs 21A, 23B–F, 27A), as well as three other character states: a convex postcranium (6 : 1), loss of presutural acrostichal setae (13 : 1), and female tergite 8 vestigial or absent (32 : 1). The polarity and/or distribution of the three latter characters could, however, be altered by the inclusion of additional outgroups.

All species of Miltogrammmine and Paramacronychiinae have a simple acrophallus (Fig. 23), except the miltogrammine *Senotainia trifida* Pape, in which it is tripartite (Pape, 1989). In the Sarcophaginae the acrophallus is generally a complex tripartite structure (Pape, 1989). Pape (1992, 1996) suggested that the presence of a simple acrophallus in some Sarcophaginae represents a secondary loss because the tripartite acrophallus (e.g. Figs 6C, 12D, 22G) has apparently evolved only twice in the family. Our phylogeny supported this hypothesis in that well-developed lateral styli (55 : 1) originated once, at the base of the Sarcophaginae (Fig. 27A), and were secondarily lost (or at least strongly modified) multiple times (Fig. 27), in *Titanogrypa alata* (Aldrich) (Fig. 22B, C), *Ravinia derelicta* (Walker) (Fig. 16G), and *Ravinia effrenata* (Walker) (Fig. 16H) as well as in *Blaesoxipha* (*Gigantotheca*) *plinthopyga* (Wiedemann) (Fig. 4A–D), *Blaesoxipha* (*s.s.*) *setosa* (Salem) (Fig. 4E–F), and *Blaesoxipha* (*Kellymyia*) *kellyi* (Aldrich) (Fig. 4G–I).

The topology of the weighted tree corresponded to that of Wells *et al.* (2001) at the generic and subfamily level, but the trees did not coincide at the subgeneric level within *Sarcophaga*, most likely because of incomplete taxon sampling.

Tricharaea

This clade, represented by *Tricharaea* (*Sarothromyia*) *simplex*, was the sister group to all remaining Sarcophaginae and was defined on four homoplasious character states (Fig. 27A). The basal position of this clade relative to other Sarcophaginae was assumed by some previous authors (Roback, 1954; Lopes, 1982b) and Pape's, (1994) phylogenetic analysis supported that placement. The larvae of *Tricharaea* also have a vestigial, mostly membranous labrum and a clypeal arch situated far from the parastomal bar (Lopes, 1982b).

Five character states, all homoplasious, supported the monophyly of all Sarcophaginae except *Tricharaea* (Fig. 27A).

Ravinia clade

The monophyly of a clade consisting of *Oxysarcodexia* and *Ravinia* was supported in the weighted analysis by a uniquely derived character state, the male mid femoral ctenidium with flattened spines (29 : 1) (Figs 24A, 27A). This clade is the only one other than the *Tricharaea* clade with a desclerotized strip between the basiphallus and distiphallus (45 : 1) (Fig. 27A). This state can be seen as an intermediate state between the complete absence of a demarcation between basiphallus and distiphallus in the Miltogrammmine and Paramacronychiinae (Fig. 23) and the distinct hinge in most other Sarcophaginae (e.g. Figs 11A, 13A, 21A). *Oxysarcodexia* and *Ravinia* have been considered closely related (Roback, 1954; Downes, 1955; Lopes, 1982b) and were sister groups in Pape's (1994) phylogenetic analysis. The *Ravinia* clade is also supported by some first-instar larval character states, particularly the festoon-like configuration of the oral ridges (Downes, 1955; Lopes, 1982b; Leite & Lopes, 1987; Lopes & Leite, 1987; Pape, 1996).

Oxysarcodexia was monophyletic (Fig. 27A), supported by a uniquely derived apomorphy, the lateral triangular extension of the phallic tube above the vesica (49 : 1) (Fig. 12A), and by four homoplasious character states (Fig. 27A).

The monophyly of *Ravinia* was supported by eight apomorphies including the presence of hillae (66 : 1) (Figs 14, 15), uniquely derived in this genus.

Relationships amongst the eight *Ravinia* species included in the analysis were well resolved except for a basal trichotomy (Fig. 27A). The clade including *Ravinia errabunda, R. derelicta*, and *R. effrenata* was supported by three character states, two of which are uniquely derived: the absence of a membranous bladder (67 : 1) (e.g. Figs 14G, 15F) and absence of a groove (68 : 1) (Fig. 15C, F) on the hillae. These three species have been previously placed (with others not included in the present study) in the subgenus (or genus) *Chaetoravinia* Townsend (Dodge, 1956; Downes, 1965; Lopes, 1969). Similarly, species of the clade *Ravinia columbiana* + *Ravinia rufipes*, supported by one apomorphy, the setose third costal sector (19 : 1), have previously been assigned to the genus *Andinoravinia* Townsend (Lopes, 1962, 1969).

The monophyly of all genera above the *Ravinia* clade was supported by a uniquely derived apomorphy: a distinct hinge between basiphallus and distiphallus (45 : 2; e.g. Figs 11A, 13A, 21A) (Fig. 27A).

Dexosarcophaga clade

A sister-group relationship between *Dexosarcophaga* Townsend and *Oxyvinia* Dodge was supported in the weighted analysis by four homoplasious character states (Fig. 27A), including one genitalic character state, a median stylus curved towards the base of the phallus (60 : 2) (Fig. 5D). Bootstrap support and relative Bremer support were low. These two genera had not previously been considered closely related. Dodge (1968) considered *Dexosarcophaga* more closely related to *Oxysarcodexia*. Lopes (1982b) included species of those two genera in two different tribes: Cuculomyiini and Raviniini, in which *Oxyvinia* was tentatively placed. Larvae of *Oxyvinia* have festoon-like oral ridges (Lopes, 1982b; Leite & Lopes, 1987; Pape, 1996) as in the *Ravinia* clade. Unfortunately, larval characters were not very informative because of large amounts of missing data, and we did not include this character in the matrix (Supporting Information Appendix S2). This is one of the regions of the tree where additional larval characters may provide additional, or alternative, resolution of generic relationships.

Although females of the *Dexosarcophaga* clade were not available to be scored in the analysis, Mello-Patiu & Pape (2000) noted an additional apomorphy to those previously listed by Pape (1996) for *Dexosarcophaga*: female tergite 8 with broad and ventrolaterally truncated halves connected medially by a narrow strip.

Dexosarcophaga and *Oxyvinia* were each supported by five homoplasious character states (Fig. 27A).

Cistudinomyia

This monotypic genus was the sister group to all Sarcophaginae above the *Dexosarcophaga* clade, based on a single character state, the presence of a window in male sternite 5 (36 : 1). This character state is variable within well-defined genera (e.g. *Ravinia* and *Blaesoxipha*) and subgenera (see Blackith, Blackith *et al.*, 1997) and this high degree of homoplasy makes this node tenuous. Our placement of *Cistudinomyia* Townsend within the subfamily did not correspond to that usually given in the literature, probably because of differing interpretations of the connection between basiphallus and distiphallus. We scored *Cistudinomyia cistudinis* as having a distinct hinge (45 : 2) (Fig. 6D). In contrast, Roback (1954) included *Cistudinomyia* in his subtribe Raviniina, with *Ravinia* and *Oxysarcodexia*, based on the absence of demarcation between the basiphallus and

distiphallus, and Pape (1994) described *C. cistudinis* as having a distinct desclerotized strip between the basiphallus and distiphallus. Although *Cistudinomyia* itself was supported by four homoplasious character states (Fig. 27A), the placement of the genus within the subfamily should be tested using additional characters or exemplar taxa.

Blaesoxipha clade

This clade included *Comasarcophaga* Hall, *Fletcherimyia* Townsend, *Blaesoxipha*, and *Spirobolomyia* Townsend. The *Blaesoxipha* clade was monophyletic in all trees in the weighted analysis (Figs 25, 27B). Its monophyly was supported by a uniquely derived apomorphy, the bent male cerci (41 : 1), and by the setae of the cercal prong differentiated into spines (42 : 1). In Pape's (1994) analysis of *Blaesoxipha* the same group of genera was considered monophyletic.

A sister-group relationship between *Comasarcophaga* and *Fletcherimyia* was supported by two apomorphies (Fig. 27B). Each of the two genera was supported by multiple apomorphies, mostly in the male genitalia (Fig. 27B). In *Fletcherimyia,* the acrophallus is distinct with the lateral styli loop-shaped (Fig. 7H) and the median stylus reduced to an opening (Fig. 7F). Roback (1954) named this 'wreath-like structure' the stemmatis and suggested a possible homology of its lateral dorsal projections (the current lateral styli) with the hillae, an autapomorphy for *Ravinia*.

Blaesoxipha was not monophyletic in any of the analyses, with *Spirobolomyia* consistently treated as the sister group to *Blaesoxipha* (*Gigantotheca*) *plinthopyga* + *Blaesoxipha* (*Kellymyia*) *kellyi,* and *Blaesoxipha* (*s.s.*) *setosa* as the sister group to these three (Figs 25, 27B). This differs from the conclusions of Pape (1994) who found *Blaesoxipha* monophyletic. The grouping of *Spirobolomyia* as sister group of *B. plinthopyga* + *B. kellyi* was supported by four apomorphies (Fig. 27B) including the setosity of the median occipital sclerite (9 : 1), female tergite 7 absent or vestigial (31 : 1), a pair of dome-shaped protuberances on male sternite 5 (35 : 1), and the absence of demarcation between the juxta and the phallic tube (52 : 0) (Fig. 4A, G).

Pape (1994) homologized the structures on each side of the median stylus (e.g. the two digitate structures of *B. plinthopyga*, Fig. 4B) with the lateral styli of other sarcophagine species. However, our SEM study revealed that those structures are not connected with the sperm duct (e.g. Fig. 4C, D) as are the lateral styli of other species (e.g. Figs 13H, 15G), and an interpretation of the digitate structures as part of the median stylus may therefore be more parsimonious. Even though Pape misinterpreted those lateral structures as lateral styli, their presence could still be

synapomorphic for *Blaesoxipha* spp. Pape (1994) also stated that the lateral styli were fused to each other through a ventromedian bridge (a median plate-like structure) proximal to the median stylus, but this bridge was not found in the species included in the present analysis. The median bridge is visible in many *Blaesoxipha* – especially in subgenus *Acanthodotheca* – and more detailed studies are needed to decide whether this structure will define all or part of the genus. Therefore, two of the five apomorphies used by Pape (1994) to support the monophyly of *Blaesoxipha* – the flattened structures lateral to the median stylus and the median bridge – require reinterpretation, based on methods such as SEM and serial sectioning, for a thorough assessment of the monophyly of the genus. Roback (1954: plate 20, p. 144, figs 281–284) also misinterpreted the lateral styli.

The relationships and limits of the genera in the *Blaesoxipha* clade remain unresolved. Roback (1954) originally placed *Kellymyia* close to *Spirobolomyia* based on the shape of male sternite 5. Downes (1965) included *Fletcherimyia* and *Spirobolomyia* as subgenera of *Blaesoxipha* but omitted *Comasarcophaga*. Lopes (1982b) divided the species included in *Blaesoxipha* by Pape (1994) into two tribes. Pape (1994) treated *Blaesoxipha* as a large genus divided into ten subgenera. Our analysis did not support the monophyly of *Blaesoxipha*; however, our exemplar set included only three of the ten subgenera and a subset of the characters (e.g. characters 41 and 42) used by Pape (1994). A comprehensive phylogenetic analysis incorporating all subgenera, as well as *Spirobolomyia* would be required to resolve the limits of *Blaesoxipha*.

Peckia clade

This clade included *Engelimyia* Lopes, *Peckia*, *Sarcodexia* Townsend, and *Titanogrypa* and was defined by two homoplasious character states (Fig. 27B). The monophyly of the individual genera was supported in the weighted analysis (Figs 25, 27B).

Engelimyia, represented by *Engelimyia inops*, was defined on six apomorphies, five of which were male genitalic character states (Fig. 27B). The position of this genus within the Sarcophaginae has not previously been clearly defined. Lopes (1982b) noted that the phallus was similar to that of *Rafaelia* Townsend but because of differences in head morphology and as no material of the first instar larva was available to him, he did not reach a taxonomic conclusion. In a recent revision of *Engelimyia*, Pape & Mello-Patiu (2006) showed that the four species included in this genus probably fall into two sister-group pairs and that detailed comparative morphological studies or molecular data are needed

for further testing and corroboration of their relationships.

Peckia was supported by seven character states, including three states of male sternite 5 (36 : 0, 39 : 1, 40 : 1). Roback (1954) identified the lateral extension of the phallic tube in *Peckia chrysostoma* (Wiedemann) as 'apparent' harpes pending additional morphological evidence as to their homology. Although the position and point of origin of those lateral extensions agree with our definition, they do not spread ventromedially over the base of the lateral styli (Fig. 13A, B) as, for example, is the case in species of *Sarcophaga* (Fig. 18A, C, F). Therefore, we do not consider them to be true harpes.

