Fly

A standardized nomenclature and atlas of the male terminalia of Drosophila melanogaster

--Manuscript Draft--

Manuscript Number:	KFLY-2019-0013R1
Full Title:	A standardized nomenclature and atlas of the male terminalia of Drosophila melanogaster
Article Type:	Brief Communication
Manuscript Classifications:	Courtship; Drosophila Research Resources; fertilization; Phylogenetics; reproduction
Corresponding Author:	Virginie Courtier-Orgogozo Institut Jacques Monod Paris, FRANCE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Institut Jacques Monod
Corresponding Author's Secondary Institution:	
First Author:	Gavin Rice
First Author Secondary Information:	
Order of Authors:	Gavin Rice
	Jean R. David
	Yoshitaka Kamimura
	John P. Masly
	Alistair P. Mcgregor
	Olga Nagy
	Stéphane Noselli
	Maria Daniela Santos Nunes
	Patrick O'Grady
	Ernesto Sánchez-Herrero
	Mark L. Siegal
	Masanori J. Toda
	Mark Rebeiz
	Virginie Courtier-Orgogozo
	Amir Yassin
Order of Authors Secondary Information:	
Manuscript Region of Origin:	FRANCE
Abstract:	Animal terminalia represent some of the most diverse and rapidly evolving structures in the animal kingdom, and for this reason have been a mainstay in the taxonomic description of species. The terminalia of Drosophila melanogaster, with its wide range of experimental tools, have recently become the focus of increased interest in the fields of development, evolution, and behavior. However, studies from different disciplines have often used discrepant terminologies for the same anatomical structures. Consequently, the terminology of genital parts has become a barrier to integrating results from different fields, rendering it difficult to determine what parts are being referenced. We formed a consortium of researchers studying the genitalia of D.

	melanogaster to help establish a set of naming conventions. Here, we present a detailed visual anatomy of male genital parts, including a list of synonymous terms, and suggest practices to avoid confusion when referring to anatomical parts in future studies. The goal of this effort is to facilitate interdisciplinary communication and help newcomers orient themselves within the exciting field of Drosophila genitalia.

A standardized nomenclature and atlas of the male terminalia of *Drosophila melanogaster*

Gavin Rice[1], Jean R. David[2], Yoshitaka Kamimura[3], John P. Masly[4], Alistair P. Mcgregor[5], Olga Nagy[6], Stéphane Noselli[7], Maria Daniela Santos Nunes[5], Patrick O'Grady[8], Ernesto Sánchez-Herrero[9], Mark L. Siegal[10], Masanori J. Toda[11]*, Mark Rebeiz[1]*, Virginie Courtier-Orgogozo[6]*, Amir Yassin[12]*

*Corresponding authors: hutian@lemon.plala.or.jp, rebeiz@pitt.edu, virginie.courtier@ijm.fr, yassin@mnhn.fr

[1] Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, United States of America; @gavinrrice, @RebeizLab

[2] Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE), UMR 9191, CNRS ,IRD, Univ.Paris-Sud, Université Paris-Saclay, Gif sur Yvette, 91198 cedex, France

[3] Department of Biology, Keio University, Hiyoshi, Yokohama 2238521, Japan

[4] Department of Biology, University of Oklahoma, 730 Van Vleet Oval, Norman, OK 73019; @MaslyLab

[5] Department of Biological and Medical Sciences, Oxford Brookes University, Gipsy Lane, Oxford, OX3 0BP, UK; @Nunes_lab

[6] CNRS UMR7592, Institut Jacques Monod, Université de Paris, Paris, France; @Biol4Ever

[7] Universite C te d'Azur, CNRS, INSERM, iBV, Nice, France

[8] Department of Entomology, Cornell University, Ithaca, NY

[9] Centro de Biología Molecular Severo Ochoa (C.S.I.C.-U.A.M.), Universidad Autónoma de Madrid, Nicolás Cabrera 1, Cantoblanco, 28049 Madrid, Spain

[10] Center for Genomics and Systems Biology, Department of Biology, New York University

[11] Hokkaido University Museum, Hokkaido University, Sapporo 060-0810, Japan

[12] Institut de Systématique, Evolution et Biodiversité, UMR7205, Centre National de la Recherche Scientifique, MNHN, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, 75005 Paris, France

Abstract

Animal terminalia represent some of the most diverse and rapidly evolving structures in the animal kingdom, and for this reason have been a mainstay in the taxonomic description of species. The terminalia of *Drosophila melanogaster*, with its wide range of experimental tools, have recently become the focus of increased interest in the fields of development, evolution, and behavior. However, studies from different disciplines have often used discrepant terminologies for the same anatomical structures. Consequently, the terminology of genital parts has become a barrier to integrating results from different fields, rendering it difficult to determine what parts are being referenced. We formed a consortium of researchers studying the genitalia of *D. melanogaster* to help establish a set of naming conventions. Here, we present a detailed visual anatomy of male genital parts, including a list of synonymous terms, and suggest practices to avoid confusion when referring to anatomical parts in future studies. The goal of this effort is to facilitate interdisciplinary communication and help newcomers orient themselves within the exciting field of *Drosophila* genitalia.

Keywords: genitalia, terminalia, anatomy, *Drosophila melanogaster,* nomenclature

Introduction

Insect terminalia, which usually encompass the male and female genitalia and analia, are among the most diverse and complex morphological structures (Eberhard 1985). In *Drosophila*, they have been the subjects of three research disciplines that led to different terminologies. The earliest of these is *ontogeny*, which aimed at identifying the segmental origin of the different structures and how they sexually differentiate during development from the larval genital disc in *D. melanogaster*. It is thanks to this discipline that the 'traditional terminology' was established (Dobzhansky 1930; Ferris 1950; Bryant 1978) and continues to be used by contemporary developmental biologists (Chatterjee et al. 2015). Most of the terms currently annotated in FlyBase (www.flybase.org) are based on the traditional system.

The second discipline was *phylogenetics*, which aimed at describing the diversity of terminalia among drosophilids in order to group species according to their similarities in these structures. The earliest comparative studies (Hsu 1949; Okada 1954) standardized the 'traditional terminology' in *Drosophila* systematics (e.g., Bock and Wheeler 1972). However, following the publication of McAlpine's (1981) *Manual of Nearctic Diptera*, an effort to standardize morphological terms of putatively homologous structures across the Diptera emerged (Grimaldi 1987, 1990). Subsequently, the resulting 'revised terminology' was widely accepted by *Drosophila* systematists (McEvey 1990; Vilela and Bächli 1990; Zhang and Toda 1992), although some terms, such as parameres, paraphyses or gonopods, remained problematic because they sometimes refer to structures not related by clear homology in different species (Hu and Toda 2001; Bächli et al. 2004).

Recently, a third discipline, *functional morphology*, has emerged, aiming at understanding the role that each genital structure may play during copulation (Acebes et al. 2003; Kamimura 2010; Polak and Rashed 2010; Frazee and Masly 2015; LeVasseur-Viens et al. 2015; Mattei et al. 2015, Tanaka et al. 2018). With advanced techniques such as laser surgery and tomography scanning, this approach has enhanced our understanding of the functional roles of genital anatomy. However, researchers in this discipline used a mixture of traditional and revised terminologies (Kamimura and Polak 2011) that can lead to confusion as community members from different disciplines assimilate the literature.

Believing that the breadth and richness of the three research disciplines offers a unique opportunity for integrative biology, the purpose of the current paper is to unify terminology of *Drosophila* male terminalia (Figure 1). As a group of researchers working on different aspects of *Drosophila* terminalia, we think that a unified system would facilitate exchanges between research fields. Although some researchers highlighted the usefulness of the traditional system in providing meaningful English terms rather than obscure Latin-derived names (e.g., clasper vs. surstylus), the majority opted for the phylogenetic tradition which captures homology relationships between species. Consequently, we provide an update of the terminalia terminology found in FlyBase. For the problematic terms (parameres, paraphyses, and gonopods), we relied on Sinclair's (2000) and Cumming and Wood's (2017) revisions of Diptera terminalia nomenclature to propose new terms not previously used in *Drosophila* biology (namely, pregonites, postgonites, and gonocoxites). Although we restricted our revision to male terminalia, we do so with the intention to address female terminology later.

Results and Discussion

A visual atlas of adult D. melanogaster male terminalia

In much of the past literature, genital morphology was rendered by hand-drawings, and the names of different parts were indicated by lines pointing to each structure. As new researchers join this growing field, it can be quite difficult to grasp the exact extent of a structure based on these drawings. In order to make the revised nomenclature as useful as possible, we provide here a visual guide to this terminology which shows both drawings and cuticle images that outline the full extent of each named part (Figures 2, 3). It is important to note that the exact size and shape of these structures, such as the epandrial posterior lobe, can vary within *D. melanogaster* (Liu et al. 1996; McNeil, Bain and Macdonald 2011). In Table 1, we propose a unified nomenclature of the various anatomical elements containing definitions and references to previously used terms. Conversely, Table 2 provides correspondence from previously used terms to the unified nomenclature. Although the current set of nomenclature is centered around *D. melanogaster*, we have adopted general terms such that most should also apply to other Drosophilidae species.

