Comparative anatomy of the thoracic muscles of bees (Hymenoptera: Apoidea) (#124653)

First submission

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Original primary research within Aims and Scope of the journal.
Research question well defined, relevant & meaningful. It is stated how research fills an identified knowledge gap.

The submission should clearly define the research question, which must be relevant and meaningful. The knowledge gap being investigated should be identified, and statements should be made as to how the study contributes to filling that gap.

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The investigation must have been conducted rigorously and to a high technical standard. The research must have been conducted in conformity with the prevailing ethical standards in the field.

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The data on which the conclusions are based must be provided or made available in an acceptable discipline-specific repository. The data should be robust, statistically sound, and controlled.

Conclusions are well stated, linked to original research question & limited to supporting results.

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Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
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Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Comparative anatomy of the thoracic muscles of bees (Hymenoptera: Apoidea)

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Bees exhibit remarkable anatomical diversity, with phenotypic traits that reflect broad evolutionary patterns and specific adaptations. Understanding these patterns requires examining key anatomical features, such as thoracic musculature, which drives morpho-functional variation and underscores their extensive phenotypic diversity. The thorax (or 'mesosoma,' as it can be referred to in the context of bees and other ~~aculeate~~ Hymenoptera) serves as a locomotion center housing muscles responsible for leg, wing, and intertagmatal articulation movements. Despite the role of the thoracic musculature in the flight mechanics of bees, detailed comparative studies are limited to accounts of individual species or small subsets of muscles, leaving gaps in understanding muscular variation and phylogenetic significance. To address this, we conducted detailed dissections of 13 species, representing six bee families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, and Melittidae) and ~~three~~ apoid wasp taxa (Bembicidae, Crabronidae, Philanthidae), selected to capture a broad range of morphological and phylogenetic diversity. Our results revealed high conservation in mesosomal musculature, with only 16 of 58 muscle groups showing significant variation, primarily in origin points, suggesting a balance between functional constraints and evolutionary flexibility in muscle attachment. Phylogenetically relevant changes were investigated by coding 17 morphological characters, revealing potential synapomorphies for bees or certain lineages. These include the dorsomedial origin of Idlm1 (M. prophragma-occipitalis) in Meliponini, as evident in species such as Melipona quadrifasciata and Tetragonisca fiebrigi, suggesting a shared derived trait for this tribe. Additionally, the extended origin of IIIscm2 is observed in Andrenidae, Colletidae, and Halictidae, indicating closer evolutionary relationships among these families. Bee-specific modifications, including the non-separation of IItpm7b and IItpm7c by the mesepisternal ridge, distinguished bees from most apoid wasps, interpreted here as a potential synapomorphy for bees. Additional variations, such as the ventral origin of Ivlm3 in select lineages and the branched morphology of IIpcm4, suggest independent evolutionary shifts potentially linked to biomechanical demands. These findings underscore the evolutionary stability and phylogenetic value of bee mesosomal musculature, revealing a conserved framework punctuated by lineage-specific adaptations that may correlate with ecological traits.

Comparative anatomy of the thoracic muscles of bees (Hymenoptera: Apoidea)

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ABSTRACT

Bees exhibit remarkable anatomical diversity, with phenotypic traits that reflect broad evolutionary patterns and specific adaptations. Understanding these patterns requires examining key anatomical features, such as thoracic musculature, which drives morpho-functional variation and underscores their extensive phenotypic diversity. The thorax (or 'mesosoma,' as it can be referred to in the context of bees and other aculeate Hymenoptera) serves as a locomotion center housing muscles responsible for leg, wing, and intertagmatal articulation movements. Despite the role of the thoracic musculature in the flight mechanics of bees, detailed comparative studies are limited to accounts of individual species or small subsets of muscles, leaving gaps in understanding muscular variation and phylogenetic significance. To address this, we conducted detailed dissections of 13 species, representing six bee families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, and Melittidae) and three apoid wasp taxa (Bembicidae, Crabronidae, Philanthidae), selected to capture a broad range of morphological and phylogenetic diversity. Our results revealed high conservation in mesosomal musculature, with only 16 of 58 muscle groups showing significant variation, primarily in origin points, suggesting a balance between functional constraints and evolutionary flexibility in muscle attachment. Phylogenetically relevant changes were investigated by coding 17 morphological characters, revealing potential synapomorphies for bees or certain lineages. These include the dorsomedial origin of Idlm1 (M. prophragma-occipitalis) in Meliponini, as evident in species such as *Melipona quadrifasciata* and *Tetragonisca fiebrigi*, suggesting a shared derived trait for this tribe. Additionally, the extended origin of IIIscm2 is observed in Andrenidae, Colletidae, and Halictidae, indicating closer evolutionary relationships among these families. Bee-specific modifications, including the non-separation of IItpm7b and IItpm7c by the mesepisternal ridge, distinguished bees from most apoid wasps, interpreted here as a potential synapomorphy for bees. Additional variations, such as the ventral origin of Ivlm3 in select lineages and the branched morphology of IIpcm4, suggest independent evolutionary shifts potentially linked to biomechanical demands. These findings underscore the evolutionary stability and phylogenetic value of bee mesosomal musculature, revealing a conserved framework punctuated by lineage-specific adaptations that may correlate with ecological traits.