The weighted analysis placed *Sarcodexia* as the sister group of *Titanogrypa* (Fig. 27B). The clade was supported by a larval apomorphy: first instar larva with an elongate clypeal arch reaching the parastomal bar (1 : 1), as well as one male genitalic character state. The larval character was scored only for *Titanogrypa alata* and *Sarcodexia lambens* (Supporting Information Appendix S1). Three species of *Peckia* and one of *Titanogrypa*, not included in the analysis, namely *Peckia* (*s.s.*) *pexata* (Wulp), *Peckia* (*Euboettcheria*) *anguilla* (Curran & Walley), *Peckia* (*Euboettcheria*) *gulo* (Fabricius), and *Titanogrypa* (*Cucculomyia*) *larvicida* Lopes all show this character state (Lopes, 1943, 1982b; Méndez & Pape, 2002), whereas *Peckia* (*Pattonella*) *intermutans*, included in the matrix, does not. Further analysis of the distribution of this character state in the clade is needed.

Roback (1954) grouped *Sarcodexia* with the genera *Paraphrissopoda* Townsend and *Euboettcheria* Townsend (both included in *Peckia* by Pape, 1996) but his grouping was primarily based on the possession of 'median filaments'. According to Roback (1954), those filaments resemble the lateral styli but have a different origin; however, we found little evidence of differences in homology between the structures. Roback may have named the structures differently simply because he did not see a median stylus in the species examined. The acrophallus of *Sarcodexia lambens* and all species of *Peckia* examined are similar in that the median stylus is reduced to an opening flanked by tubular lateral styli (Figs 13D, H, 17E–G). Leite & Lopes (1989) also misidentified the lateral styli of *S. lambens* and confused them with the small spiralling tubes found within the apex of the juxta (Fig. 17C). They noted the reduced median stylus (Fig. 17D, E, G) and the presence of a rounded vesica but it is not clear if they were referring to the small sclerite located at the anterodorsal side of the distiphallus (Fig. 17A).

The monophyly of *Titanogrypa* was supported in the weighted analysis by two uniquely derived apomorphies: a basiphallus with a dorsal hump (48 : 1)

(Fig. 22D) and the presence of white setae laterally on the scutellum (15 : 1) (Fig. 27B). The two included species also have five additional apomorphies that are homoplasious.

Microcerella

This clade, represented by *Microcerella spinigena* (Rondani), was supported by seven apomorphies (Fig. 27B), only one of which was a male genitalic character state: the reduced, oval postgonal apodeme (44 : 1). The characteristic shape of the phallic vesica (Fig. 11E) is found in many species of this genus (e.g. Lopes, 1981). Lopes (1982a, b) suggested a sister-group relationship between his tribes Microcerellini and Sarcophagini mainly based on the well-sclerotized mandibles and the complete clypeal arch of the first instar larvae.

Boettcheria

Dahlem & Downes (1996) revised the Nearctic species of this genus, but without a cladistic analysis. In our analysis, *Boettcheria* Parker was the sister group of *Lepidodexia* + *Sarcophaga* based on two apomorphies. The genus itself was supported by seven apomorphies including the uniquely derived vesica with more than three lobes (64 : 4) (Fig. 5A). The presence of a ventromedian pad of short bristles on the male hind trochanter (25 : 1) and of a posteromedian row of spines in both sexes (26 : 1) was noted by Dahlem & Downes (1996). However, they noted that the reduction of the anteroventral bristles on the male hind femur to one or two distal bristles (24 : 1) and a vesica with more than three lobes (64 : 4) (they specified 'vesica trilobed and complex' for the genus as a whole) are unique to *Boettcheria latisterna*. Lopes (1982b) included the subtribe Boettcheriina in the Sarcophagini but considered that the group may be related to the tribe Microcerellini.

The anterior juxtal process of Roback (1954) is visible in Figure 5B, ventral to the median stylus. According to Roback (1954), this sclerotized process, present only in subtribe Boettcheriina, is associated with the well-developed median process of that group. Our observations suggest that this process is a prolongation of the juxta, not fused to the base of the median stylus.

Sarcophaga clade

A sister-group relationship between *Lepidodexia* and *Sarcophaga* was supported in the weighted analysis (Fig. 25), based on the possession of two uniquely derived apomorphies: the juxta fused with the median stylus (51 : 1) (Fig. 18C, E, F) and the presence of harpes (69 : 1) (Figs 10D, F, 18C, F, 20B, E), along with one homoplasious character state: pointed harpes (72 : 1) (Figs 18C, 19A, 27B). However, the delimitation of the harpes and the scoring of these characters were difficult in some species, particularly within *Lepidodexia*. Roback (1954) considered true harpes present only in the species of his *Sarcophaga* group, which did not include, amongst others, *Lepidodexia* or *Helicobia*. However, he considered the three genera closely related, mainly because of the similar form of the lateral styli and phallic tube and the point of attachment of the median stylus.

In the weighted analysis *Lepidodexia* was supported by three homoplasious character states; species relationships within *Lepidodexia* were well resolved, with most clades supported by multiple apomorphic states, even though most were homoplasious (Fig. 27B). The monophyly of *Lepidodexia* (*Johnsonia*) was based on five apomorphies. A setose CuA$_1$ (21 : 1) is a uniquely derived apomorphy for *Lepidodexia* (*Johnsonia*) *setosa* (Aldrich) + *Lepidodexia* (*Johnsonia*) *elegans* (Coquillett).

Pape (1995, 1996) based the monophyly of his broad concept of *Lepidodexia* on similarities in phallic morphology, one of which is the presence of a characteristic spinous lobe proximal to the vesica (see Fig. 10A–C, E, F). Similarly, Lopes (1979, 1984) proposed a list of characters for his tribe Johnsoniini and its subtribes mainly based on female and first instar larval features. We did not include all of those characters in the present study because of uncertain homologies, and a revision of *Lepidodexia*, including detailed morphological study of the male genitalia and incorporating female and larval characters, is needed.

The exemplar species of *Sarcophaga* formed a monophyletic group supported by nine apomorphies (Fig. 27B), including three uniquely derived character states: the lateral stylus coiled at base (57 : 1) (Fig. 18D) and a bifurcate median stylus (62 : 1) (Fig. 18D, F, H) with no opening to the sperm duct (61 : 1) (Fig. 18C–H).

Relationships within *Sarcophaga* were poorly resolved in the unweighted analysis but resolution was greater in the weighted analysis. *Helicobia*, previously treated as a separate genus, was monophyletic but was nested within *Sarcophaga* in both analyses. Therefore, we consider *Helicobia* a synonym of *Sarcophaga*, and treat it as a subgenus of *Sarcophaga*.

The subgeneric classification of Pape (1996) largely corresponds to the generic classification of the tribe Sarcophagini of Verves (1986) and Povolný & Verves (1997), divided into 15 subtribes and more than 750 species. Their classification was based on a comparative analysis of morphological and ecological features of the species of Sarcophagini as outlined by Rohdendorf (1965) and Lopes (1982b). It was not a cladistic analysis, and monophyly of the included taxa was not tested. Roback (1954) also treated *Sarcophaga* as a

large single genus and included more than 180 species. The 45 species included in the phylogenetic tree of his *Sarcophaga* group were mostly Nearctic and represented 20 of the subgenera recognized by Pape (1996). Sugiyama & Kano (1984) proposed a classification of the tribe Sarcophagini to address generic limits within the Oriental Sarcophaginae. They corroborated the monophyly of *Sarcophaga* with a phylogenetic analysis based on five genitalic characters of 112 species from 35 of the subgenera recognized by Pape (1996). Wells *et al.* (2001) corroborated the monophyly of *Sarcophaga* – although with a very limited taxon sampling – but their study was not intended as a broad phylogenetic analysis and included only seven species of *Sarcophaga*.

Given the limited sample of species studied by the above authors, as well as in the present study, a more comprehensive study of *Sarcophaga* species, including phylogenetic analyses of a matrix including more exemplars, is clearly needed to identify additional and, hopefully, less homoplasious characters that will help to resolve relationships within this huge genus.

Aside from *Helicophagella* (Blackith *et al.*, 1997) and *Liopygia* Enderlein in part (Wells *et al.*, 2001), the monophyly of the 133 subgenera included in *Sarcophaga* has never been cladistically tested. As an indication of the need for these analyses, of the three subgenera represented by multiple species in our analysis, neither *Mehria* nor *Neobellieria* was monophyletic (Fig. 28), and *Helicobia* was monophyletic but had not previously been treated as a subgenus of *Sarcophaga*.

The bifurcate median stylus with no exit opening and the coiled base of the lateral stylus are visible on all specimens of *Helicobia* studied (Figs 8D–H, 9C), as well as in published illustrations of other *Helicobia* species (Tibana, 1981; Tibana & Mello, 1992). The monophyly of *Helicobia* was supported by seven apomorphies.

The monophyly of the clade *Sarcophaga* (*Neobellieria*) *bullata* Parker + *Sarcophaga* (*Neobellieria*) *polistensis* Hall was supported by two apomorphies: female tergite 8 absent or vestigial (32 : 1) and male sternite 5 forming a dome-shaped window anteriorly (37 : 1) (Fig. 28B). However, two other species currently assigned to *Neobellieria* (*Sarcophaga* (*Neobellieria*) *triplasia* Wulp and *Sarcophaga* (*Neobellieria*) *semimarginalis* Hall) were placed elsewhere in *Sarcophaga*, as sister groups to other subgenera; thus *Neobellieria*, as currently defined, is polyphyletic. A sister relationship between *S.* (*N.*) *bullata* + *S.* (*N.*) *polistensis* and *Sarcophaga* (*Tolucamyia*) Dodge was supported by three character states of the male phallus, one of which, phallic vesica with round lobes bearing thorn-like spines (65 : 3), was uniquely derived (Fig. 28B).

CONCLUSION AND RECOMMENDATIONS

This study was the first attempt at a broad phylogenetic analysis of the Sarcophaginae using a range of morphological characters. Male genitalia provided apomorphies for almost every node in the tree, confirming the value of this complex structure as a source of phylogenetic characters. This was especially true in the *Sarcophaga* clade, supported by three male genitalic characters, all unique apomorphies (Fig. 27B). Similarly, the monophyly of, and some relationships within, the *Ravinia* clade were supported mainly by male genitalic characters (Fig. 27A).

Other morphological character sets also provided apomorphies at several levels (Figs 27, 28). Despite comprising a minor fraction of the total character set, larval character states provided apomorphies for one node within the *Peckia* genus-group (Fig. 27B). Female character states supported the clades *Sarcophaga*, (*S.* (*Tolucamyia*) + *S.* (*Neobellieria*)), and (*S.* (*N.*) *bullata* + *S.* (*N.*) *polistensis*) despite the fact that female characters were not scored for several species in the analysis.

This study provides a preliminary hypothesis of sarcophagine relationships that should be tested by the incorporation of additional morphological characters and/or exemplar species. Future research should also test this phylogenetic hypothesis using molecular character sets.

By allowing the identification of several monophyletic groups, the current study also provides direction for future revisionary and phylogenetic studies at levels below the subfamily.

The analysis suggested that *Blaesoxipha*, as currently defined, is not monophyletic relative to *Spirobolomyia*. This is in conflict with the conclusions of Pape (1994) who considered *Blaesoxipha* monophyletic. More exemplars and more characters will be required to determine which hypothesis is best corroborated.

Although *Lepidodexia* and *Sarcophaga* were both monophyletic and were sister groups in this analysis, both genera are in need of revision. Both genera are large and contain several subgenera (Pape, 1996) whose monophyly and phylogenetic relationships have not been tested cladistically. This is especially true within *Sarcophaga*, where only one of the subgenera represented by more than one species was shown to be monophyletic (*Helicobia*); neither *Neobellieria* nor *Mehria* was monophyletic, and this may be the case with many other subgenera.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Data matrix of morphological characters used in the study. ?, missing data; -, inapplicable data; A, polymorphic 0/1; B, polymorphic 1/2; *, type species of genus or subgenus.
Appendix S2. Characters used in phylogenetic analysis.