The male terminalia of *D. melanogaster* corresponds to the entire set of external structures in the distal half of the male abdomen (Figures 1–3), i.e. segments 8–10. It derives from the genital disc, which comprises three primordia: a reduced Abdominal segment 8 primordium, which in females gives rise to most genital structures but in males gives rise only to a miniature eighth tergite (here termed the epandrial anterodorsal phragma, see below); an Abdominal segment 9 primordium, forming the male genitalia, and the Abdominal segment 10 primordium, making the analia (Keisman et al., 2001). During development, the *D. melanogaster* male genitalia rotates 360 degrees clockwise, causing the internal organs to loop around the gut; this rotation and thus the dorsal/ventral designation of the genitalia varies within Diptera (Suzanne et al. 2010). We dissected and imaged adult cuticle preparations of a *D. melanogaster* wild type strain (Canton S), and provide cuticle images as well as drawings of the distinct parts in Figures 1–3. To maximize clarity, we present each part both in isolation and in the context of intact tissue, and we indicate the outlines of each anatomical component (Figures 2, 3).

We have subdivided the terminalia into two parts, periphallic structures, which are secondarily connected to the intromittent organ (Figure 2) and the phallic structures, which comprise the intromittent organ and structures directly connected to it (Figure 3). These two classes are easily separable via dissection in the adult.

Periphallic portions of the terminalia

Periphallic structures comprise the cercus (former anal plate), the epandrium (former genital arch), the pair of surstyli (former claspers) and the subepandrial sclerite (former pons) that connects the surstyli to the other periphallic structures (Figure 1). Although periphallic structures are not directly involved in transferring sperm, several of them (cercus, surstylus, and epandrial posterior lobe) have been implicated in grasping onto the female during copulation (Robertson 1988; Eberhard and Ramirez 2004; Jagadeeshan and Singh 2006; Kamimura 2010; Kamimura and Mitsumoto 2011; Mattei et al. 2015; Frazee and Masly 2015; LeVasseur-Viens et al. 2015). Additionally, although many of these structures do not show obvious boundaries in *D. melanogaster*, they are far more complex in its close relatives, suggesting that there are natural subdivisions of these structures in some species. For example, while the ventral margin of the cercus forms a relatively flat cuticle in *D. melanogaster,* it bears a lobe-like extension in *D. bipectinata* that affects copulatory success (Polak and Rashed 2010; Kamimura and Polak 2011). Furthermore, the dorsal and ventral parts of the cercus accumulate distinct levels of *engrailed* in *D. melanogaster* (Fig. 3F in Sánchez et al. 1997).

Subdivision and nomenclature of phallic parts

During copulation, several parts of the male genitalia enter the female vagina: the aedeagus, part of the phallapodeme, the ventral and dorsal postgonites, and the aedaegal sheath (Kamimura 2010; Mattei et al. 2017). All of these structures except the phallapodeme form the intromittent organ or phallus. The aedeagus is perhaps the most complex structure of the male genitalia of *D. melanogaster:* it is covered with cuticular projections and its shape varies broadly between closely related species (see Figures 13, 14, 16, 17 of Tsacas et al. 1971; Yassin and Orgogozo 2013). The postgonites are flexible relative to the aedeagus; they move progressively during mating and have

been implicated in producing copulatory wounds in females (Kamimura 2010). The aedeagal sheath surrounds the aedeagus and the postgonites dorso-laterally. It also moves outwards during mating. The movement of the postgonites and the aedeagal sheath may be induced through the complex musculature found in the phallus (Figure 5; Kamimura 2010).

The hypandrium is a large structure that surrounds the phallus ventrally. It can be broken down into several identifiable substructures. We consider the posterior part to be fused gonocoxites (see below) and divide each gonocoxite into two parts lateral and median. The fused median gonocoxites host a pair of large bristles (Taylor 1989; Nagy et al. 2018). They connect to the phallus through via the two pregonites. Each pregonite displays two to three smaller bristles.

Justifying the separation/individuality of parts

It is important to note that the boundary of each anatomical element is based largely on defined cuticular ridges observed in the adult. However, some key parts lack clear boundaries with other adjacent tissues. Examples include the epandrial posterior lobe, cercal ventral and dorsal lobes, and sub-parts of the hypandrium. We envision that in these cases, a careful analysis of cellular formation during development will be necessary to precisely define the boundaries of separate parts. Experiments that map the spatial expression patterns of regulatory genes such as transcription factors further support the boundaries of each anatomical element, and could motivate further refinements into smaller sub-parts (Sánchez et al. 1997; Vincent et al. 2019).

Implications of our system to the terminological debate within Diptera

The term "surstylus" has been proposed by Crampton (1923) to refer to the clasping organs that are associated with the dorsal compartment of the genitalia (*i.e.* epandrium) in Eremoneuran (Higher) Diptera to which *Drosophila* belongs. In non-Eremoneuran (Lower) Diptera and in other related insect orders, the clasping organs consist of appendices, the gonopods, consisting of two substructures, the gonocoxites and the gonostyli, that are associated with the ventral compartment of the genitalia (*i.e.* the hypandrium). Crampton's view, which would later be called the "surstylar concept" (Zatwarnicki 1996), postulates that the gonostyli have been lost whereas the gonocoxites

remain associated with the hypandrium in Eremoneurans. This view has a wide acceptance among Dipterologists (Yeates and Wiegmann 1999; Sinclair 2000; Cumming and Wood 2017), as well as between Drosophila systematists who have opted for a revised terminology (Grimaldi 1990; Hu and Toda 2001; Bächli et al. 2004). For example, the term gonopod, whereas used for different structures in *D. melanogaster* (see Tables 1 and 2), has always been applied to ventral structures associated with the phallic portions.

However, alternative hypotheses for the origin of the Eremoneuran dorsal claspers exist, *i.e.* the "gonostylar concept", postulating that Eremoneuran dorsal claspers are homologous to the ventral gonopods (mostly to the gonostyli) of non-Eremoneuran Diptera (reviewed in Zatwarnicki 1996). Zatwarnicki (1996) further considered the subepandrial sclerite（*medandrium* in Zatwarnicki 1996） to be homologous to the gonocoxites. Although evaluating these concepts goes far beyond the scope of our paper, we believe that further research in *Drosophila* could ultimately help elucidating the origin of the Eremoneuran dorsal claspers. For example, Abd-B mutants in *D. melanogaster* genital disc transform the phallic structures, as well as a part of the surstylus (clasper), into a leg (Estrada and Sanchez-Herrero 2001). This supports the idea that the ventral parts of the Eremoneuran genitalia are of appendicular origin (as the name gonopod, *i.e.* genital leg, would suggest), but it also suggests that a part (not the whole) of the surstylus might be of appendicular origin. Further mapping of transcription factors expression in the different compartments of the male terminalia between *D. melanogaster* (e.g., Vincent et al. 2019) and other non-Eremoneuran Diptera could shed light on the deep homology between these structures. At the time being, and because our major aim is to unify terms used by *Drosophila* biologists, we have opted here for the terminology based on the "surstylar concept", and we hope that this would prompt further research on these questions.

Incorporating the new standardized terminology into diverse ongoing studies

The revised terminology described here should facilitate cross-disciplinary synthesis of our knowledge of genital function, development, and evolution. We have worked with the FlyBase team to integrate these terms into their anatomy ontology (Costa et al. 2013; Thurmond et al. 2019).

Although we focused on the *D. melanogaster* terminalia, a standardized terminology is vital/crucial for the ease of comparing various species. Thus, it is our hope that these terms will facilitate descriptions of homologous and novel structures in other insect species. It was important for us to include as much of the community of researchers working on *Drosophila* genital morphology as possible to reach consensus in the definition and deployment of this terminology. We suggest that when publishing studies that name these structures, authors use the terms of the revised terminology, while parenthetically citing alternate synonyms such as familiar terms, e.g. surstylus (clasper). For those who would like to use familiar terms (perhaps for the purpose of continuity with previous publications), we would strongly recommend that the revised terminology is presented parenthetically, e.g. clasper (surstylus). This way, the broader scientific community can understand and integrate results with as few barriers to comprehension as possible.

Studies of *Drosophila* genitalia have provided examples of large-scale differences between males and females, vital taxonomic traits to distinguish species from one another, and important factors in the reproductive incompatibility between species. Yet, the complexity of the genitalia itself presents barriers to the study of these fascinating anatomical parts. This problem has been aggravated by variability in nomenclature, which has further impeded entry into this field. The revision and visual atlas of male genital structures provided here will hopefully allow for increased communication across a range of disciplines and welcome new scientists to this growing field.

Materials and Methods

A Canton S line of *Drosophila melanogaster* (Bloomington # 64349) was used for all imaging. Adult males were dissected in 100% EtOH with micro-forceps and mounted in PVA Mounting Medium (BioQuip). For Figure 1A, the sample was imaged at 500× magnification with a digital microscope VHX 2000 (Keyence) using lens VH-Z20R/W. For Figure 1B and 1C digital images were taken at different depths of focus using a Dino-Lite® Microscope Eyepiece Camera (AM7025X, AnMo Electronics Corporation) on an Olympus BX50 microscope and stacked with CombineZP 1.0 (https://combinezp.software.informer.com/). For Figures 2 and 3, samples were imaged at 16× magnification on a Leica M205C microscope with a Leica DFC425 camera or at 20×

magnification on a Leica DM 2000 with a Leica DFC540 C camera. Images from the former microscope were Z stack-compiled with the Leica Application Suite to allow for optimal focus. Images of the epandrial anterodorsal phragma, epandrial dorsal lobe, epandrial posterior lobe, epandrial ventral lobe, subepandrial sclerite, cercal dorsal lobe, cercal ventral lobe, lateral gonocoxite, median gonocoxite, transverse rod, and hypandrial phragma were modified in Adobe Photoshop via the eraser tool to isolate full parts along sutures to provide the clearest view of each part in its entirety. Photoshop was used because dissection of the various parts would be difficult.