KEYWORDS: Apidae, flight, mesosoma, morphology, systematics.

INTRODUCTION

Like other arthropods, bees have their bodies covered by a chitinous exoskeleton featuring articulated parts that are moved internally by specialized muscles with well-defined attachment points (Snodgrass, 1927, 1935). The roles played by these muscles, in coordination with their cuticular attachment points, vary across body regions, reflecting regional specialization that makes each tagma suited for specific tasks, with differing degrees of musculature specialization (Snodgrass, 1935). The prominent thorax of a bee is the main tagma responsible for movement, because it has two pairs of wings and three pairs of legs attached to it, as is also the case in other flying insects (Snodgrass, 1927, 1935; Matsuda, 1970), whereby muscles play a crucial role in fulfilling the demands of flight (Dudley, 2000; Dickinson, 2006; Iwamoto, 2011). In aculeate Hymenoptera, such specialization is especially pronounced: the enlargement of the mesothorax correlates with the activity of indirect flight muscles, which are essential for rapid wing beats (Chapman, 2013). In these insects, the mesosoma, the thoracic tagma fused to the first abdominal segment, supports complex locomotor adaptations (Vilhelmsen et al., 2010).

Classical morphological techniques, especially detailed dissections, remain essential for studying the complex skeletomuscular system of insects and documenting the muscle structure. In this context, "The skeleto-muscular mechanisms of the honey bee" (Snodgrass, 1942) stands out as a landmark, offering unmatched direct access to muscle attachment points, fiber orientation, and tissue features. Detailed studies made for a single species (e.g., Snodgrass, 1942; Daly 1964; Mikó et al., 2007; Aibekova et al., 2022, 2025), and comparative investigations (e.g., Wille, 1956; Matsuda, 1970; Vilhelmsen et al., 2010; Meira & Gonçalves, 2021) have enabled more accurate identification of homologous structures vital for interpreting morphological diversity across taxa of bees and other taxa of Hymenoptera. Recently, traditional methods have been supplemented by modern imaging techniques, such as micro-computed tomography (CT scanning), which provides non-invasive, high-resolution three-dimensional reconstructions of internal anatomy (e.g., Friedrich & Beutel, 2008; Friedrich et al., 2014; Willsch et al., 2020; Aibekova et al., 2022, 2025; Meira et al., 2024). While CT scanning uncovers details that were previously unreachable without destructive methods, its interpretations rely on the careful foundation set by traditional dissections. Dissections confirm digital imaging by verifying anatomical features and clarifying ambiguities in CT data, ensuring precise identification of muscle groups and their arrangements. The synergy between these approaches, where classical dissections anchor and enhance the reliability of modern imaging, creates a robust knowledge base that underpins comparative research, enabling systematic documentation of anatomical variation across bee lineages and supporting inferences about their functional and evolutionary significance.

There are about 21,000 known bee species (Ascher & Pickering, 2020) inhabiting nearly all terrestrial habitats, and exhibiting an impressive size range (Messer, 1984; Michener, 2007) from less than 2 mm (e.g., some *Euryglossina* [Colletidae: Euryglossinae]) to nearly 40 mm (e.g., *Megachile pluto* [Megachilidae: Megachilini]). Their species diversity is also reflected by a wide variety of shapes, sizes, and life histories (Roubik, 1989; Michener, 2007; Danforth et al., 2019), resulting from the diversification of bees since the early Cretaceous (Almeida et al., 2023). For instance, while flight adaptation is a key feature of winged insects, many bee species thrive in environments with limited light, such as dense vegetation or high-altitude regions, and some have evolved flightlessness (Michener, 2007). Comparing the anatomical variation of thoracic muscles among bees can yield important insights into how these differences relate to their diverse ecological and biological strategies in the future.