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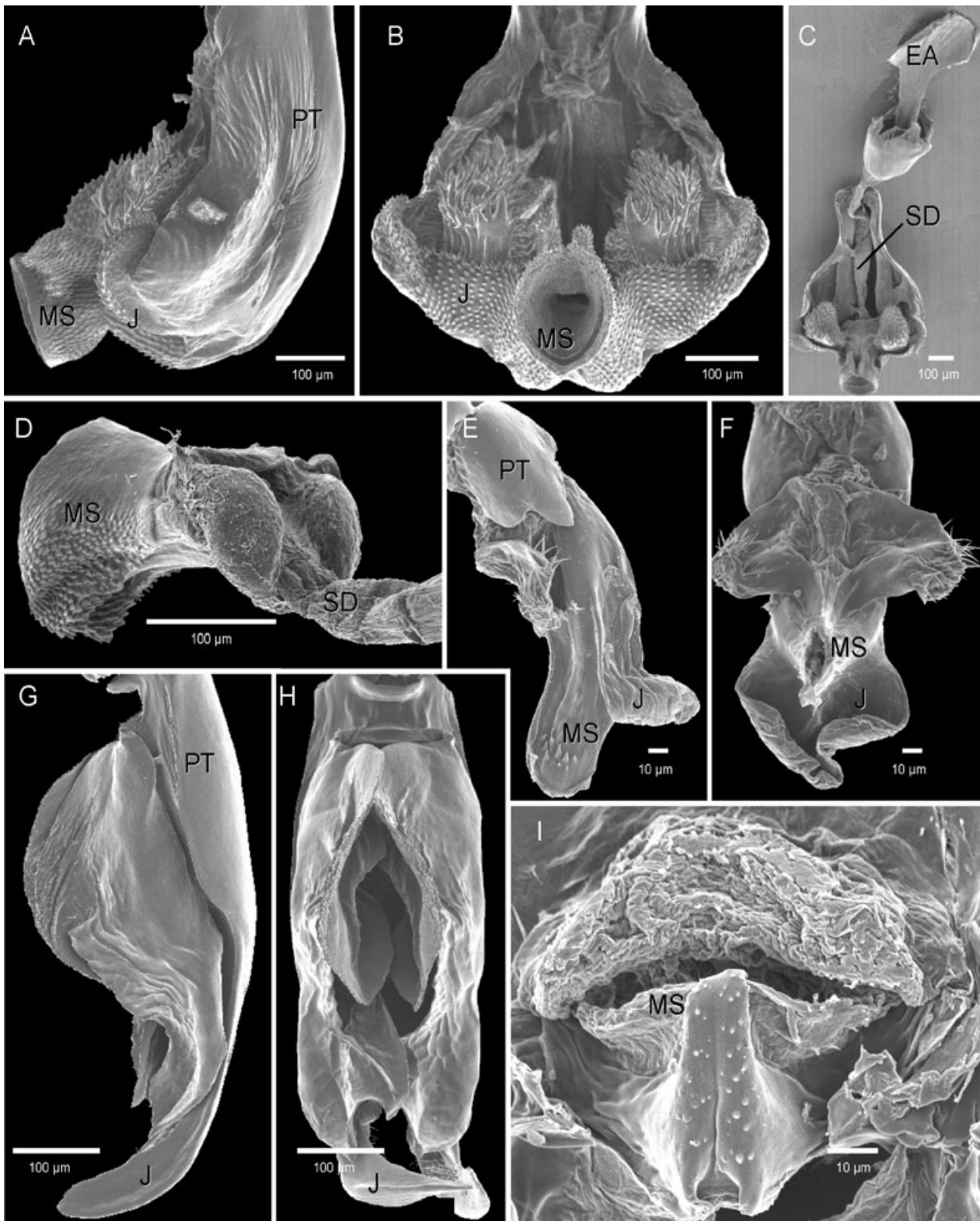
Figure 4. A–D, *Blaesoxipha* (*Gigantotheca*) *plinthopyga*: A, distiphallus, lateral; B, distiphallus, anterior; C, dissected phallus, anterodorsal; D, median stylus and sperm duct, posteroventral. E–F, *Blaesoxipha* (*s.s.*) *setosa*: E, distiphallus, lateral; F, distiphallus, anterior. G–I, *Blaesoxipha* (*Kellymyia*) *kellyi*: G, distiphallus, lateral; H, distiphallus, anterior; I, median stylus. Abbreviations: EA, ejaculatory apodeme; J, juxta; MS, median stylus; PT, phallic tube; SD, sperm duct.

Figure 5. A–B, *Boettcheria latisterna*: A, distiphallus, lateral; B, styli, anterior (arrow = anterior juxtal process of Roback, 1954). C–F, *Dexosarcophaga transita*: C, styli, anterior; D, distiphallus, lateral; E, styli, lateral; F, median stylus, anterior. Abbreviations: J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.

Figure 6. A–C, *Comasarcophaga texana*: A, distiphallus, lateral; B, distiphallus, anterior; C, styli, anterior. D–F, *Cistudinomyia cistudinis*: D, distiphallus, lateral; E, distiphallus, anterior; F, styli anterior. Abbreviations: J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.
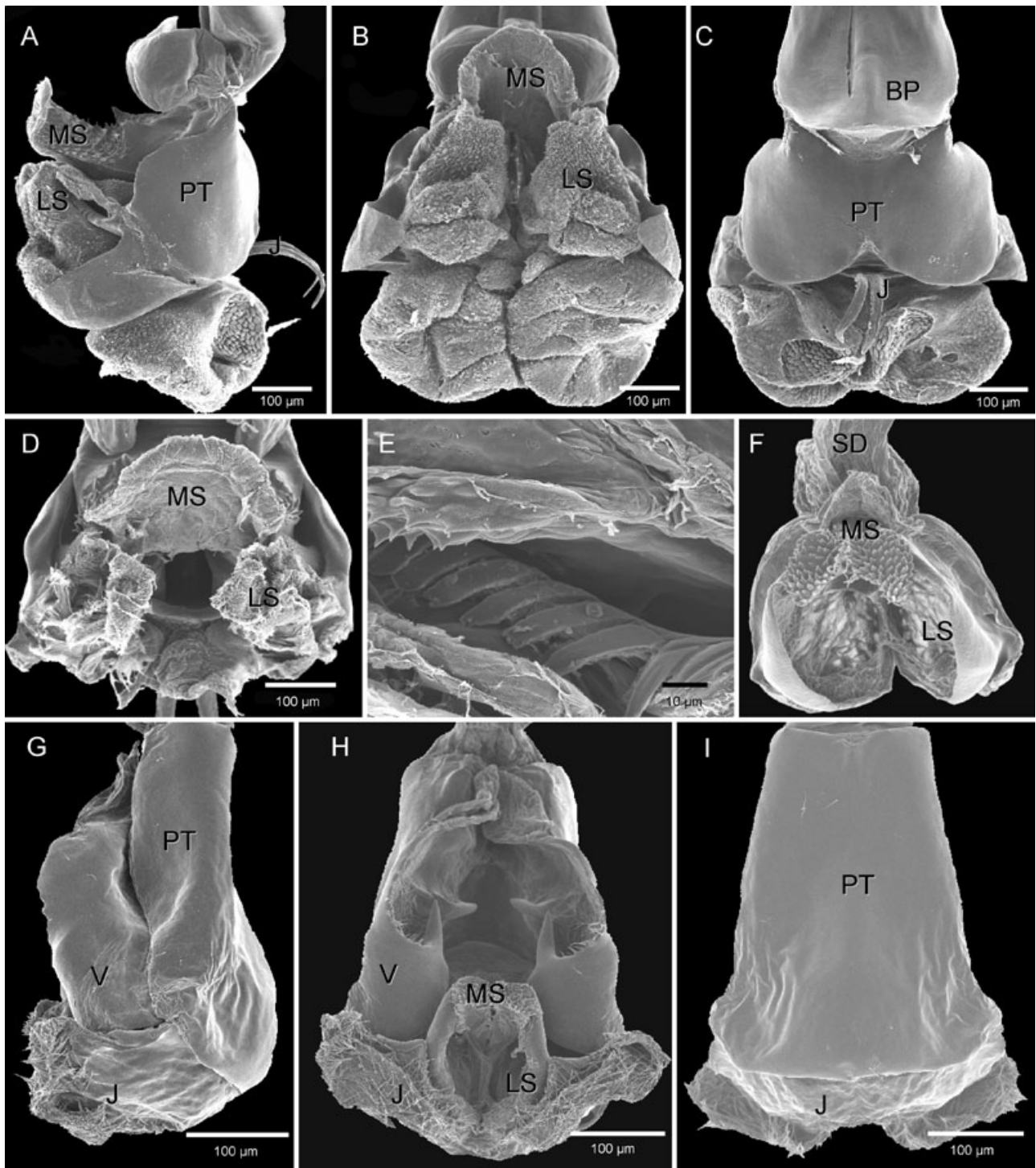
Figure 7. A–E, *Engelimyia inops*: A, distiphallus, lateral; B, distiphallus, anterior; C, distiphallus, posterior; D, distiphallus, anterior; E, lateral styli. F–I, *Fletcherimyia fletcheri*: F, styli; G, distiphallus, lateral; H, distiphallus, anterior; I, distiphallus, posterior. Abbreviations: BP, basiphallus; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; SD, sperm duct; V, vesica.
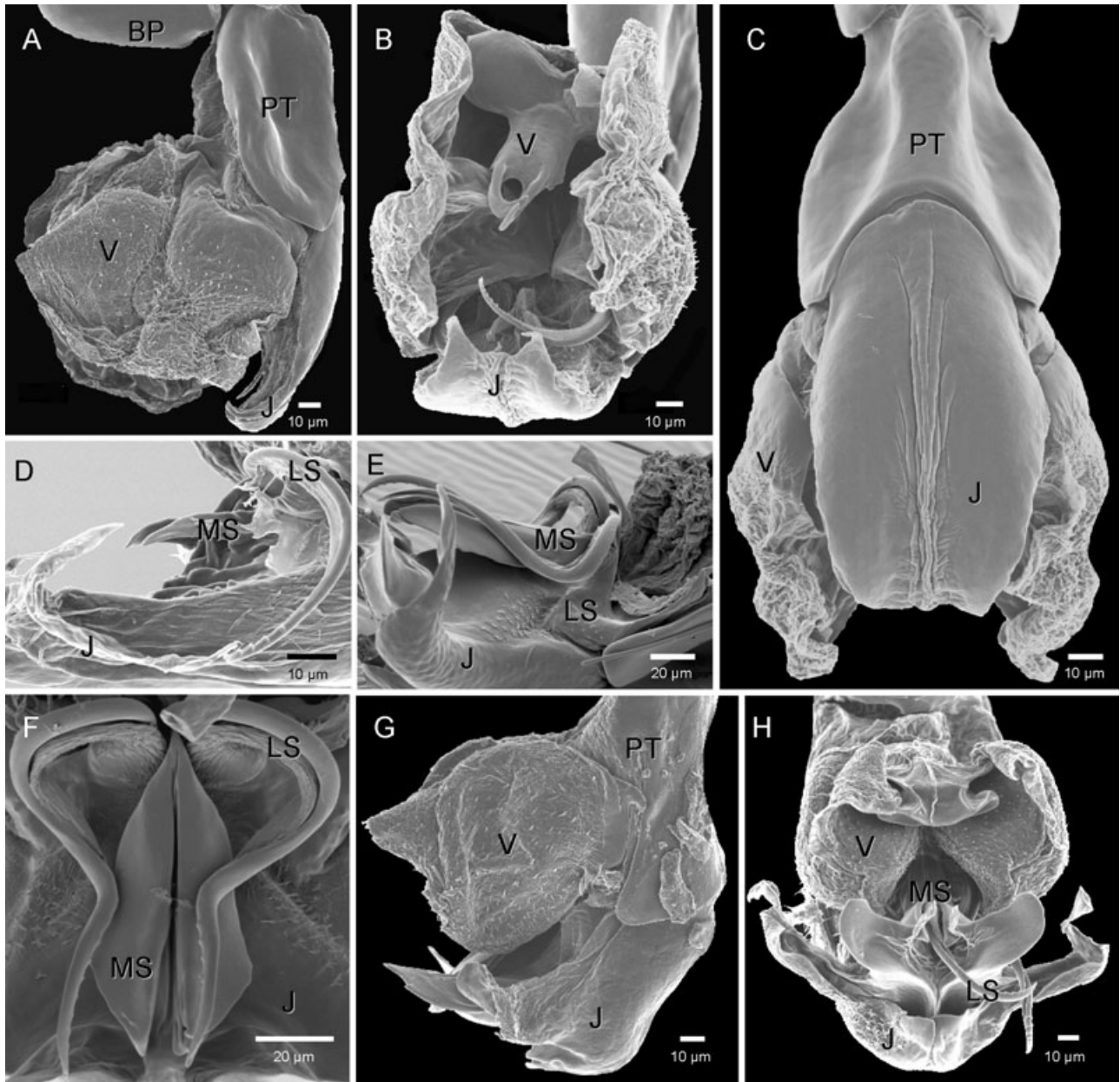
Figure 8. A–D, *Sarcophaga* (*Helicobia*) *morionella*: A, distiphallus, lateral; B, distiphallus, anterior; C, distiphallus, posterior; D, styli, lateral. E–H, *Sarcophaga* (*Helicobia*) *rapax*: E, styli, lateral; F, styli, dorsal; G, distiphallus, lateral; H, distiphallus, anterior. Abbreviations: BP, basiphallus; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.
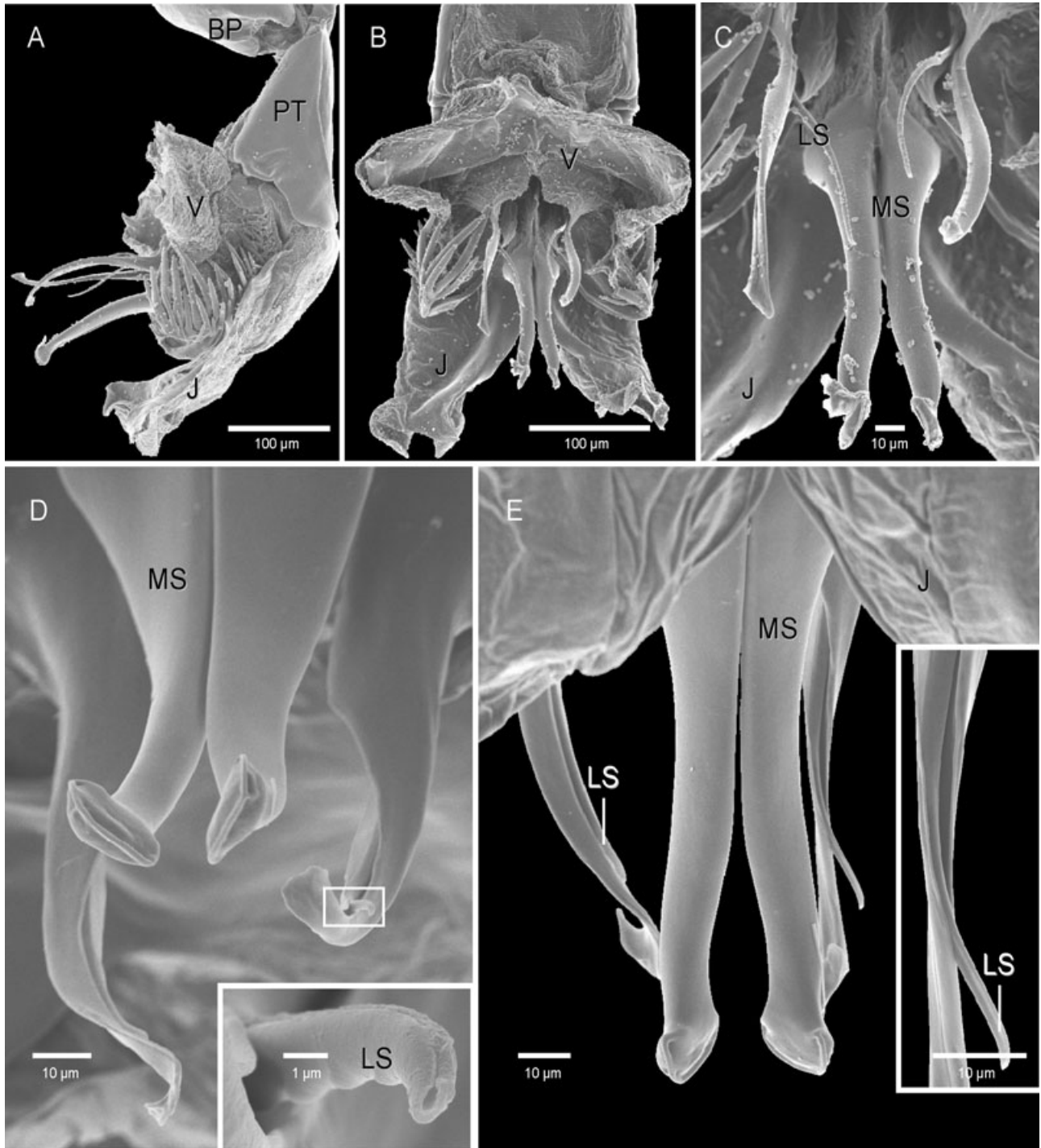
Figure 9. *Sarcophaga* (*Helicobia*) *surrubea*: A, distiphallus, lateral; B, distiphallus, anterior; C, styli, anterior; D, apex of styli, anterior; E, lateral styli within their sheaths, posterior. Abbreviations: BP, basiphallus; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.
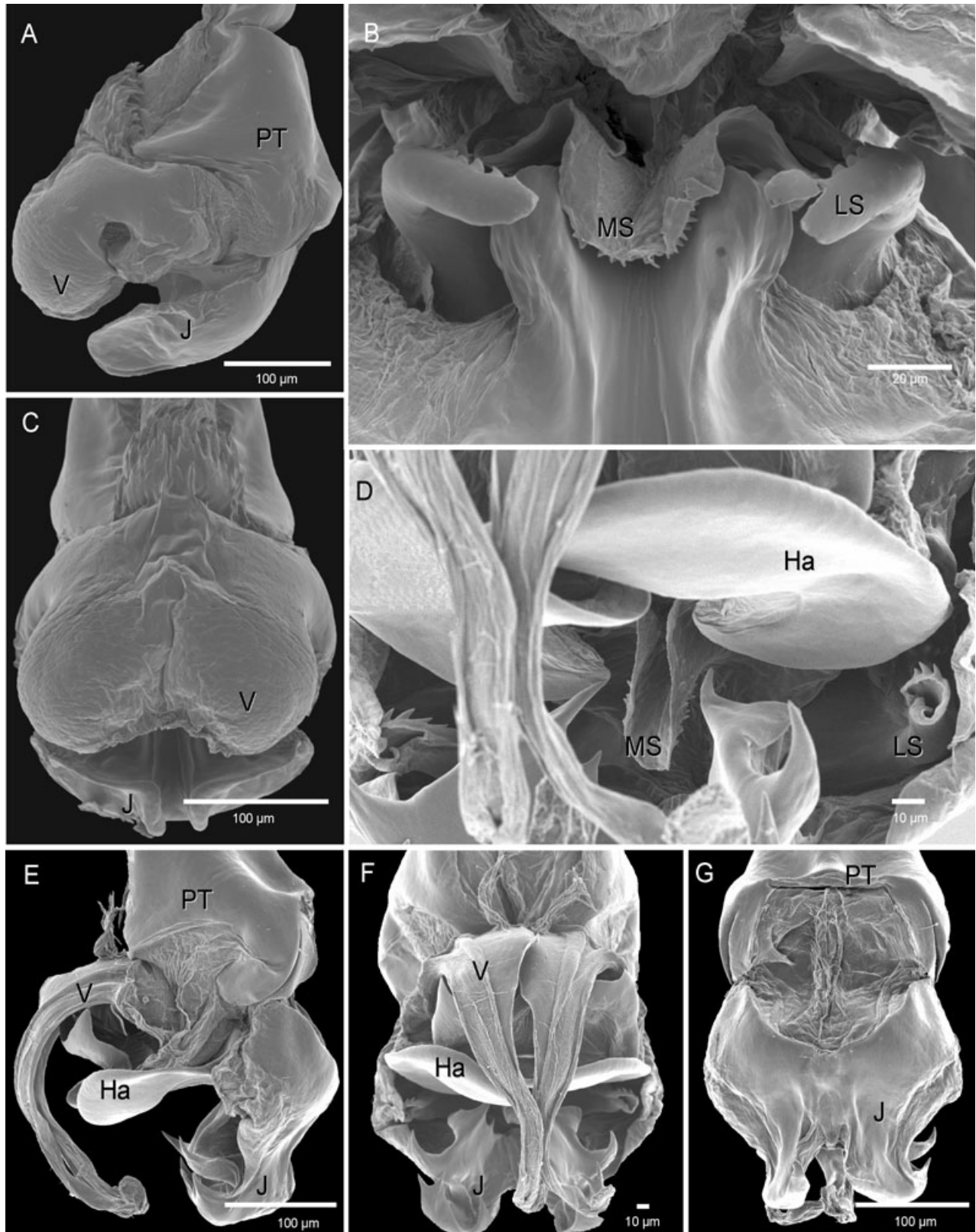
Figure 10. A–C, *Lepidodexia* (*s.s.*) *tetraptera*: A, distiphallus, lateral; B, styli, anterior; C, distiphallus, anterior. D–G, *Lepidodexia* (*Notochaeta*) *woodi*: D, styli, anterolateral; E, distiphallus, lateral; F, distiphallus, anterior; G, distiphallus, posterior. Abbreviations: Ha, harpes; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.