Acknowlegements:

We thank Clare Pilgrim and Steve Mangold for working with us to integrate our terminology into the FlyBase anatomy ontology, as well as the reviewers and Tadeusz Zatwarnicki for their useful comments.

Disclosure of potential conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

Funding was provided by European Research Council (FP7/2007-2013 Grant Agreement no. 337579) to VC, by the National Institutes of Health (FP4/2014-2019 GM107387) to MR, by the Agence Nationale de la Recherche (ANR-18-CE02-0008) to AY, by the National Institutes of Health (R35GM118170) to MLS.

247 **References**

248

249 Acebes A, Cobb M, Ferveur JF. (2003). Species-specific effects of single sensillum ablation on
250 mating position in Drosophila. Journal of Experimental Biology, 206: 3095-3100
251 Bächli G, Vilela CR, Andersson Escher, S., & Saura, A. (2004) The Drosophilidae (Diptera) of
252 Fennoscandia and Denmark. Fauna Entomologica Scandinavica, vol. 39, 362 pp. Brill, Leiden.
253 Bock IR & Wheeler, MR (1972) The Drosophila melanogaster Species Group. The University of
254 Texas Publication 7213:1-102.
255 Bryant P (1978). Pattern formation in imaginal discs. In M. Ashburner and T. R. F. Wright, editors,
256 The Genetics and Biology of Drosophila, volume 2c, chapter 22, pages 229--335. Academic Press,
257 1978.
258 Bryant P and Hsei B (1977). Pattern Formation in Asymmetrical and Symmetrical Imaginal Discs of
259 Drosophila melanogaster. American Zoologist. 17:595-611
260 Chassagnard MT (1988) Esquisse phylogénétique du genre Zaprionus Coq. (Diptera: Drosophilidae)
261 et description de trois nouvelles espèces afrotropicales. Naturaliste can. (Rev. Eeol. Syst.), 115: 305-
262 322.
263 Chatterjee RN, Chatterjee P, Kuthe S, Acharyya-Ari M, & Chatterjee R (2015). Intersex (ix)
264 mutations of Drosophila melanogaster cause nonrandom cell death in genital disc and can induce
265 tumours in genitals in response to decapentaplegic (dppdisk) mutations. *Journal of genetics*, *94*(2),
266 207-220.
267 Costa M, Reeve S, Grumbling G & Osumi-Sutherland D (2013). The Drosophila anatomy ontology.
268 Journal of Biomedical Semantics. 4:32
269 Crampton GC (1923) The Genitalia of Male Diptera and Mecoptera Compared with Those of Related
270 Insects, from the Standpoint of Phylogeny. Transactions of the American Entomological Society
271 48(3) pp. 207-225
272 Cumming JM, & Wood DM (2017) Adult morphology and terminology. In: Kirk-Spriggs, A. H.,
273 Sinclair, B. J., editors. Manual of Afrotropical Diptera. v. 1. Introductory chapters and keys to diptera
274 families. Suricata 4, South African National Biodiversity Institute: Pretoria; pp. 89–133.

Dobzhansky T (1930) Studies on the intersexes and supersexes in Drosophila melanogaster. (in Russian, with English summary). Izvestia Biuro po evgenike 8:91-158.

Eberhard, WG (1985). *Sexual selection and animal genitalia*. Harvard University Press.

Eberhard W, Ramirez N (2004) Functional Morphology of the Male Genitalia of Four Species of *Drosophila*: Failure to Confirm Both Lock and Key and Male-Female Conflict Predictions. Annals of the Entomological Society of America, 97(5) 1007–1017.

Estrada B, Sanchez-Herrero E (2001) The Hox gene Abdominal-B antagonizes appendage development in the genital disc of Drosophila. Development 128, 331-339.

Ferris GF (1950). External morphology of the adult. In M. Demerec, editor, Biology of Drosophila, chapter 5, pages 368-419. Cold Spring Harbor Laboratory Press, facsimile edition, 1994.

Frazee, SR, Masly JP, (2015) Multiple sexual selection pressures drive the rapid evolution of complex morphology in a male secondary genital structure. Ecology and Evolution. 5(19):4437-4450.

Grimaldi, DA (1987) Phylogenetics and taxonomy of Zygothrica (Diptera: Drosophilidae). Bulletin of the American Museum of Natural History 186:103-268.

Grimaldi, DA 1990. A phylogenetic, revised classification of genera in the Drosophilidae (Diptera). Bulletin of the American Museum of Natural History 197:1-139.

Hsu, TC (1949) The external genital apparatus of male Drosophilidae in relation to systematics. The University of Texas Publication 4920:80-142.

Hu, Y.-G. & Toda, MJ (2001) Polyphyly of Lordiphosa and its relationships in Drosophilinae (Diptera: Drosophilidae). Systematic Entomology 26:15-31.

Jagadeeshan S, Singh RS (2006) A time-sequence functional analysis of mating behaviour and genital coupling in Drosophila: role of cryptic female choice and male sex-drive in the evolution of male genitalia. Journal of Evolutionary Biology, 19: 1058–1070.

Kamimura Y (2010). Copulation anatomy of Drosophila melanogaster (Diptera: Drosophilidae): wound-making organs and their possible roles. *Zoomorphology*, *129*(3), 163-174.

Kamimura Y & Mitsumoto H (2011) Comparative copulation anatomy of the *Drosophila melanogaster* species complex (Diptera: Drosophilidae). Entomological Science 14: 399-410.

Kamimura Y, Polak M (2011) Does surgical manipulation of Drosophila intromittent organs affect insemination success? Proc. R. Soc. B 278:815-816.

Keisman EL, Christiansen AE, & Baker, BS (2001). The sex determination gene *doublesex* regulates the A/P organizer to direct sex-specific patterns of growth in the *Drosophila* genital imaginal disc. Dev Cell 1(2):215-225.

Kopp AV, True JR (2002) Evolution of male sexual characters in the Oriental Drosophila melanogaster species group. Evolution & Development 4:4, 278–291

LeVasseur-Viens H, Polak M, Moehring AJ (2015) No evidence for external genital morphology affecting cryptic female choice and reproductive isolation in Drosophila. Evolution 69(7):1797-80

Liu J, Mercer JM, Stam LF, Gibson GC, Zeng ZB, and Laurie ZB (1996) Genetic analysis of a morphological shape difference in the male genitalia of Drosophila simulans and D. mauritiana. Genetics 142: 1129-1145.

Mattei AL, Riccio ML, Avila FW, Wolfner MF (2015) Integrated 3D view of postmating responses by the Drosophila melanogaster female reproductive tract, obtained by micro-computed tomography scanning. Proceedings of the National Academy of Sciences 112 (27), 8475-8480.

Mattei AL, Kamimura Y, Wolfner MF. (2017). Intimate intimas: positioning of copulatory organs in mating Drosophila. Mol. Repro. Devt., 84:1117.

McAlpine JF (1981) Morphology and terminology-adults. In J. F. McAlpine et al., Manual of Nearctic Diptera, vol 1, chapter 2. Agriculture Canada Monograph No. 27. Ottawa: Minister of Supply and Services.

McEvey, SF (1990) New species of Scaptomyza from Madagascar and Mauritius with a note on terminology (Diptera: Drosophilidae). Annales de la Société entomologique de France 26(N.Ser.):51-64.

McNeil C, Bain C, Macdonald S (2011) Multiple Quantitative Trait Loci Influence the Shape of a Male-Specific Genital Structure in *Drosophila melanogaster*. G3: Genes|Genomes|Genetics 1(5): 343-351.

328 Nagy O, Nuez I, Savisaar R, Peluffo AE, Yassin A, Lang M, Stern DL, Matute D, David JR, Courtier-
329 Orgogozo V (2018) Correlated evolution of two copulatory organs via a single cis-regulatory
330 nucleotide change. Current Biology 28 (21), 3450-3457.
331 Okada T (1954) Comparative morphology of the drosophilid flies. I. Phallic organs of the
332 melanogaster group. Kontyu 22:36-46.
333 Okada T (1956) Systematic study of Drosophilidae and allied families of Japan. Gihodo Co., Ltd.,
334 Tokoyo.
335 Okada T (1963) Cladogenetic differentiation of Drosophilidae in relation to material compensation.
336 Mushi, 37: 79-100.
337 Polak M, & Rashed A 2010 Microscale laser surgery reveals adaptive function of male intromittent
338 genitalia. Proc. R. Soc. B 277, 1371 – 1376.
339 Robertson HM, (1988) Mating asymmetries and phylogeny in the Drosophila melanogaster species
340 complex. Pac. Sci. 42: 72–80
341 Salles H (1947) Sobre a Genitalia dos Drosofilidios (Diptera): I. Drosophila melanogaster E. D.
342 simulans. Summa Brasiliensis Biologiae (15): 1-73.
343 Sánch z L, Casares F, Gorfinkiel N, Guerrero I (1997) The genital disc of Drosophila melanogaster.
344 II. Role of the genes hedgehog, decapentaplegic and wingless. Development Genes and Evolution
345 207:229–241
346 Sánchez L, Guerrero I (2001) The development of the Drosophila genital disc. BioEssays 23:698–
347 707.
348 Sinclair, BJ (2000) Morphology and terminology of Diptera male terminalia. In: Papp, L. and Darvas,
349 B. (eds), Contributions to a Manual of Palaearctic Diptera, Vol. 1, pp. 53-74. Science Herald,
350 Budapest.
351 Suzanne M, Petzoldt A, Spéder P, Coutelis J, Steller H, and Noselli S (2010) Coupling of apoptosis
352 and L/R patterning controls stepwise organ looping. Current Biology 20:19, 1773-1778.
353 Tanaka, KM, Kamimura, Y, & Takahashi, A (2018) Mechanical incompatibility caused by
354 modifications of multiple male genital structures using genomic introgression in *Drosophila*.
355 Evolution 72: 2406-2418.