The investigation of the bee mesosomal skeletomusculature from a comparative perspective to highlight anatomical variation in this complex is limited to a single study, which only examined ten muscles (Wille, 1956). There are between 57 (Snodgrass, 1942) and 58 (Meira et al., 2024) muscle groups in a bee mesosoma (intrinsic mesosomal muscles, plus coxal muscles originating in the mesosoma), meaning that several dozen muscles remain unstudied from a comparative perspective. Recent advances in morphological analysis and the terminological standardization efforts by Meira et al. (2024) have helped align bee morphological terminology with broader developments in Hymenoptera, making further research in this area both timely and essential for expanding our understanding of this system.

This study aims to systematically assess and document the diversity of mesosomal muscles across bee lineages, quantifying anatomical variation and exploring its potential functional and phylogenetic significance. By mapping muscular configurations across a taxonomic range, this research aims to determine whether internal mesosomal anatomy exhibits conserved patterns or reflects lineage-specific adaptations. Furthermore, this study contributes to filling a longstanding gap in hymenopteran morphological research by providing a detailed comparative framework for internal thoracic (or mesosomal) musculature in bees.

MATERIALS & METHODS

Taxon sampling

Representative specimens of 10 bee species, representing all major lineages of bees were sampled to explore the detailed structure of the mesosomal musculature: *Oxaea flavescens* Klug and *Psaenythia bergii* Holmberg [Andrenidae]; *Apis mellifera* Linnaeus, *Melipona quadrifasciata* Lepeletier, *Schwarziana quadripunctata* (Lepeletier), and *Tetragonisca fiebrigi* (Latreille) [Apidae]; *Tetraglossula anthracina* (Michener) [Colletidae]; *Oragapostemon divaricatus* (Vachal) [Halictidae]; *Lithurgus huberi* Ducke [Megachilidae]; and *Hesperapis carinata* Stevens [Melittidae]. This sampling encompasses a considerable range of morphological variation, with body sizes ranging from 4 to 22 mm (Fig. 1), and phylogenetic diversity that covers all the extant bee diversity (according to current hypotheses, e.g., Almeida et al. [2023]). Three apoid wasps were selected and investigated in detail for comparison with the bee morphology: *Steniolia duplicata* [Bembicidae], *Trypoxylon lactitarse* de Saussure, 1867 [Crabronidae], and *Trachypus boharti* Rubio-Espina 1975 [Philanthidae]. Voucher specimens of the taxa investigated in this study and the dissected specimens are deposited in the *Coleção Entomológica "Prof. J.M.F.Camargo"* (RPSP), Universidade de São Paulo, Ribeirão Preto, Brazil.

Preparation of the specimens

The specimens were killed in a cyanide jar and transferred to a vial containing the fixative Dietrich fixative, a formaldehyde-based solution. These specimens remained in Dietrich for about three days, then preserved in absolute ethanol for long-term storage. Ethanol-preserved specimens were stained with B-I2E (2.5%) for 30 minutes immediately before dissection to ensure optimal muscle visibility. The specimens were washed in 100% ethanol to remove the remaining excess stain and carried to the dissection step for about 30 minutes. The B-I_2E (2,5%) solution is prepared by adding 8.33 mL of I_2E (15% w/v) to 25 mL of Phosphate buffer and 16.7 mL of bi-distilled water (modified from Dawood et al., 2021) and the I_2E = alcoholic iodine (15% w/v) solution can be prepared by combining 15 mL of pure I_2 to 100 mL of absolute ethanol (modified from Li et al., 2016). Staining with 2% iodine was used to facilitate muscle visualization. As the iodine staining is not permanent, the procedure was repeated when necessary.