Figure 11. A–D, *Oxyvinia xanthophora*: A, distiphallus, lateral; B, distiphallus, anterior; C, styli, anterolateral; D, styli, anterior. E–F, *Microcerella spinigena*: E, distiphallus, lateral; F, styli, anterior. Abbreviations: BP, basiphallus; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.
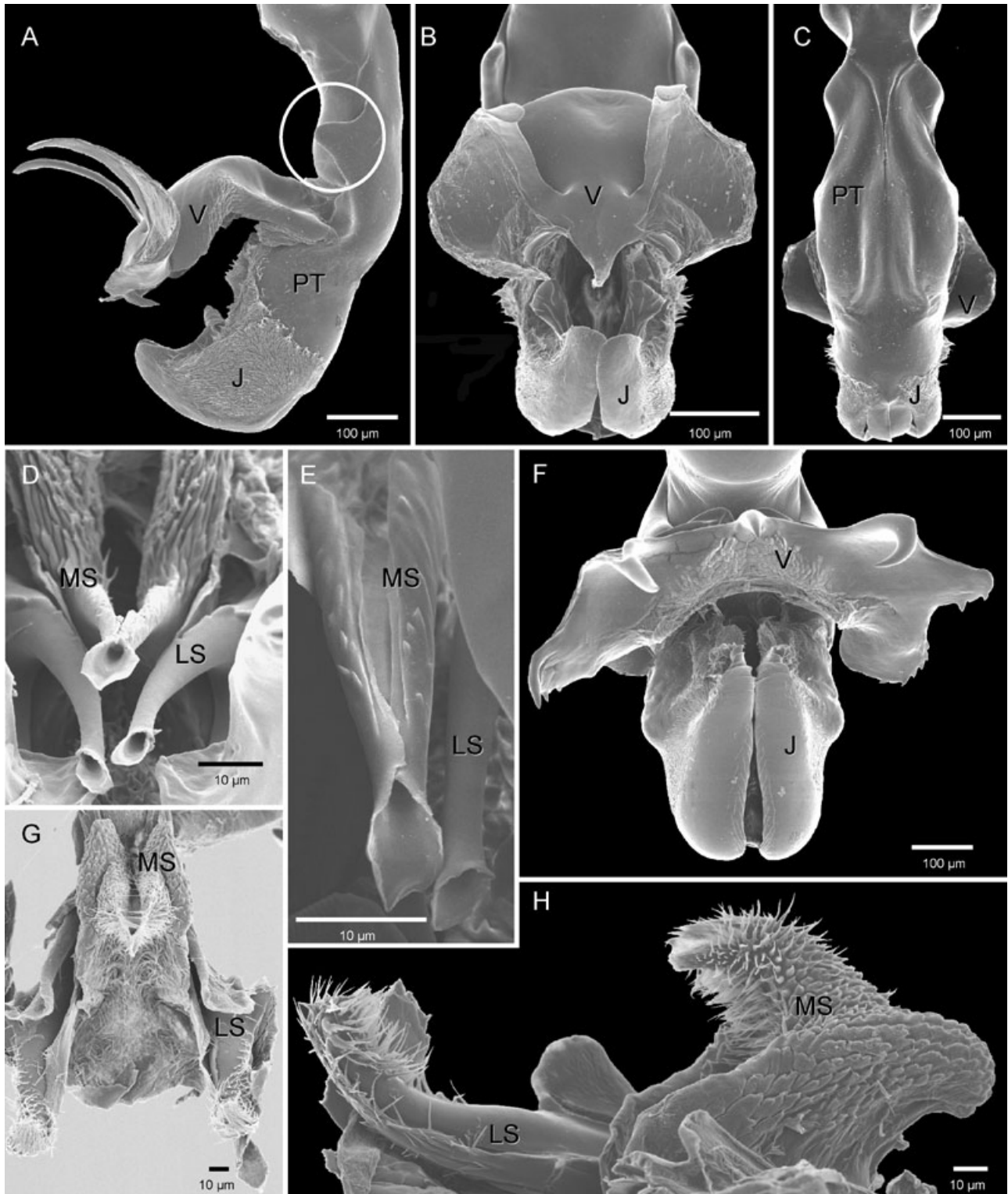
Figure 12. A–E, *Oxysarcodexia* sp.: A, distiphallus, lateral. Lateral extension above vesica (character 62) is circled; B, distiphallus, anterior; C, distiphallus, posterior; D, styli, anterior; E, styli, dorsal. F–H, *Oxysarcodexia timida*: F, distiphallus, anterior; G, styli, anterior; H, styli, lateral. Abbreviations: J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.