Taylor, BJ (1989) Sexually Dimorphic Neurons in the Terminalia of Drosophila Melanogaster: I. Development of Sensory Neurons in the Genital Disc During Metamorphosis, Journal of Neurogenetics, 5:3, 173-192, DOI: 10.3109/01677068909066207

Thurmond J, Goodman JL, Strelets VB, Attrill H, Gramates LS, Marygold SJ, Matthews BB, Millburn G, Antonazzo G, Trovisco V, Kaufman TC, Calvi BR, and the FlyBase Consortium. (2019) FlyBase 2.0: the next generation. Nucleic Acids Research 47(D1): D759--DD765.

Tsacas L, Bocquet C, Daguzan M, & Mercier A (1971) Comparaison des genitalia males de *Drosophila melanogaster*, de *Drosophila simulans*et de leurs hybrides (Dipt. Drosophilidae). Annales de la Société entomologique de France 7:75-93.

Vilela, CR & Bachli, G. (1990) Taxonomic studies on Neotropical species of seven genera of Drosophilidae (Diptera). Mitteilungen der Schweizerische Entomologischen Gesellschaft 63:1-332.

Vincent BJ, Rice GR, Wong GM, Glassford WJ, Downs KI, Shastay JL, Charles-Obi K, Natarajan M, Gogol M, Zeitlinger J, Rebeiz M. (2019) An atlas of transcription factors expressed in the Drosophila melanogaster pupal terminalia. Biorxiv. doi: https://doi.org/10.1101/677260

Wheeler MR. (1960) Sternite modification in males of the Drosophilidae (Diptera). Annals of the Entomological Society of America, 53: 133-137.

Wheeler MR. (1987) Drosophilidae (Chap. 95). In J. F. McAlpine (ed.), Manual of Nearctic Diptera 2: 1011-1018. Research Branch Agriculture Canada monogr. 28. Ottawa: Minister Supply and Services Canada.

Yassin A & Orgogozo V. (2013) Coevolution between Male and Female Genitalia in the Drosophila melanogaster Species Subgroup. PLoS ONE 8:e57158.

Yeates, D.K. & Wiegmann, B.M. (1999) Congruence and controversy: toward a higher-level phylogeny of Diptera. *Annual Review of Entomology* 44: 397–428.

Zatwarnicki T (1996) A new reconstruction of the origin of eremoneuran hypopygium and its implications for classification (Insecta: Diptera). Genus 7: 103–175.

381 Zhang, W & Toda, MJ. (1992) A New Species-subgroup of the Drosophila immigrans Species-group
382 (Diptera, Drosophilidae), with Description of Two New Species from China and Revision of
383 Taxonomic Terminology. Japanese Journal of Entomology 60:839-850.

Figures:

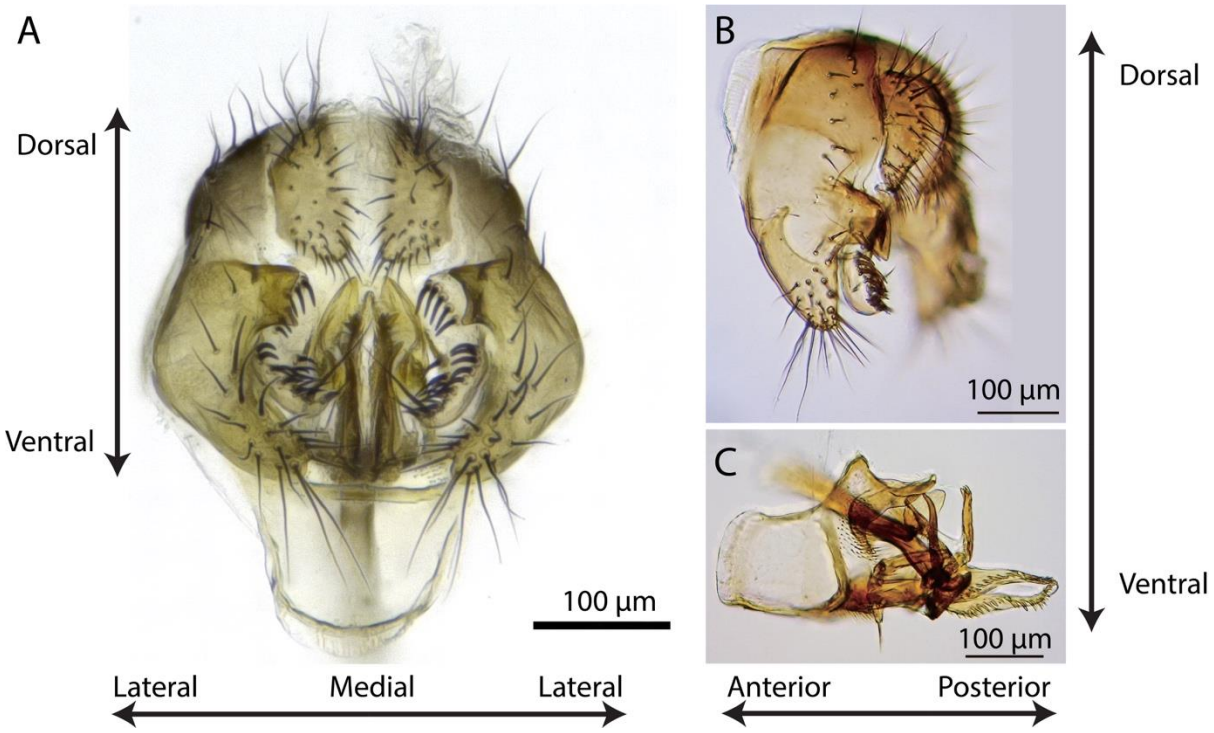


Figure 1. (A) Light microscope preparation of the entire male terminalia of *D. melanogaste*r Canton S. Scale bar is 100 µm. (B) Caudolateral view of the periphallic structures. (C) Ventrolateral view of the phallic structures. Scale bars are 100 µm. Note that the exact size and shape of terminalia structures, such as the epandrial posterior lobe, vary within *D. melanogaster* (Liu et al. 1996; McNeil, Bain and Macdonald 2011).

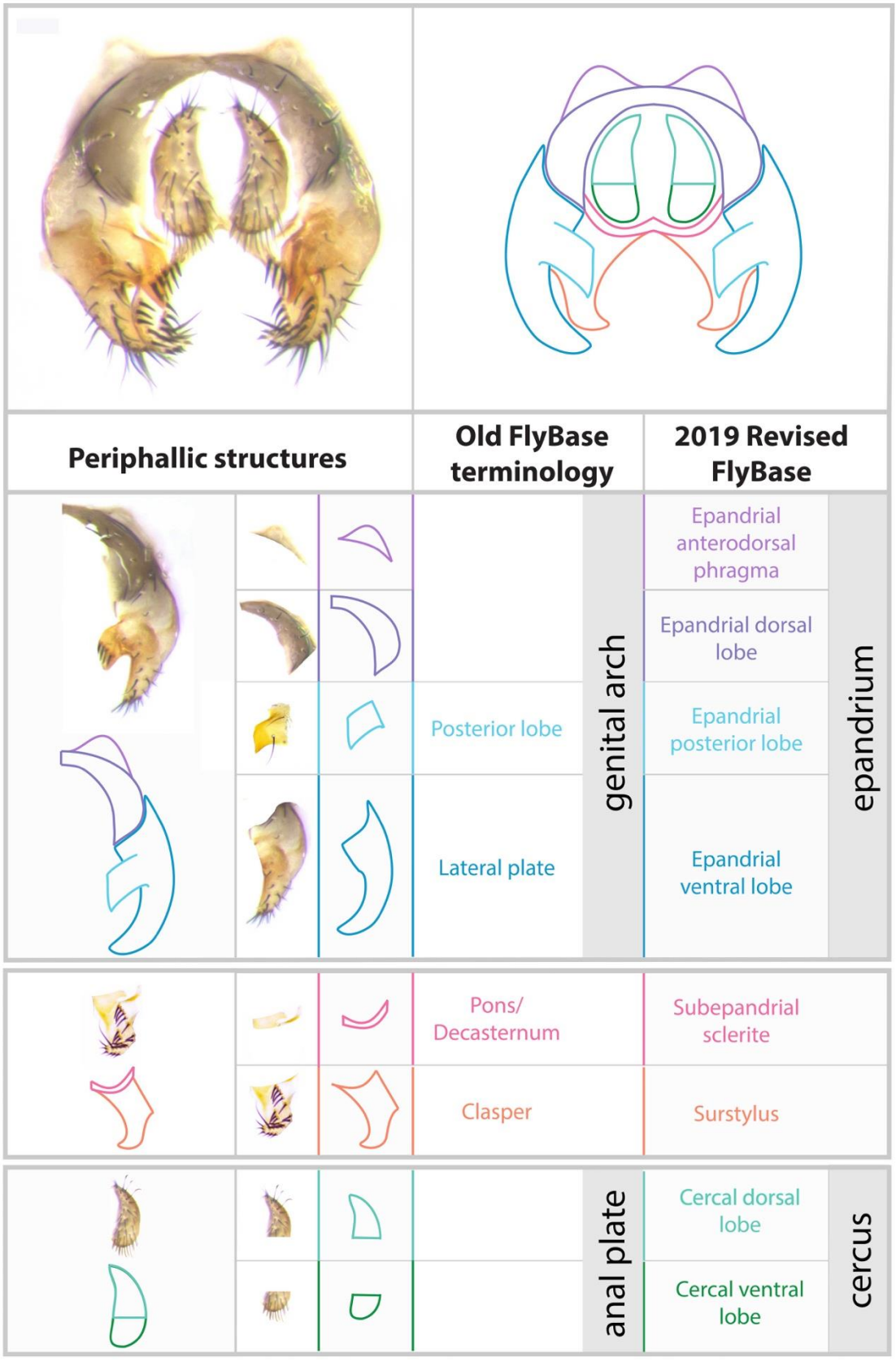


Figure 2. Visual atlas of periphallic structures. Light microscopy images (Canton S strain) and diagrams representing the broad divisions and substructures of epandrium and cercus. The images are

oriented dorsal (top) to ventral (bottom). Previous FlyBase terms are on the left and 2019 revised terms are on the right.