Dissection techniques

Each dissection began with an incision along the margins of the mesoscutum, which was carefully removed to expose the indirect flight muscles (Fig. 3a). Dissections were performed using microforceps and fine scalpels under a stereomicroscope to preserve delicate muscle attachments. Each muscle was meticulously dissected and photographed, before removal to progressively access deeper structures, documenting muscle attachments to skeletal elements and their spatial arrangements. This systematic documentation ensured a comprehensive record of the structural relationships and morphological details of the musculature. Next, an incision was made to remove the propectus (Fig. 2f), allowing access to the prothoracic musculature, including muscles associated with the forelegs and their articulation with the mesothorax and head. The study then proceeds posteriorly, examining the mesothoracic and metathoracic muscles (Fig. 4), focusing on the intrinsic and extrinsic muscle groups responsible for wing movement and structural support. Throughout this posterior dissection, the gradual removal of muscles continues, ensuring that each newly exposed structure is documented and analyzed in situ before proceeding further.

Visualization and image acquisition

The musculature was observed in Petri dishes containing absolute ethanol filled with fine sand substrate to prevent the material from moving excessively. We used a Leica M205c stereomicroscope with transmitted and incident light. Images were taken with a Leica DFC450 camera using bright field illumination. The stack of images was obtained with Helicon Focus software (Helicon Soft Ltd.).

Terminology

The terminology for the muscles and skeleton primarily follows Meira et al. (2024). The propectus is understood as the complex including the propleuron plus prosternum (per Snodgrass, 1942). The function of each muscle group mainly follows the interpretations of Snodgrass (1942), with additions by Mikó et al. (2007) and Vilhelmsen et al. (2010). Additional literature concerning the morphology of the mesosomal extrinsic musculature of several hymenopteran taxa was reviewed to contextualize the findings (Snodgrass, 1942; Vilhelmsen, 2000a, 2000b, Vilhelmsen et al., 2010; Mikó et al., 2007; Friedrich & Beutel, 2008; Willsch et al., 2020; Yoder et al., 2010; Aibekova et al., 2022; Lieberman et al., 2022), as summarized in supplementary Table S1.

Character coding and optimization

Based on the comparative study, we ~~built~~ characters, coded as binary, to assess the homology hypotheses implied by the similarities and variations found. The resulting data matrix was cladistically analyzed to display the joint phylogenetic signal present therein. Individual character state transformations were estimated using Fitch optimization (Fitch, 1971) in Winclada software version 1.00.08 (Nixon, 2002) under both unambiguous and ACCTRAN schemes, with the latter prioritizing early character transformations consistent with bee phylogeny. For the character transformations, a summary tree including all 10 bee species of this study was prepared in Mesquite (Maddison and Maddison, 2007) by pruning the phylogenetic hypotheses of Almeida et al. (2023) for the 10 species of bees, complemented by the tree of Sann et al. (2018) to represent the three taxa of apoid wasps sampled.

RESULTS

DESCRIPTION OF THE SKELETOMUSCULATURE

The variation of the mesosomal musculature of Apoidea is described in detail below based on ten species of bees and three apoid wasps. The exemplars of these 13 taxa that were analyzed in this comparative research have the same number of muscle groups, although variations in size, relative position in relation to other muscles and sclerites, and their position of insertion and/or origin were observed for 16 of 58 studied muscles (intrinsic leg muscles were not studied). In instances where some degree of variation was documented, this is indicated by the “**VAR**” annotation at the muscle description; likewise, if the observed variation was used as the basis for character construction (see next section, below), the character number is indicated too. In contrast, no significant variation was documented for 35 muscle groups, and a concise summary is provided for those too; the lack of variation in such cases is indicated by the “**INVAR**” annotation in the muscle description.

***Idlm1**, M. prophragma-occipitalis* (Fig. 2a-b) [**VAR**: variation coded into character 1 (Fig. 10), below]: one of the five elevators of the head. It originates dorsomedially or dorsolaterally from the prophragma of bees, but always lateral to **Idlm2** (Figs. 2a, b) and inserts dorsolaterally on the postocciput. All three apoid wasps have Idlm1 located dorsolaterally on the prophragma, lateral to **Idlm2**, inserting on the dorsolateral areas of the postocciput. There is variation related to the origin point of this muscle (the insertion point does not vary in Apoidea). In *M. quadrifasciata*, *T. fiebrigi*, *S. quadripunctata*, and apoid wasps, the muscle origin is dorsomedially (Fig. 2a-b) located on the prophragma. In contrast, the remaining bees have the muscle origin of **Idlm1** dorsolaterally (Fig. 7a) located on the prophragma. The morphology of Idlm1 observed in Meliponini suggests that the morphology of **Idlm1** might be a synapomorphy for the tribe.