Figure 13. A–D, *Peckia* (*s.s.*) *chrysostoma*: A, distiphallus, lateral; B, distiphallus, anterior; C, styli and part of vesica, anterior (part of vesica removed); D, styli, anterior. E–H, *Peckia* (*Pattonella*) *intermutans*: E, distiphallus, lateral; F, distiphallus, anterior; G, styli, posteroventral; H, styli, anterior. Abbreviations: BP, basiphallus; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; SD, sperm duct; V, vesica.
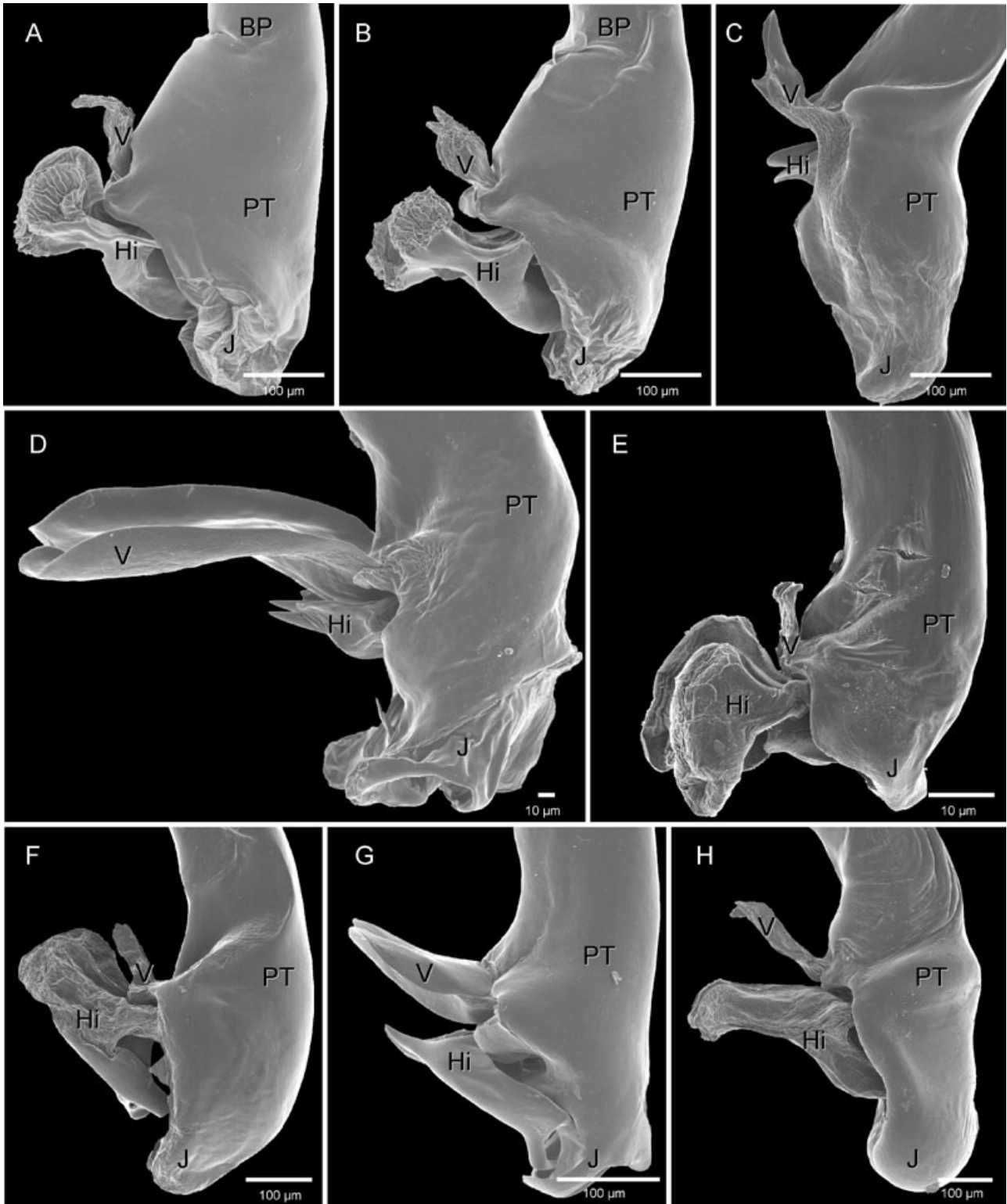
Figure 14. *Ravinia*. Distiphallus, lateral: A, *Ravinia columbiana*; B, *Ravinia rufipes*; C, *Ravinia errabunda*; D, *Ravinia effrenata*; E, *Ravinia heithausi*; F, *Ravinia pernix*; G, *Ravinia derelicta*; H, *Ravinia querula*. Abbreviations: BP, basiphallus; Hi, hillae; J, juxta; PT, phallic tube; V, vesica.
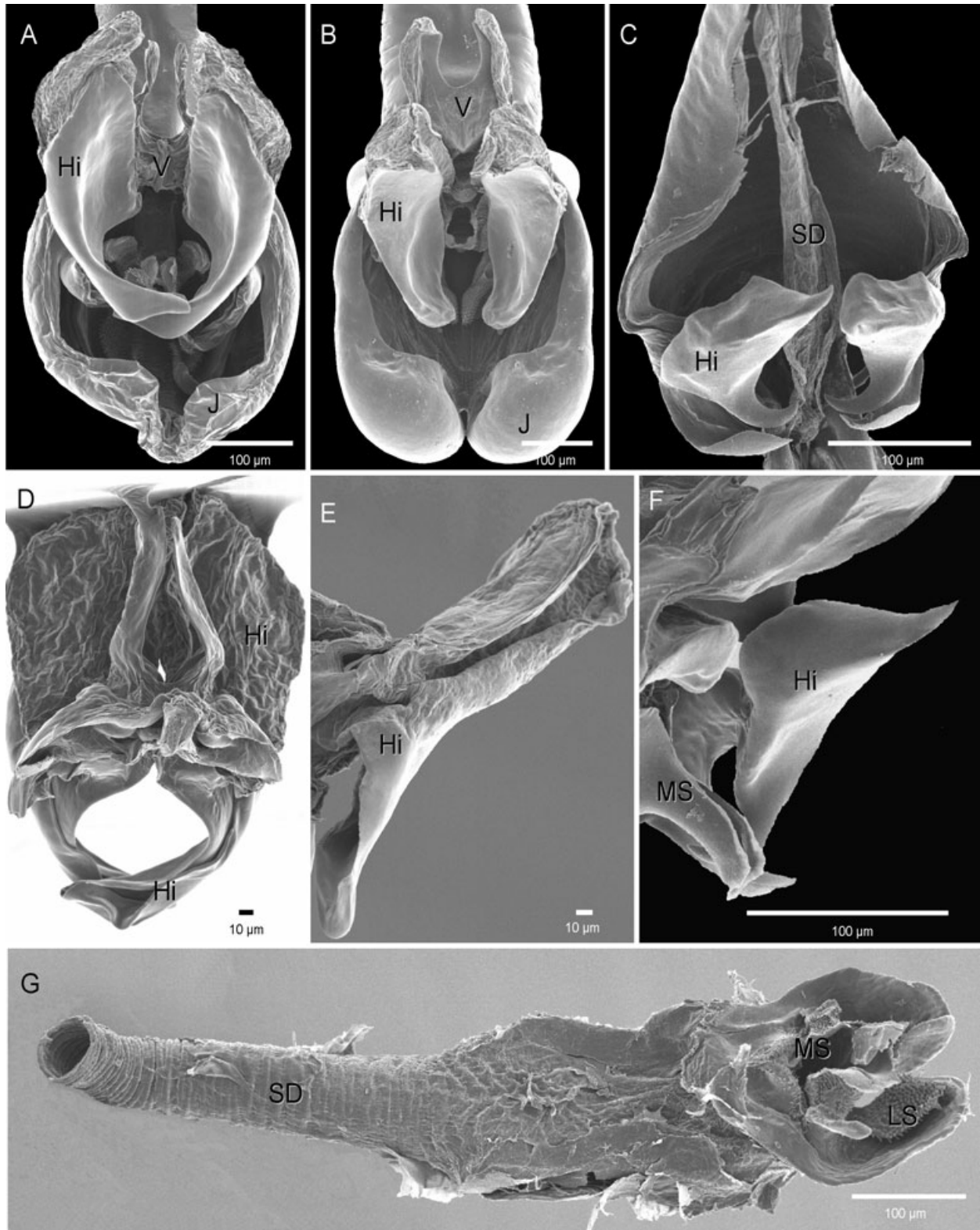
Figure 15. *Ravinia*. Distiphallus, anterior: A, *Ravinia pernix*; B, *Ravinia querula*. C, *Ravinia derelicta* (phallic tube opened to show sperm duct); D, *Ravinia pernix,* hillae, posterior; E, *Ravinia querula,* left hilla, interior; F, *Ravinia derelicta*, left hilla, interior; G, *Ravinia pernix* (phallic tube removed to show sperm duct). Abbreviations: Hi, hillae; J, juxta; LS, lateral stylus; MS, median stylus; SD, sperm duct; V, vesica.