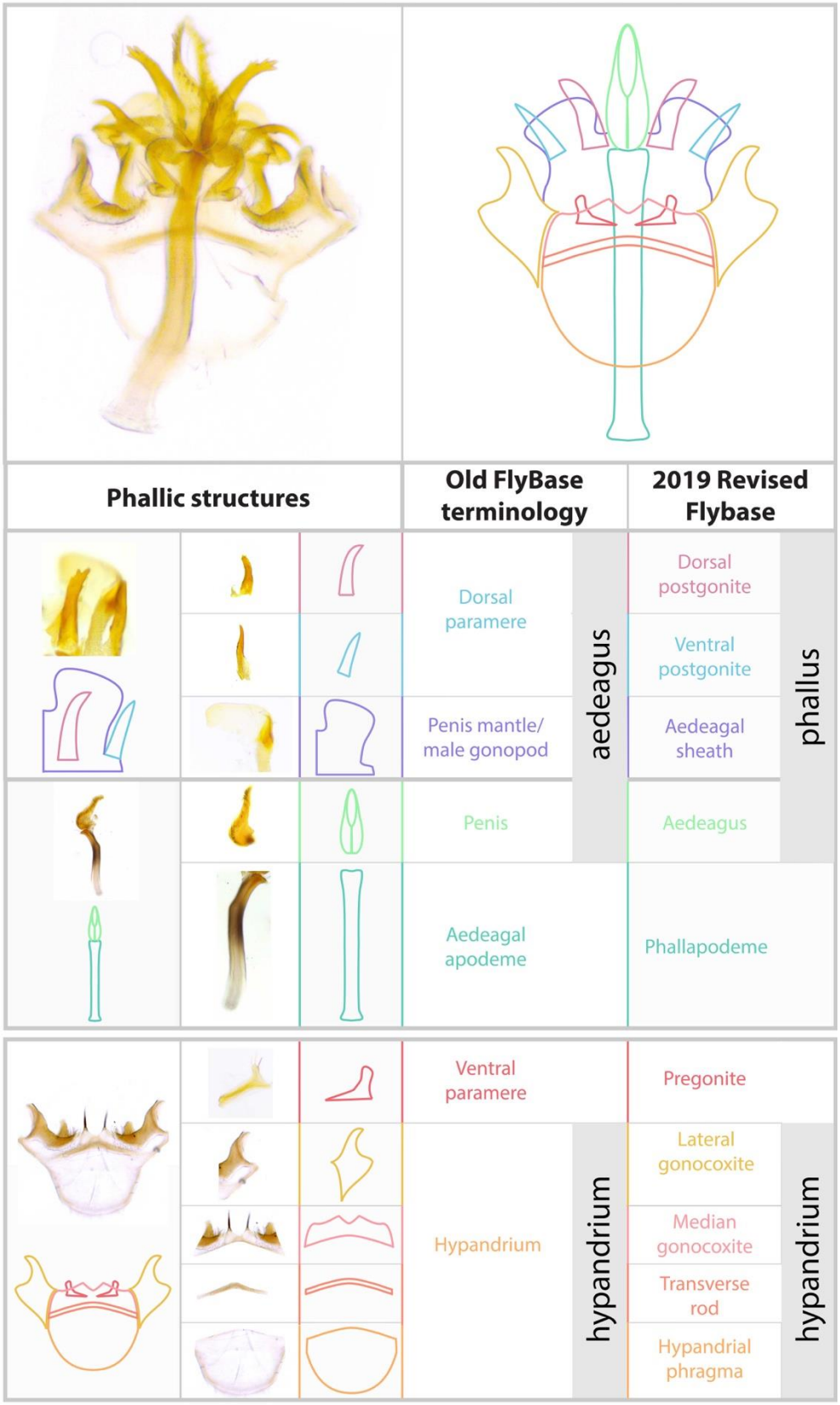
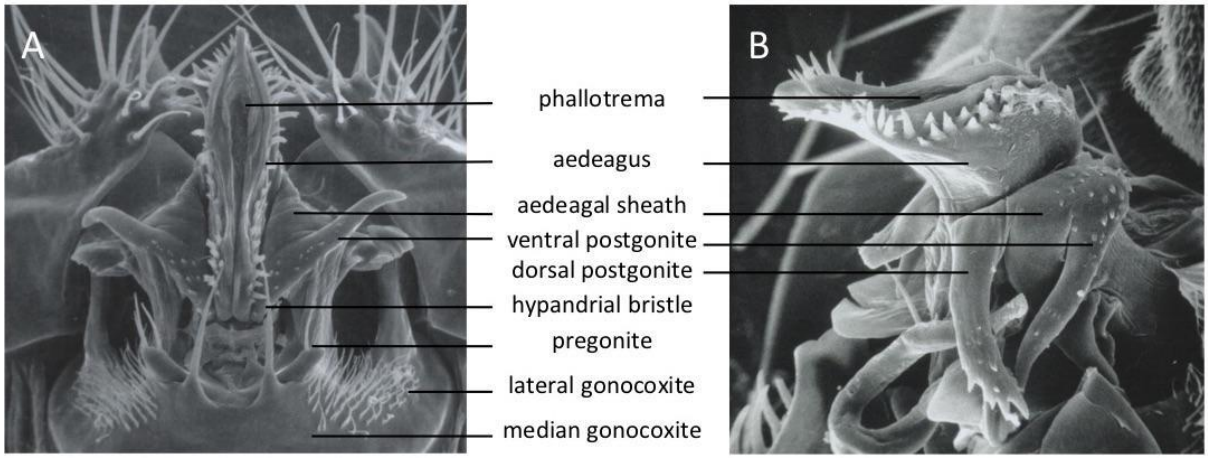


Figure 3. Visual atlas of phallic structures. Light microscopy images (Canton S strain) and diagrams representing the broad divisions and substructures of phallus and hypandrium. The images are

oriented posterior (top) to anterior (bottom). Previous FlyBase terms are on the left and the 2019 revised terms are on the right.



402

403

404 Figure 4. Scanning electron micrographs of the phallic structures in (A) ventral and (B) lateral views,

405 from L. Tsacas' collection at the National Museum of Natural History, Paris (Courtesy of the

406 Museum).

407

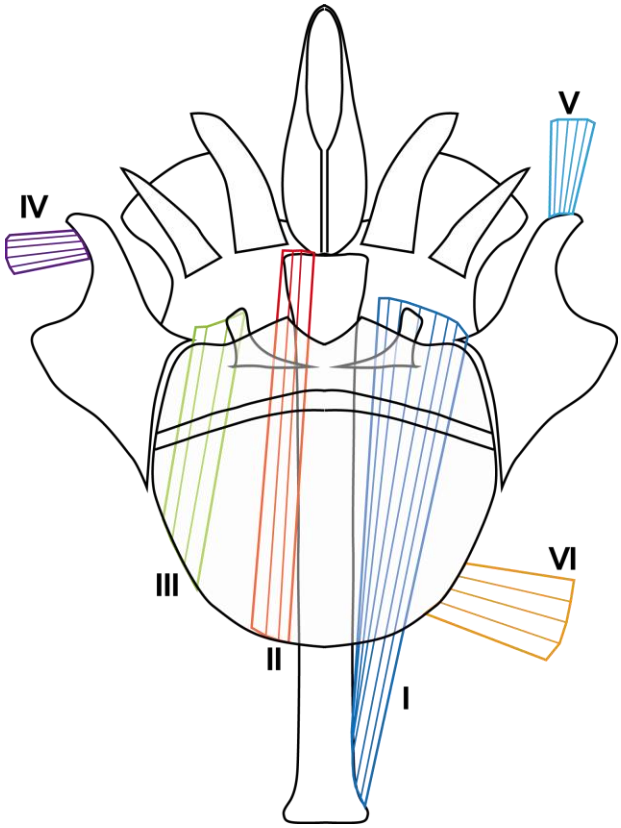


Figure 5. Musculature of the phallic structures. Same diagram of cuticular parts as in Fig. 3 (ventral view). Muscles are indicated in distinct colors and numbered I to VI (Kamimura 2010). These muscles are bilateral. For sake of clarity, muscles are shown either on the left or on the right side of the diagram. See Table 1 for muscles description.

Tables

Table 1. Definition of the terms in the standardized nomenclature.