***Idlm2**, M. pronoto-occipitalis* (Fig. 2a, b) [**INVAR**]: one of the five elevators of the head. The origin point is dorsomedially located on the posterior margin of the pronotum, and the insertion is dorsomedially located on the postocciput.

***Itpm1**, M. pleurocrista-occipitalis* (Fig. 2b, d) [**INVAR**]: one of the five elevators of the head. It originates from the propleuron (dorsal propleural margin) and inserts dorsolaterally on the postocciput through a shared tendon with the branched **Itpm2** (**Itpm2a** and **Itpm2b**, see below).

***Itpm2**, M. propleuro-occipitalis* (Fig. 2d) [**INVAR**]: one of the five elevators of the head. Branched muscle (**Itpm2a**, M. propleuro-occipitalis dorsal and **Itpm2b**, M. propleuro-occipitalis ventral); both branches originating from the ventral propleura area and inserting dorsolaterally to the foramen magnum on the postocciput through a shared tendon with **Itpm1**.

***Idvm9**, M. profurca-occipitalis* (Fig. 2b, c) [**INVAR**]: one of the five elevators of the head. Large muscle broadly originating from the profurca (anterodorsal and posterodorsal profurcal lamellae on the posterior profurcal branch), inserting dorsolaterally to the foramen magnum (close to the insertion of **Itpm1** and **Itpm2**) on the postocciput.

***Ivlm3**, M. profurca-tentorialis* (Fig. 2c) [**VAR**: variation coded into character 2 (Fig. 10), below]: the only depressor of the head. Large muscle whose origin can be located either on the dorsal (Fig. 2c) or the ventral (Fig. 7b) surface of the posterior profurcal branch, and the insertion is located ventrolaterally on the postocciput. Two bee species (*O. divaricatus* [Halictidae] and *H. carinata* [Melittidae]) have this ventral origin, while the remaining bees and apoid wasps have a dorsal origin.

***Idlm5**, M. pronoto-phragmalis anterior* (Fig. 2a) [**INVAR**]: a depressor of the pronotum. Broad and short muscle originating laterally from the inner surface of the pronotum and inserting laterally on the prophragma of the mesoscutum.

***Idvm5**, M. pronoto-cervicalis anterior* (Fig. 2a) [**INVAR**]: the elevator of the propleuron. Branched muscle (**Idvm5a**, M. pronoto-cervicalis anterior primus and **Idvm5b**, M. pronoto-cervicalis anterior secundus) with two well-separated origins on the pronotum: one dorsolateral (**Idvm5a**) and one dorsomedial (**Idvm5b**). The insertion of both branches is located on the cervical apodeme of the propleuron.

***Itpm3**, M. pronoto-pleuralis anterior* (Fig. 2a) [**INVAR**]: protractor of the propectus. Broad pronotal muscle, with its origin dorsomedially on the pronotum, close to the origin of **Idvm5b**, and its insertion on the anterior lamella of the dorsal propleural margin.

***Itpm4**, M. pronoto-apodemalis anterior* (Fig. 2a) [**INVAR**]: protractor of the propectus. Muscle originating anterolaterally from the pronotum and inserting distally on the propleural arm of the propleuron.

***Itpm5**, M. pronoto-apodemalis posterior* (Fig. 2a) [**INVAR**]: protractor of the propectus. Muscle originating posterolaterally from the pronotum and inserting distally on the propleural arm of the propleuron.

***Ivlm1**, M. profurca-cervicalis* (Fig. 2c) [**INVAR**]: the adductor of the propleuron. Muscle originating from the anteromedian profurcal process of the prosternum and inserting posteriorly on the cervical apodeme of the propleuron.

***Ivlm7**, M. profurca-mesofurcalis* (Figs. 3d, 4c) [**VAR**: variation coded into characters 3 and 4 (Fig. 10), below]: the retractor of the propectus. Intersegmental muscle originating broad (Figs. 3d, 4c, 8d) or narrowly (Fig. 7c) from the mesofurcal bridge of the meso-metafurca and inserting in one (Fig. 7e) or two scars (Fig. 7d) on the posterior surface of the profurca at the prosternum. Only *A. mellifera* and *T. fiebrigi* (Apidae) have a narrow origin on the mesofurcal bridge. Three species, *H. carinata* (Melittidae), *O. divaricatus* (Halictidae), and *L. huberi* (Megachilidae), have two insertion points on the profurca.