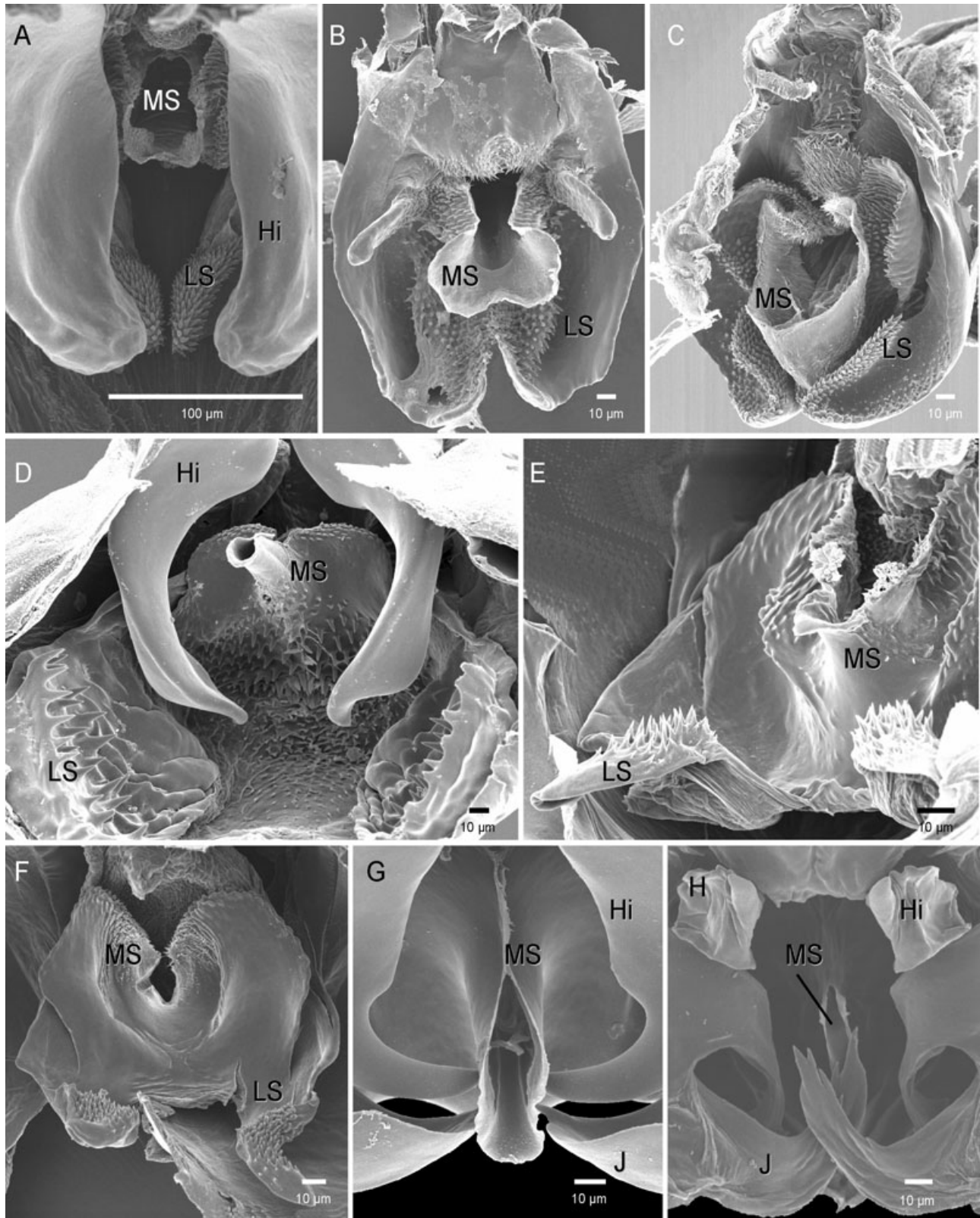
Figure 16. *Ravinia*. Styli: A, *Ravinia querula*; B, *Ravinia pernix*; C, *Ravinia heithausi*; D, *Ravinia errabunda*; E, *Ravinia rufipes*; F, *Ravinia columbiana*; G, *Ravinia derelicta*; H, *Ravinia effrenata*. Abbreviations: Hi, hillae; J, juxta; LS, lateral stylus; MS, median stylus.
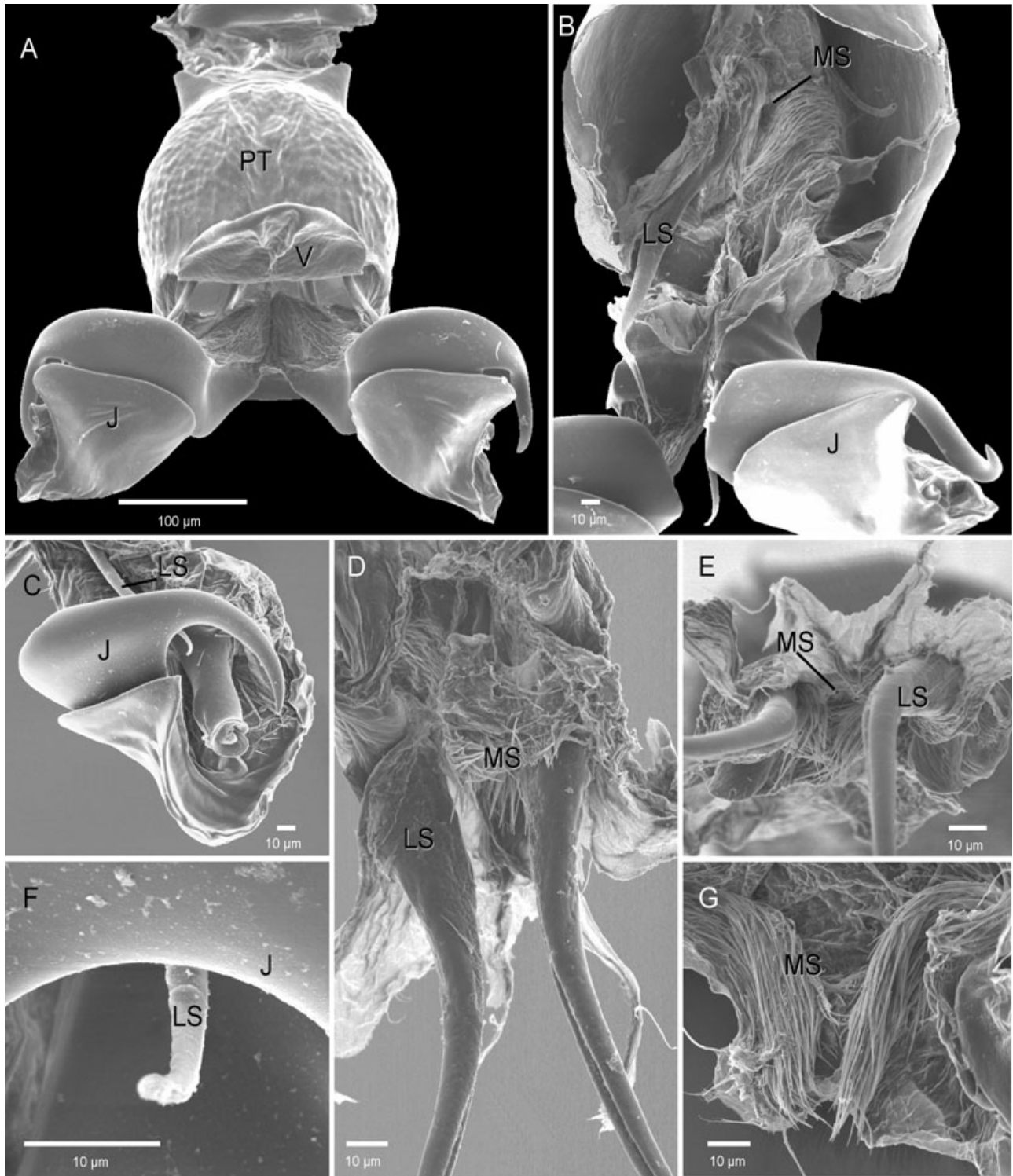
Figure 17. *Sarcodexia lambens*. A, distiphallus, anterior; B, distiphallus, anterior (vesica and phallic tube removed); C, lateral stylus at apex of juxta arm; D, styli, dorsal; E, styli, anterior; F, tip of lateral stylus; G, median stylus. Abbreviations: J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.
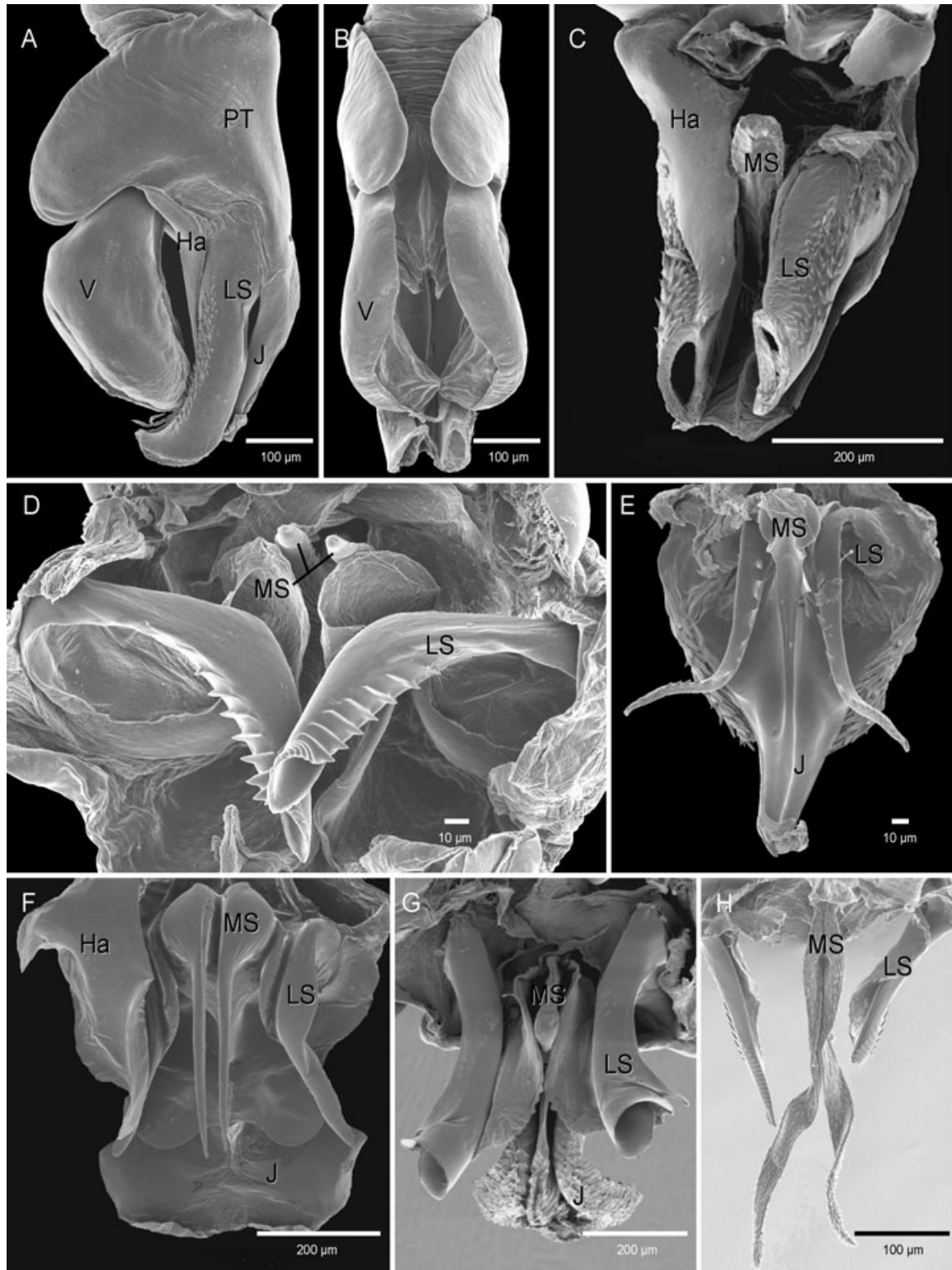
Figure 18. A–C, *Sarcophaga* (*s.s.*) *carnaria*: A, distiphallus, lateral; B, distiphallus, anterior; C, distiphallus, anterior (left harpes and vesica removed). Styli, (harpes and vesica removed). D, *Sarcophaga* (*Parasarcophaga*) *taenionota*. E, *Sarcophaga* (*Phytosarcophaga*) *destructor*. F, *Sarcophaga* (*Wohlfahrtiopsis*) *johnsoni* (right harpes preserved). G, *Sarcophaga* (*Mehria*) *houghi*. H, *Sarcophaga* (*Seniorwhitea*) *orientalis*. Abbreviations: Ha, harpes; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.