Parts
Male analia (FBbt:00004825). Definition: The entire set of external structures in the distal half of the male abdomen, i.e. segment 10, that makes up the anal apparatus (*cerci* and anus). It develops from the male genital disc.
Male genitalia (FBbt:00004828). Definition: Set of internal and external structures originating from segments 8--9, that makes up the genital apparatus. It develops from the male genital disc.
Male terminalia (FBbt:00004835). Definition: The entire set of external structures in the distal half of the male abdomen, i.e. segments 8--10, that makes up the *male genitalia* and *male analia*. It develops from the male genital disc.
Sclerites
Aedeagal sheath (FBbt:00004845) FlyBase synonyms: male gonopod, male paramere, penis mantle. Definition: A membranous process that dorsally connects to the two posterior sides of the *hypandrium*, embracing the *aedeagus* and both pairs of *postgonites*. Synonyms: phallus envelope (Tsacas et al. 1971), penis mantle (Salles 1947, Bryant and Hsei 1977), gonopod (Hu and Toda 2001, Kamimura 2010), posterior paramere (Okada 1954, Bock and Wheeler 1972), dorsal arch (Bächli et al. 2004).
Aedeagus (FBbt:00004852) FlyBase synonyms: penis. Definition: A tubular organ with a single external opening called *phallotrema*. The aedeagus is entirely membranous and laterally covered with fringe-like, irregular rows of long and blunt scales. Synonyms: penis (Salles 1947, Ferris 1950, Bryant and Hsei 1977), phallus (Tsacas et al. 1971).
Cercal dorsal lobe (FBbt:00048379) FlyBase synonyms: New term. Definition: Dorsal portion of the cercus bearing long thin *cercal dorsal bristles*.
Cercal ventral lobe (FBbt:00048380) FlyBase synonyms: New term. Definition: Ventral portion of the cercus bearing short rigid *cercal ventral bristles*. Synonyms: secondary clasper (Hsu 1949).
Cercus (FBbt:00004844) FlyBase synonyms: anal plate. Definition: Paired tergite that lies immediately lateral to the anus in males. There are two of these in a male individual. Synonyms: abdominal tergite 10 (Salles 1947); anal plate (Salles 1947, Hsu 1949, Ferris 1950, Tsacas et al. 1971, Bryant and Hsei 1977), cercus (Hu and Toda 2001, Bächli et al. 2004).
Dorsal postgonite (FBbt:00048381) FlyBase synonyms: New term. Definition: Dorsal branch of the postgonite, covered with tiny scales. Synonyms: dorsal branch of basal process (Kamimura 2010), dorsal paramere (Bryant and Hsei 1977).
Epandrial anterodorsal phragma (FBbt:00048382) FlyBase synonyms: New term. Definition: thin sclerite connecting the *epandrium* to abdominal tergites 6 and 7. Synonyms: abdominal tergite 8 (Salles 1947), phragma (Hsu 1949, Ferris 1950, Tsacas et al. 1971, Bryant and Hsei 1977).

Epandrial dorsal lobe (FBbt:00048383)
FlyBase synonyms: New term.
Definition: Dorsal portion of the *epandrium* above the *epandrial posterior lobe*. The two dorsal lobes are fused into a single sclerite. It contains about 8 long thin bristles.

Epandrial posterior lobe (FBbt:00004841)
FlyBase synonyms: posterior lobe.
Definition: Lobe on the posterior region of the *epandrium*. It is posterior to the *epandrial ventral lobe* and mostly covers the *surstylus*.
Synonyms: posterior process (Salles 1947, Hsu 1949), posterior lobe (Ferris 1950, Tsacas et al. 1971, Bryant and Hsei 1977), dorsal branch of the ventral epandrial lobe (Bächli et al. 2004)), lateral lobe (Sánchez and Guerrero 2001), ventral lobe (Eberhard and Ramirez 2004), ventral process (Eberhard and Ramirez 2004).

Epandrial ventral lobe (FBbt:00004842)
FlyBase synonyms: lateral plate.
Definition: Lobe ventral to the *epandrial dorsal lobe* and anterior to the *epandrial posterior lobe*. It contains about 22 long thin bristles.
Synonyms: lateral plate (Bryant and Hsei 1977), pouch (Salles 1947), toe (Hsu 1949), epandrial ventral lobe (Bächli et al. 2004).

Epandrium (FBbt:00004839)
FlyBase synonyms: genital arch, abdominal tergite 9.
Definition: Horseshoe-shaped tergite which, dorsally, surrounds the male cerci. It contains about 30 *epandrial bristles* on each side. The left and right sides of the epandrium are connected by the *subepandrial sclerite*. The ventral part of each side of the epandrium is divided into an *epandrial ventral lobe* and an *epandrial posterior lobe*.
Synonyms: abdominal tergite 9 (Salles 1947), genital arch (Salles 1947, Hsu 1949, Ferris 1950, Tsacas et al. 1971), epandrium (Tsacas et al. 1971, Hu and Toda 2001, Bächli et al. 2004).

Gonocoxite (FBbt:00048384)
FlyBase synonyms: New term.
Definition: Part of the *hypandrium* posterior to the *hypandrial transverse rod*. It is posteriorly protruded into the *lateral gonocoxite* and the *median gonocoxite*.
Synonyms: abdominal sternite 9 (Ferris 1950), novasternum (Okada 1954, Tsacas et al. 1971), gonopod (Bächli et al 2004).

Hypandrial phragma (FBbt:00048385)
FlyBase synonyms: New term.
Definition: Part of the *hypandrium* anterior to the *hypandrial transverse rod*.
Synonyms: intersegmental phragma (Ferris 1950), ventral fragma (Okada 1954), ventral phragma (Tsacas et al. 1971), hypandrium (Bächli et al. 2004), hypandrial apodeme (Kamimura 2010).

Hypandrial transverse rod (FBbt:00048386)
FlyBase synonyms: New term.
Definition: Sclerotized line extended transversally from left to right that separates the *hypandrium* into the *gonocoxite* and the *hypandrial phragma*.
Synonyms: transversal thickening of the hypandrium (Salles 1947), scleoritzed rod (Tsacas et al. 1971).

Hypandrium (FBbt:00004847)
FlyBase synonyms: abdominal sternite 9.
Definition: The male ninth abdominal sternum which extends beneath the phallus. Its posterior ends are dorsally connected to the *aedeagal sheath*. The hypandrium is composed of the *gonocoxite* posteriorly, the *hypandrial phragma* anteriorly and the *hypandrial transverse rod* in between.
Synonyms: novasternum (Wheeler 1960, Okada 1963), hypandrium (Salles 1947, Tsacas et al. 1971, Zhang and Toda 1992, Hu and Toda 2001).

Lateral gonocoxite (FBbt:00004849)
FlyBase synonyms: hypandrial process.
Definition: Lateral part of the *gonocoxite*. It comprises a sclerotized pocket into which the female ovipositor inserts during copulation. It is connected with *epandrium* (*epandrial ventral lobe* and *epandrial posterior lobe*) via two pairs of muscle bundles (*male genital muscles IV* and *male genital muscles V*).
Synonyms: outer process (Salles 1947), lateral process (Okada 1954), lateral expansion (Tsacas et al. 1971), paramere (Wheeler 1987, Grimaldi 1990).

Median gonocoxite (FBbt:00048387)
FlyBase synonyms: New term.

Definition: Medial part of the *gonocoxite*, which bears the *hypandrial bristle*. The two gonocoxites are medially fused into a single sclerite bearing the two hypandrial bristles.

Periphallic sclerite (FBbt:00048388)
FlyBase synonyms: New term.
Definition: Sclerites that are used during mating to grasp the female oviscapt from the outside. They include the *epandrium*, the *surstyli* and the *cerci*.

Phallapodeme (FBbt:00003524)
FlyBase synonyms: aedeagal apodeme, basal apodeme of penis.
Definition: Long, slender apodeme extending from the base of the *phallus* into the body.
Synonyms: aedeagal apodeme (Hu and Toda 2001; Bächli et al. 2004)), basal apodeme of penis (Ferris 1950, Okada 1954), penis apodeme (Salles 1947, Bryant and Hsei 1977), phallapodeme (Tsacas et al. 1971)

Phallotrema (FBbt:00048389)
FlyBase synonyms: New term.
Definition: External opening of the aedeagus.
Synonyms: gonopore (Grimaldi 1987, Chassagnard 1988, Zhang and Toda 1992, Bächli et al. 2004), phallotreme (Eberhard and Ramirez 2004), secondary gonopore (Sinclair 2000).

Phallic sclerites (FBbt:00048390)
FlyBase synonyms: New term.
Definition: Sclerites that are used during mating to penetrate or facilitate penetrating the female vagina. They include the *phallus*, the *phallapodeme* and the *hypandrium*.
Synonyms: penis apparatus (Bryant and Hsei 1977).

Phallus (FBbt:00004850)
FlyBase synonyms: aedeagus.
Definition: The main part of the *male genitalia* used for intromission. The distal portion, through which the male ejaculates, is the *aedeagus*. The basal portion consists of a pair of bifurcate processes called *postgonites* and the *aedeagal sheath*.
Synonyms: aedeagus. Note that the aedeagus corresponds to another anatomical part in the new nomenclature.

Postgonite (FBbt:00004854)
FlyBase synonyms: dorsal paramere.
Definition: Bifurcate process on the basal portion of the *phallus*. The *dorsal postgonite* is covered with tiny scales, whereas the *ventral postogonite* has a texture similar to gooseflesh. The dorsal and ventral postgonites are parallel to the aedeagus at rest and spread laterally during erection.
Synonyms: posterior paramere (Okada 1954, Tsacas et al. 1971), dorsal gonapophysis (Salles 1947), basal process (Hu and Toda 2001), inner paraphysis (Bächli et al. 2004).

Pregonite (FBbt:00004855)
FlyBase synonyms: ventral paramere.
Definition: A pair of lobes arising from and attached to the *hypandrium*, anterior to the phallus. They bear small *pregonal bristles*.
Synonyms: hypandrial process (Bryant and Hsei 1977), paramere (Hu and Toda 2001), paraphysis (Grimaldi 1990), anterior paramere (Okada 1954, Bock and Wheeler 1972), ventral gonapophysis (Salles 1947).

Subepandrial sclerite (FBbt:00004840)
FlyBase synonyms: pons, decasternum.
Definition: A bridge-like sclerite that internally connects the two sides of the *epandrium* beneath the anus.
Synonyms: abdominal sternite 10 (Salles 1947), decasternum (Okada 1956; Bächli et al. 2004), bridge (Salles 1947, Tsacas et al. 1971), pons (Bryant and Hsei 1977).

Surstylus (FBbt:00004843)
FlyBase synonyms: clasper.
Definition: Paired hook-shaped sclerotized lobe that extends ventrally from the *subepandrial sclerite* and surrounds the phallus. It contains 25 thorn-like bristles (*surstylar teeth*) in a curved band and one *long surstylar bristle* at the end.
Synonyms: inner lobe of tergite 9 (Ferris 1950), coxopodite (Ferris 1950), clasper (Salles 1947), primary clasper (Hsu 1949), forceps (Tsacas et al. 1971), surstylus (Hu and Toda 2001, Bächli et al. 2004).

Ventral postgonite (FBbt:00048391)
FlyBase synonyms: New term.
Definition: Ventral branch of the postgonite, covered with tiny scales.
Synonyms: ventral branch of basal process (Kamimura 2010), ventral paramere (Bryant and Hsei 1977).

Setation

Cercal bristle (FBbt:00048392)
FlyBase synonyms: New term.
Definition: Any bristle that is part of the *cercus*. There are nearly 40 of these.

Cercal dorsal lobe bristle (FBbt:00048393)
FlyBase synonyms: New term.
Definition: Any bristle that is part of the *cercal dorsal lobe*. They are longer and less rigid than the c*ercal ventral lobe bristle*.

Cercal ventral lobe bristle (FBbt:00048394)
FlyBase synonyms: New term.
Definition: Any bristle that is part of the *cercal ventral lobe*. They are shorter and more rigid than the *cercal dorsal lobe bristle*.

Epandrial bristle (FBbt:00048395)
FlyBase synonyms: New term.
Definition: Any bristle that is part of the *epandrium*. There are 30 of these.

Epandrial dorsal lobe bristle (FBbt:00048396)
FlyBase synonyms: New term.
Definition: Any bristle that is part of the *epandrial dorsal lobe*. There are 8 of these.

Epandrial ventral lobe bristle (FBbt:00048397)
FlyBase synonyms: New term.
Definition: Any bristle that is part of the *epandrial ventral lobe*. There are 22 of these on each lobe.

Hypandrial bristle (FBbt:00004472)
Definition: Long bristle located on the *median gonocoxite*. There are two of these on the *hypandrium*.

Hypandrial hair (FBbt:00004473)
Definition: Fine hair on the *median gonocoxite*. There is a group of these.

Male terminalia sensillum (FBbt:00004469)
Definition: Any sensillum that is part of some *male terminalia*.

Pregonal bristle (FBbt:00048398)
FlyBase synonyms: New term.
Definition: Any bristle that is part of the *pregonite*. They are 3 of these.

Surstylar long bristle (FBbt:00004471)
FlyBase synonyms: clasper long bristle.
Definition: Single long bristle at the end of the *surstylus*. Sometimes there is more than one long bristle per surstylus.

Surstylar teeth (FBbt:00004470)
FlyBase synonyms: clasper tooth.
Definition: Thorn-like bristles of the *surstylus*. There are 25 of these arranged in a curved band.
Synonyms: clasper teeth (Kopp and True 2002), prensisetae (Grimaldi 1990).

Musculature

Male genital muscle I (FBbt:00003552)
FlyBase synonyms: penis protractor muscle.
Definition: A pair of large muscle bundles connecting the anterior end of the *phallapodeme* and the base of *aedeagal sheath + lateral gonocoxite*, which works as the protractor muscles of the *phallus* and its associated structures.
Synonyms: aedeagus protractor muscle, muscles i (Kamimura 2010).

Male genital muscle II (FBbt:00110926)
FlyBase synonyms: penis retractor muscle.
Definition: A pair of muscle bundles connecting the base of *phallus* and the *hypandrial phragma* which works as the retractor muscles of the *phallus* and its associated structures.
Synonyms: aedeagus retractor muscle, muscles ii (Kamimura 2010).

Male genital muscle III (FBbt:00048399) FlyBase synonyms: New term. Definition: A pair of muscle bundles connecting the base of *aedeagal sheath*, the *lateral gonocoxite* and the *hypandrial phragma*. Synonyms: muscles iii (Kamimura 2010).
Male genital muscle IV (FBbt:00048400) FlyBase synonyms: New term. Definition: A pair of muscle bundles connecting the *lateral gonocoxite* and the *epandrial ventral lobe*. Synonyms: muscles iv (Kamimura 2010).
Male genital muscle V (FBbt:00048401) FlyBase synonyms: New term. Definition: A pair of muscle bundles connecting the *lateral gonocoxite* below the *epandrial posterior lobe*. Synonyms: muscles v (Kamimura 2010).
Male genital muscle VI (FBbt:00048402) FlyBase synonyms: New term. Definition: A pair of muscle bundles connecting the *hypandrial phragma* and the abdominal sternite 6. Synonyms: muscles vi (Kamimura 2010).

Table 2. Table of correspondence between terms previously used in publications and term of the standardized nomenclature.

Previous terminology	**Synonym in the new nomenclature**	**Reference**
abdominal sternite 10	Subepandrial sclerite	Salles 1947
abdominal sternite 9*	Gonocoxite	Ferris 1950
abdominal sternite 9*	Hypandrium	old FlyBase terminology
abdominal tergite 10	Cercus	Salles 1947
abdominal tergite 8	Epandrial anterodorsal phragma	Salles 1947
abdominal tergite 9	Epandrium	Salles 1947
aedeagal apodeme	Phallapodeme	Hu and Toda 2001, Bächli et al. 2004
anal plate	Cercus	Salles 1947, Hsu 1949, Ferris 1950, Tsacas et al. 1971, Bryant and Hsei 1977
anterior paramere	Pregonite	Okada 1954, Bock and Wheeler 1972
basal apodeme of penis	Phallapodeme	Ferris 1950, Okada 1954
basal process	Postgonite	Hu and Toda 2001
bridge	Subepandrial sclerite	Salles 1947, Tsacas et al. 1971
cercus	Cercus	Hu and Toda 2001, Bächli et al. 2004
clasper	Surstylus	Salles 1947
clasper teeth	Surstylar teeth	Kopp and True 2002
coxopodite	Surstylus	Ferris 1950
decasternum	Subepandrial sclerite	Okada 1956; Bächli et al. 2004
dorsal arch	Aedeagal sheath	Bächli et al. 2004
dorsal branch of basal process	Dorsal postgonite	Kamimura 2010

dorsal branch of the ventral epandrial lobe	Epandrial posterior lobe	Bächli et al. 2004
dorsal gonapophysis	Postgonite	Salles 1947
dorsal paramere*	Dorsal postgonite	Bryant and Hsei 1977
dorsal paramere*	Postgonite	old FlyBase terminology
epandrial ventral lobe	Epandrial ventral lobe	Bächli et al. 2004
forceps	Surstylus	Tsacas et al. 1971
genital arch	Epandrium	Salles 1947, Hsu 1949, Ferris 1950, Tsacas et al. 1971
gonopod*	Gonocoxite	Bächli et al 2004
gonopod*	Aedeagal sheath	Hu and Toda 2001, Kamimura 2010
gonopore	Phallotrema	Grimaldi 1987, Chassagnard 1988, Zhang and Toda 1992, Bächli et al. 2004
hypandrial apodeme	Hypandrial phragma	Kamimura 2010
hypandrial process	Pregonite	Bryant and Hsei 1977
hypandrium	Hypandrial phragma	Bächli et al. 2004
inner lobe of tergite 9	Surstylus	Ferris 1950
inner paraphysis	Postgonite	Bächli et al. 2004
intersegmental phragma	Hypandrial phragma	Ferris 1950
lateral expansion	Lateral gonocoxite	Tsacas et al. 1971
lateral lobe	Epandrial posterior lobe	Sánchez and Guerrero 2001
lateral plate	Epandrial ventral lobe	Bryant and Hsei 1977
lateral process	Lateral gonocoxite	Okada 1954
male paramere	Aedeagal sheath	old FlyBase terminology
novasternum*	Gonocoxite	Okada 1954, Tsacas et al. 1971
novasternum*	Hypandrium	Wheeler 1960, Okada 1963
outer process	Lateral gonocoxite	Salles 1947
paramere*	Lateral gonocoxite	Wheeler 1987, Grimaldi 1990
paramere*	Pregonite	Hu and Toda 2001
paraphysis	Pregonite	Grimaldi 1990
penis	Aedeagus	Salles 1947, Ferris 1950, Bryant and Hsei 1977
penis apodeme	Phallapodeme	Salles 1947, Bryant and Hsei 1977
penis apparatus	Phallic sclerites	Bryant and Hsei 1977
penis mantle	Aedeagal sheath	Salles 1947, Bryant and Hsei 1977
phallotreme	phallotrema	Eberhard and Ramirez 2004
phallus	Aedeagus	Tsacas et al. 1971