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Figure 19. *Sarcophaga* spp., distiphallus. A–C, *Sarcophaga* (*Thyrsocnema*) *incisilobata*: lateral, anterior, posterior view, respectively. D–F, *Sarcophaga* (*Phytosarcophaga*) *destructor*: lateral, anterior, posterior view, respectively. G–I, *Sarcophaga* (*Seniorwhitea*) *orientalis*: lateral, anterior, posterior view, respectively. Abbreviations: Ha, harpes; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.
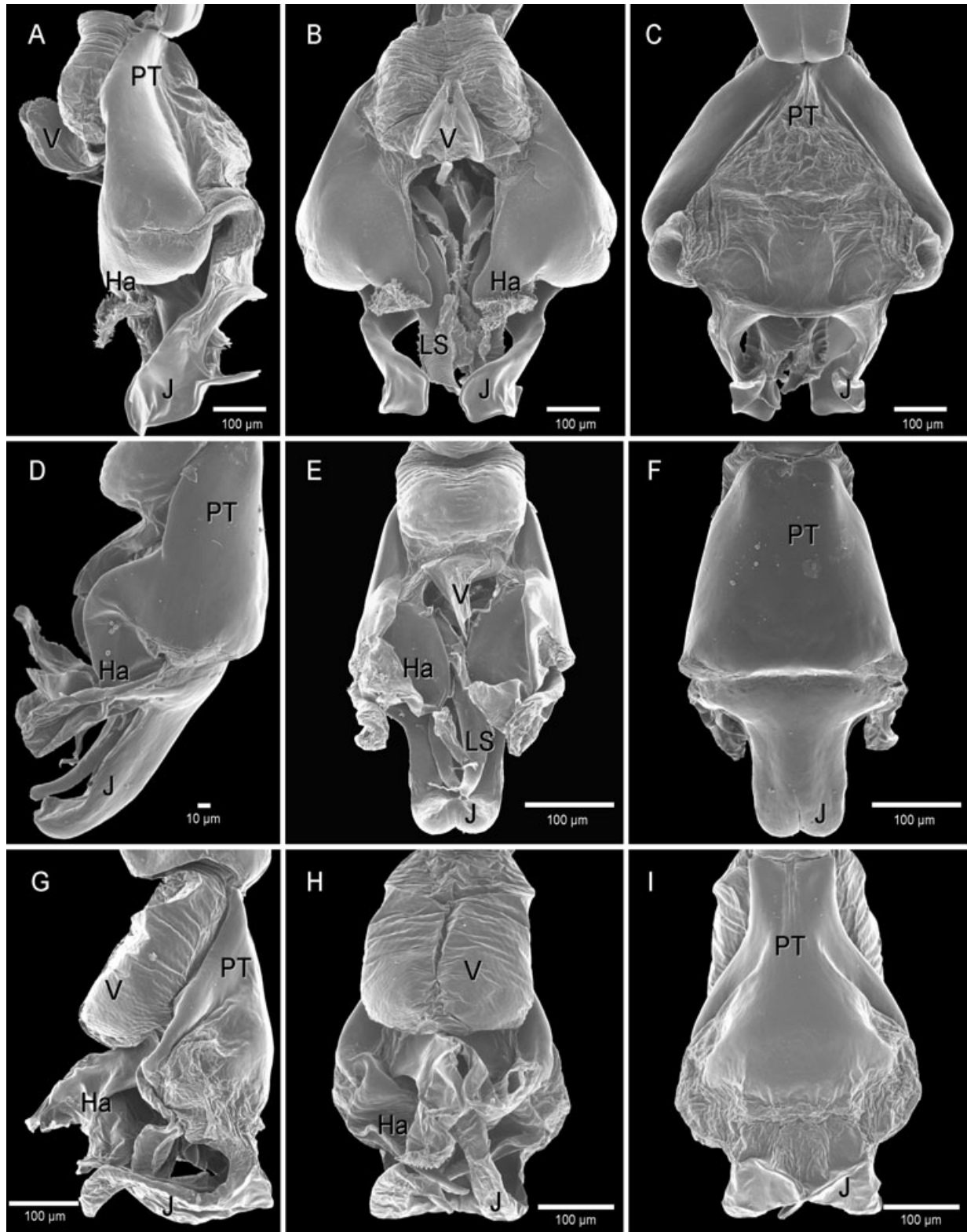
Figure 20. *Sarcophaga* spp., distiphallus. A–C, *Sarcophaga* (*Liopygia*) *ruficornis*: lateral, anterior, posterior view, respectively. D–F, *Sarcophaga* (*Heteronychia*) *haemorrhoa*: lateral, anterior, posterior view, respectively. G–I, *Sarcophaga* (*Parasarcophaga*) *taenionota*: lateral, anterior, posterior view, respectively. Abbreviations: Ha, harpes; J, juxta; LS, lateral stylus; PT, phallic tube; V, vesica.
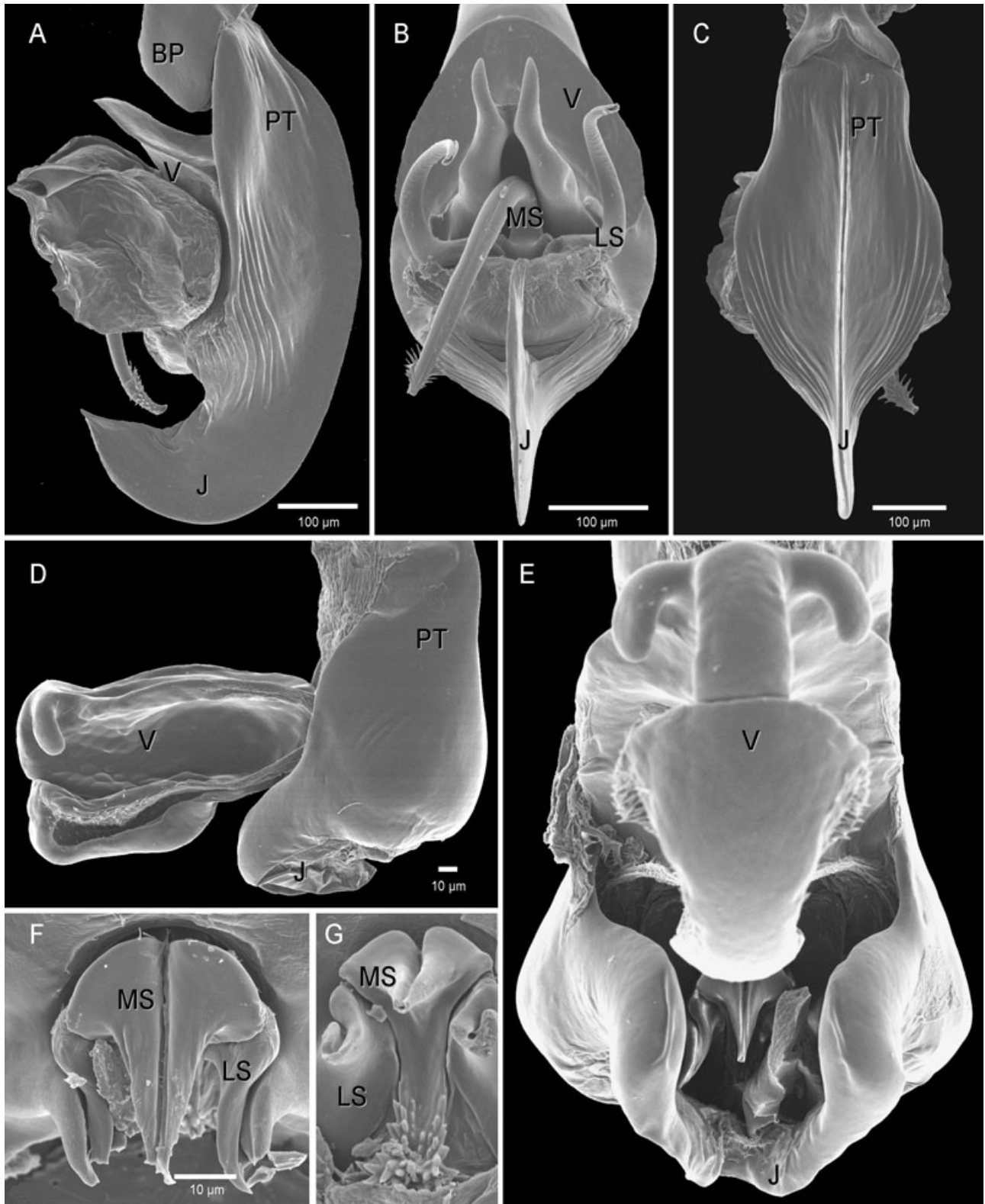
Figure 21. A–C, *Spirobolomyia singularis*: A, distiphallus, lateral; B, distiphallus, anterior (part of vesica removed); C, distiphallus, posterior. D–G, *Tricharaea (Sarothromyia) simplex*: D, distiphallus, lateral; E, distiphallus, anterior; F, styli, anterior; G, styli, apical. Abbreviations: BP, basiphallus; J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; V, vesica.
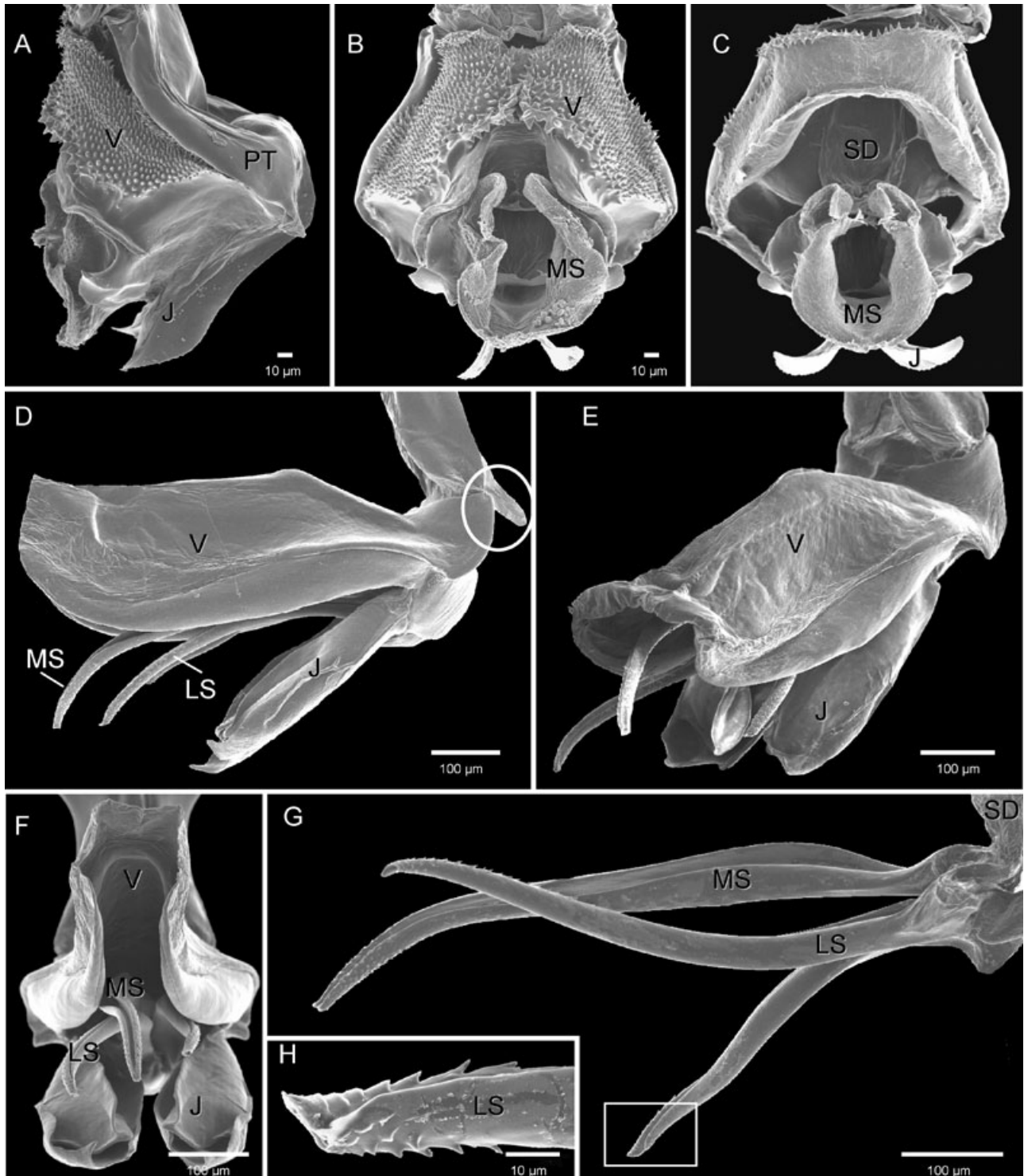
Figure 22. A–C, *Titanogrypa* (*s.s.*) *alata*: A, distiphallus, lateral; B, distiphallus, anterior; C, distiphallus, anterior (vesica removed). D–H, *Titanogrypa* (*Cucullomyia*) *placida*: D, distiphallus, lateral, dorsal hump (character 61) circled; E, distiphallus, dorsolateral; F, distiphallus, anterior; G, styli; H, tip of lateral stylus. Abbreviations: J, juxta; LS, lateral stylus; MS, median stylus; PT, phallic tube; SD, sperm duct; V, vesica.