phallus envelope	Aedeagal sheath	Tsacas et al. 1971
phragma	Epandrial anterodorsal phragma	Hsu 1949, Ferris 1950, Tsacas et al. 1971, Bryant and Hsei 1977
pons	Subepandrial sclerite	Bryant and Hsei 1977
posterior lobe	Epandrial posterior lobe	Ferris 1950, Tsacas et al. 1971, Bryant and Hsei 1977
posterior paramere*	Postgonite	Okada 1954, Tsacas et al. 1971, Bock and Wheeler 1972
posterior paramere*	Aedeagal sheath	Okada 1954
posterior process	Epandrial posterior lobe	Salles 1947, Hsu 1949
pouch	Epandrial ventral lobe	Salles 1947
prensisetae	Surstylar teeth	Grimaldi 1990
primary clasper	Surstylus	Hsu 1949
scleoritzed rod	Hypandrial transverse rod	Tsacas et al. 1971
secondary clasper	Cercal ventral lobe	Hsu 1949
secondary gonopore	Phallotrema	Sinclair 2000
toe	Epandrial ventral lobe	Hsu 1949
transversal thickening of the hypandrium	Hypandrial transverse rod	Salles 1947
ventral branch of basal process	Ventral postgonite	Kamimura 2010
ventral fragma	Hypandrial phragma	Okada 1954
ventral gonapophysis	Pregonite	Salles 1947
ventral lobe	Epandrial posterior lobe	Eberhard and Ramirez 2004
ventral paramere*	Pregonite	old FlyBase terminology
ventral paramere*	Ventral postgonite	Bryant and Hsei 1977
ventral phragma	Hypandrial phragma	Tsacas et al. 1971
ventral process	Epandrial posterior lobe	Eberhard and Ramirez 2004
*** Note that these previously used terms correspond to multiple anatomical parts in the new nomenclature.**		

425

Dear Mariana,
Thank you for coordinating the review of our manuscript. We are grateful to the reviewer for their insightful comments. We have revised the manuscript to address their concerns. We made text changes they requested throughout the manuscript. In addition, we have added a section “Implications of our system to the terminological debate within Diptera” (lines 164-194) on how our terminology more broadly relates to that of Diptera to address comments received independently by Tadeusz Zatwarnicki. Our responses to the reviewer’s comments and the changes we made to the revised manuscript, are detailed below.

Editor's comments:

Thank you for this excellent manuscript, which is quite useful to the community and appropriate for publication in FLY. There are some necessary, but easy, revisions indicated below. In addition, it might be useful to mention (briefly) some salient steps in the development of the terminalia (not simply that they come from the genital disc) - specifically that the terminalia shift in position during pupal development (rotating 360 degrees in mel, I don't know what happens other species) which affects their relationship (e.g. can introduce twists) with internal genitalia. I suggest adding the rotation because one can imagine cases where that does not go to completion, etc. causing confusion to novices as they try to figure out what they are looking at.

This is an excellent point. Many of our designations are based on dorsal/ventral orientation but in other species of Diptera with 180 degree rotation, this designation would be reversed in the adult. We have revised the manuscript to address this (lines 109-111).

ps the "philosophical point" in the review does not require revision to your ms, unless you agree with it and thus feel like doing so.

We completely understand the “philosophical point” and explain our reasoning in Reviewer 1’s section. In short, many members of the consortium prefer the traditional terminology for the same reasons as the reviewer, but as a whole we have voted and agreed to use the standardized terminology as it allows for easier comparisons between species of Drosophila.

pps the analogous manuscript on female terminalia will also be good for FLY...

Including females was discussed early on in the process but we decided to focus on males for the first manuscript. We will start on the female atlas shortly. Fly will be our top choice to publish the female atlas.

Reviewer 1:

The manuscript by Rice et al., proposing a unified terminology for structures in the male terminalia of D. melanogaster, is an important and useful addition to the literature. It is very appropriate for publication in Fly. As the authors point out, different investigators use combinations of three different terminology-systems to describe male fly terminalia in papers, etc. leading to confusion at times. The authors' systematic designations, accompanied by their clear photos and drawings, and the nice way that they set everything into historical context, should help standardize the field.

I have some suggestions (by line number) for minor modifications that I think would clarify some items. I also have a philosophical point for the authors to consider, but whether to do anything in response is entirely at their discretion.

We thank the reviewer for their thoughtful comments!

48: I have always understood the Terminalia to include analia as well as genitalia, and the paper's Table 1 says this as well. But Analia is missing from the sentence in line 48.

This is an excellent point. We have included analia in our characterization of the terminalia (line 49).

135: I think that Mattei, A.L., Kamimura, Y. and Wolfner, M.F. (2017) Intimate intimas: positioning of copulatory organs in mating Drosophila. Mol. Repro. Devt., 84:1117 might be appropriate to cite here, in addition to the Kamimura 2010 citation.

We agree. This has been included to the citation (line 139).

179: would it be possible to arrange to have the drawings (or name-table) connected to Flybase, etc. online, so people would have easy access to the new terminology

We worked with Flybase to do exactly this. Our terms will be added to Flybase in mid-September during the new release. We have added a sentence to highlight this point (line 197-198).

352: Some issues with the tables:
- "Sclerites": I always thought this term referred to the ventral plates on the abdomen (e.g. the ventral analogue of "tergites"), and most online definitions that I found are consistent with this view. In that case it does not make sense to categorize things like the aedeagal sheath, etc. as a "sclerite".

We think that the reviewer is referring to 'sternite' (the ventral equivalent of tergite), instead of 'sclerite', which refers to any sclerotized or hardened area of the insect body wall (Snodgrass, R.E. 1935. Principles of Insect Morphology, McGraw-Hill Book

Company, p. 69). We would like to keep the term “sclerite” for its wide usage in the entomological field outside of *Drosophila*.

- ***It is confusing to give a new definition for "aedeagus" since that same word is already used to name a different structure, in current terminology. Can you please find another term?***

We think the reviewer is referring to our use of aedeagus as a synonym for aedeagus. This was an error on our part. We have deleted the term aedeagus from the Synonyms section of the aedeagus row.

- ***Under "Setation" please use "bristle" not "bristles" in the titles of things like Cercal bristle, since the wording afterwards is "any bristle"… (not "Any bristles…")***

This is a great point. The following titles have been corrected in the “Setation” section of Table 1: Cercal bristle, Cercal dorsal lobe bristle, Cercal ventral lobe bristle, Epandrial bristle, Epandrial dorsal lobe bristle, Epandrial ventral lobe bristle, Pregonal bristle.

- ***I suppose it is OK to include muscles here but they are not external structures, hence not really "terminalia" in that sense.***

We understand the reviewer’s concerns. With the new terminology and figures Dr. Kamimura was able to reanalyze his previous report on the musculature of the terminalia.

Although we agree that the terminalia often refer to the external structures, we believe it is useful to consider all structures derived from A8-A10, including the internal structures as part of the terminalia. In Table 1 we have classified the external structures as either sclerites, setation, to distinguish these features from the internal musculature.

- ***It's also confusing to see two different entries for "paramere" in Table 2***

We agree that this is very confusing. We included two entries in Table 2 to reflect that the same term, paramere, has been used by previous studies to reference different structures. We want Table 2 to be a look up table for those reading previous literature so that they will be able to interpret that work in the context of our standardized literature. We have added a footnote to the table to clarify this point.

- ***At several places in Table 2, gonocoxite is mis-spelled.***

We have fixed the text in the “lateral expansion”, “lateral process”, and “outer process” rows.

Philosophy: I endorse the need for a unified terminology, and think I understand that the one that the authors chose will be best for specialists: it will systematize things well and with more precision, which in turn will make comparative studies easier to describe. But I am sorry that the authors did not choose to use the 'traditional' names, as those make it easier for non-specialists to understand intuitively. This makes the field less esoteric-sounding and lets others connect to it more easily. The tissue that I study had (and for a few people still has) an esoteric Latin name that meant nothing save to a few specialists. When we started using the common name, that resonated with others and led to much better acceptance of, and more interest in, this tissue's importance. I recognize that you are giving people an "out" - letting them use their preferred term if they put the new one into parentheses. This is a reasonable solution to the problem.

This was the first point we discussed when we first established the consortium and was one of the toughest aspects of the paper. We found that most members preferred either the traditional or revised terminology, but that they were willing to compromise on what the majority of the consortium voted for. We felt that bringing as many labs together as possible and voting was the only way to fairly determine which set to base our standardized terminology on. During our discussions we also felt that using the revised terminology would allow us to easily expand these terms to other species of *Drosophila*. Though terminologies outside of *D. melanogaster* are not the goal of this manuscript, we wanted to keep this in mind. Several members discussed that there are many lobed extensions, clasping organs, and branch-shaped structures that are produced from completely different sclerites in other species of Drosophila. We felt that keeping our definitions as expandable as possible was important for those interested in the evolution of these structures.

However, we agree that using the traditional names can resonate with others, and several of us will likely use the traditional terms while citing their revised terminology parenthetically.

Non-essential, but consider: In line 96, you mentioned that there is variation among D. melanogaster lines for some structures. Might it be useful to include a few examples in a supplementary figure? Also are some of the structures more variable between species? If so maybe note that?

We fully understand this point, but feel that the cited studies already do an excellent job of documenting the variation of these structures. We would prefer to not replicate those efforts.