Figure 23. *Macronychia aurata* (Miltogramminae): A, acrophallus, lateral. *Sarcophila* sp. (Paramacronychiinae): B, acrophallus, lateral; C, acrophallus, anterior. *Brachicoma devia* (Paramacronychiinae): D, acrophallus, lateral; E, acrophallus, anterior; F, acrophallus, anterior. Abbreviations: J, juxta; MS, median stylus; PT, phallic tube.
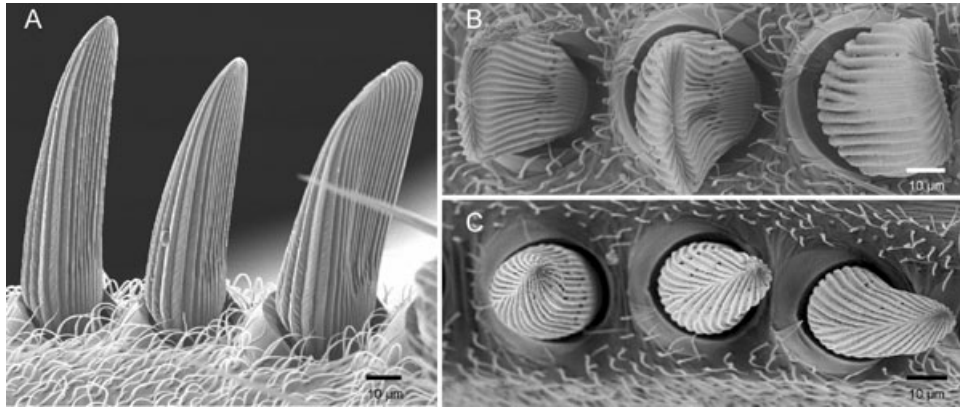
Figure 24. *Ravinia heithausi*: A, flattened spines of mid femoral ctenidium, lateral; B, ventral. *Dexosarcophaga transita*: C, rounded spines of mid femoral ctenidium, ventral.
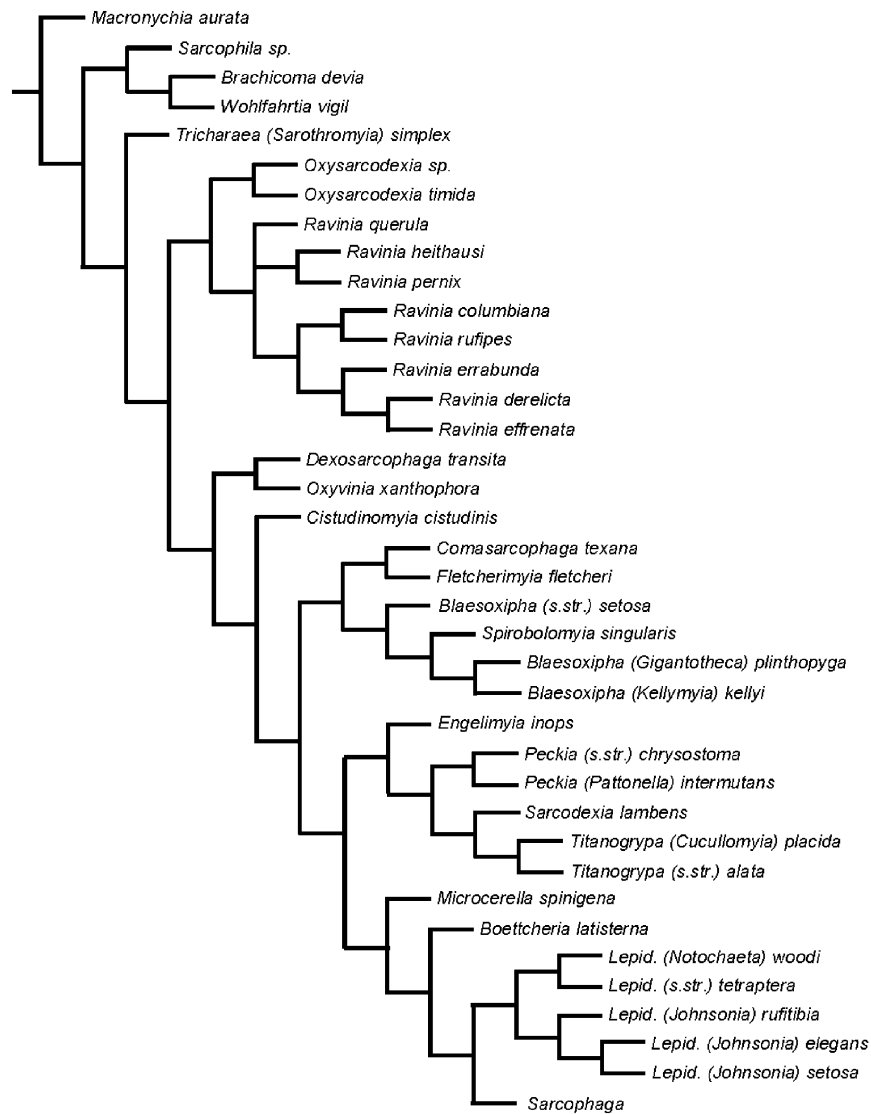
Figure 25. Strict consensus tree of 120 most parsimonious trees (length 396) obtained from analysis under implied weights (*k* = 3), not showing resolution for *Sarcophaga s.l.*
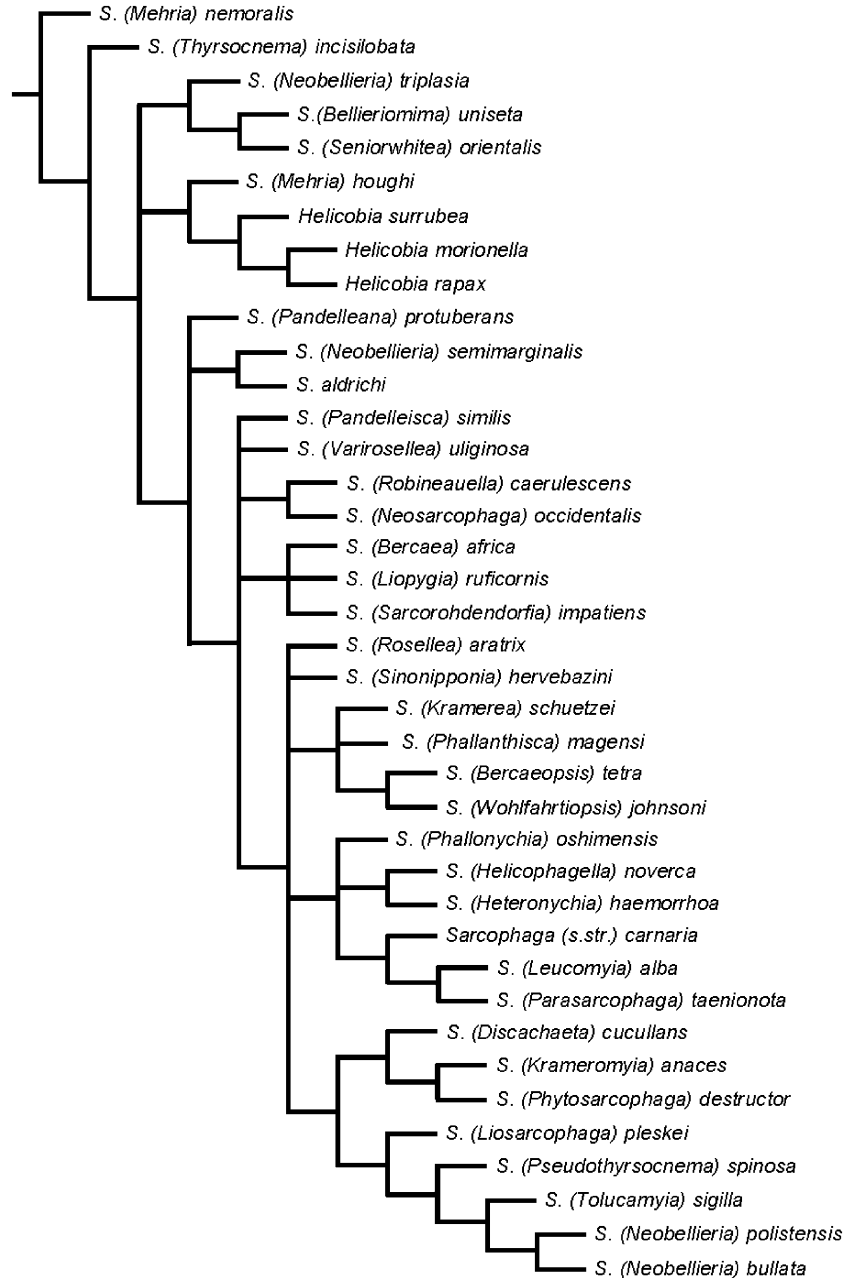
Figure 26. Strict consensus tree of 120 most parsimonious trees (length 396) obtained from analysis under implied weights ($k = 3$) showing topology within *Sarcophaga s.l.*
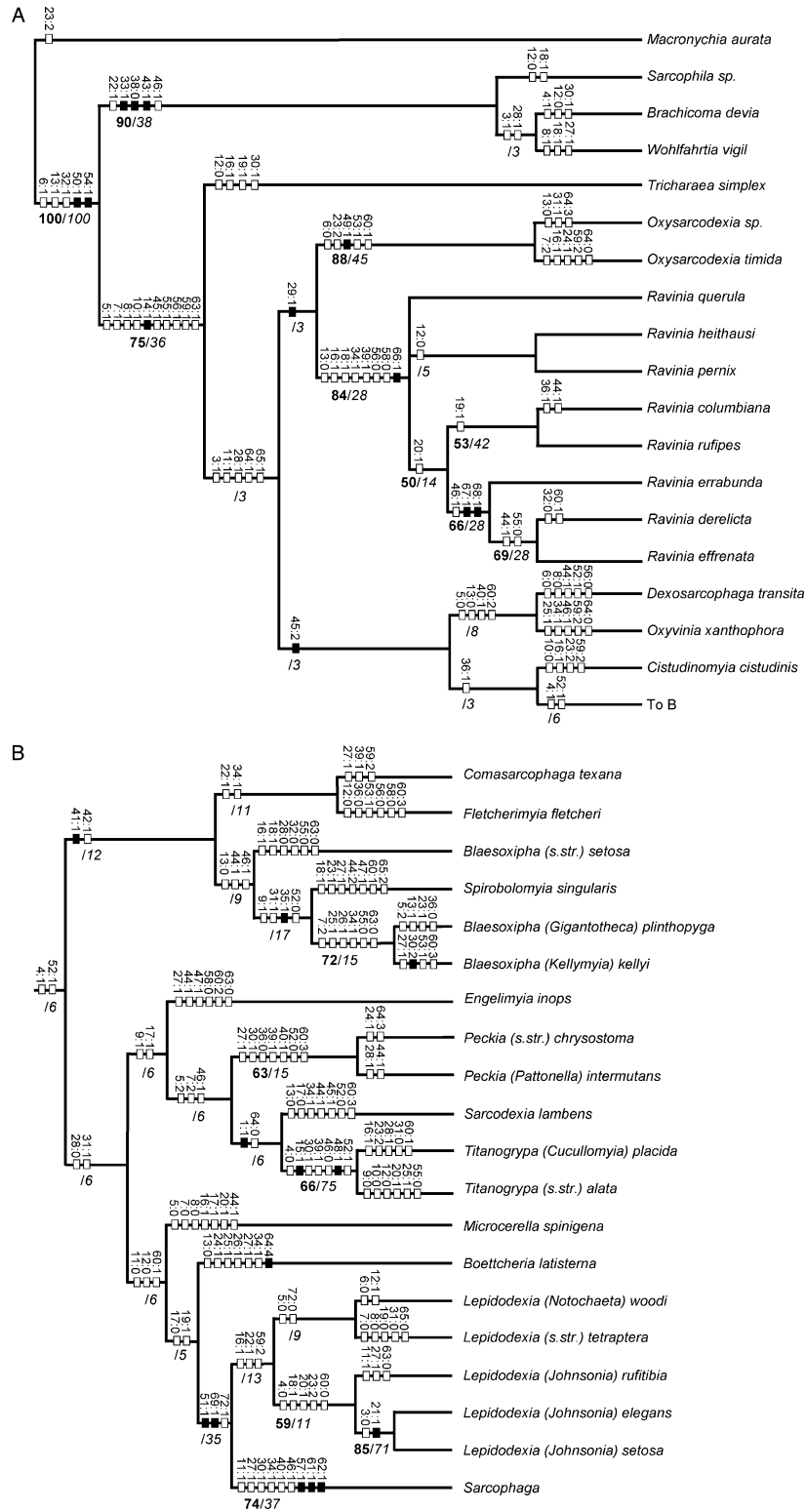
Figure 27. A, B. one of 120 most parsimonious trees obtained from analysis under implied weights ($k = 3$) showing character distribution. Black boxes, uniquely derived character states; white boxes, homoplasious character states. Bootstrap percentiles (3000 replicates) (bold) and relative Bremer support (italics) are given below branches.
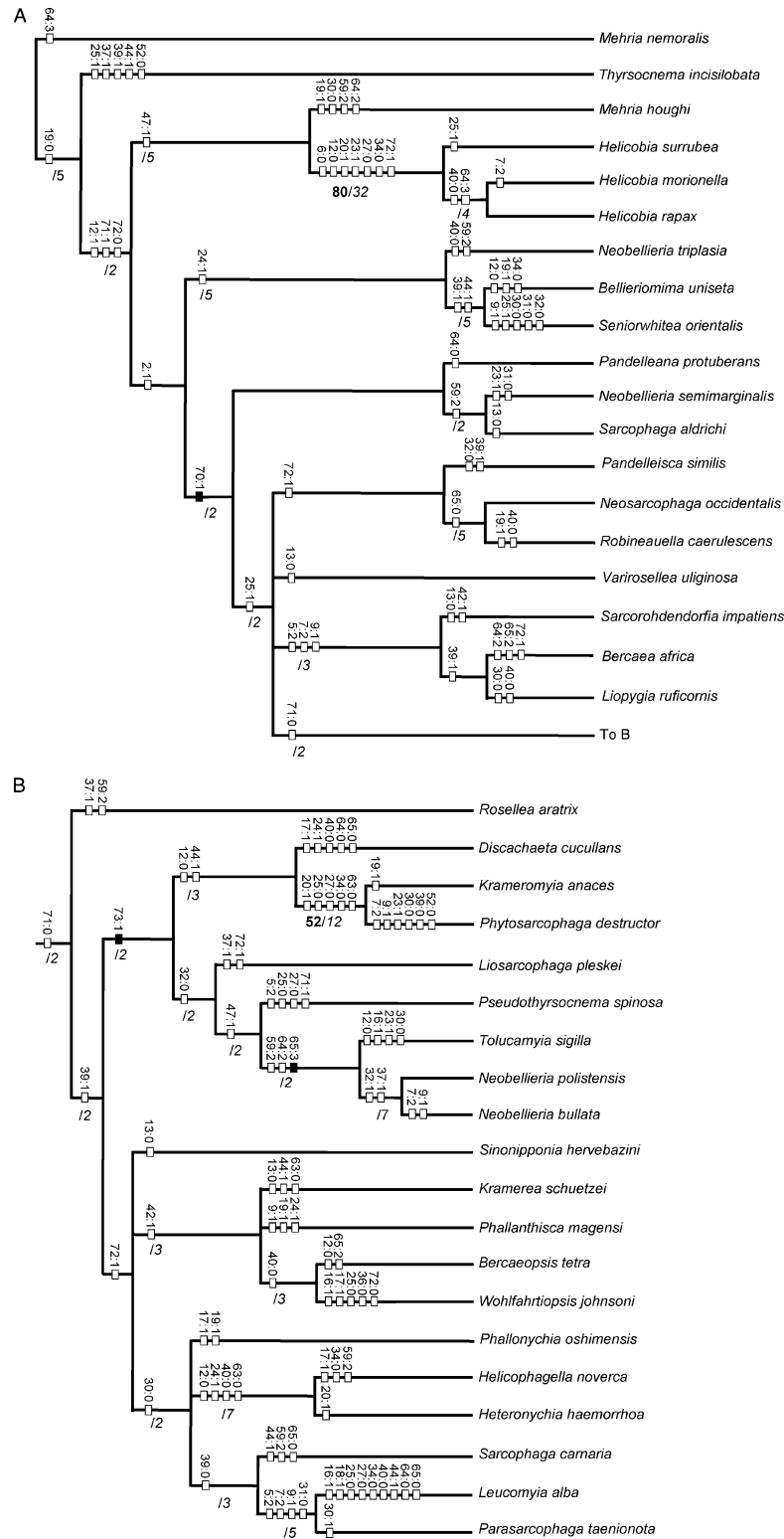
Figure 28. A, B. one of 120 most parsimonious trees obtained from the analysis under implied weights (*k* = 3) showing character distribution within *Sarcophaga s.l.* Black boxes, uniquely derived character states; white boxes, homoplasious character states. Bootstrap (3000 replicates) (bold) and relative Bremer support (italics) are given below branches.