Ipcm2, *M. procoxa cervicalis transversalis* (Fig. 2c, e) [**INVAR**]: rotator of the procoxa. Muscle originating from the anteromedian bar of the cervical apodeme of one side and inserting on the anterolateral margin of the procoxal base of the opposite side.

Ipcm4, *M. propleuro-coxalis superior* (Fig. 2e) [**INVAR**]: lateral promotor of the procoxa. Muscle originating from the anterior process of the dorsal profurcal lamella and from the ventral surface of the dorsal propleural margin. The insertion is located on the anterolateral margin of the procoxal base.

Iscm1, *M. profurca-coxalis anterior* (Fig. 2e) [**INVAR**]: medial promotor of the procoxa. Muscle originating from the prodiscrimenal lamella of the prosternum and inserting anteromedially on the procoxal base.

Idvm18, *M. pronoto-coxalis lateralis* (Fig. 2a) [**VAR**: variation coded into character 5 (Fig. 10), below]: lateral remotor of the procoxa. Long muscle that originates laterally (Fig. 7a) or dorsolaterally (Fig. 2a) from the pronotum, close to the pronotal lobe, and inserts posterolaterally on the procoxal base. The morphology of **Idvm18** observed in *A. mellifera*, in which this muscle originates laterally on the pronotum, is interpreted as an autapomorphy of the species.

Iscm3, *M. profurca-coxalis medialis* (Fig. 2a, f) [**INVAR**]: medial remotor of the procoxa. Thin muscle that originates from the sheet of the propleural arm and inserts posteromedially on the procoxal base.

Iscm4, *M. profurca-coxalis lateralis* (Fig. 2a, f) [**INVAR**]: lateral remotor of the procoxa. Broad muscle that originates from the posterodorsal profurcal lamella of the profurcal arm and inserts posterolaterally on the procoxal base.

***Iscm5**, M. prospina-coxalis* (Figs. 2a, 4d) [**VAR**: variation coded into character 6 (Fig. 10), below]: retractor of the propectus. Muscle with origin varying from restricted to the horizontal plate of the meso-metafurca (Figs. 4d, 7c, 8d) to broader and also reaching the free basal portion of mesofurcal arms (Fig. 8a). The insertion is located posteriorly on the procoxal base. The morphology of **Iscm5** observed in *L. huberi*, in which the origin reaches the free basal portion of the mesofurcal arms, is interpreted as an autapomorphy of this species.

***Iscm6**, M. profurca-trochanteralis* (Fig. 2a, f) [**INVAR**]: depressor of the protrochanter. Broad muscle originating from the sheet of the propleural arm and inserting on the depressor tendon of the protrochanter.

***IIIdlm3**, M. metascutello-scutellaris* (Fig. 3b) [**VAR**: variation coded into character 7 (Fig. 10), below]: retractor of the mesoscutellum. Muscle originating medially (Fig. 7e) or laterally (Fig. 3b) from the scutoscutellar ridge of the mesoscutellum and the insertion located medially or laterally on the anterior margin of the internal metanotal ridge. The morphology of **IIIdlm3** observed in *A. mellifera*, in which both origin and insertion of this muscle are located laterally, is interpreted as an autapomorphy of the species.

***IIdlm1**, M. prophragma-mesophragmalis* (Fig. 3a) [**INVAR**]: indirect depressor of the wing. Large dorsal longitudinal indirect flight muscle with the origin medially located on the ventral side of the mesoscutum and on the posterior face of the prophragma, and the insertion is located broadly on the anterior face of the mesophragma.

***IIdvm1**, M. mesonoto-sternalis* (Fig. 3a) [**INVAR**]: indirect elevator of the wing. Mesonotal muscle that arises laterally from the ventral side of the mesoscutum and from the posterior margin of the prophragma and inserts broadly on the mesepisternum.

***IIpspim1**, M. mesanepisterno-spiracularis* (Fig. 3c) [**INVAR**]: occlusor of the first spiracle. Muscle of the mesothorax, originating from the anterior margin of the subspiracular area and inserting on the spiracular membrane at the spiracular aperture.

***IItpm5**, M. mesonoto-pleuralis medialis* (Fig. 4a) [**INVAR**]: depressor of the mesoscutellum. Muscle originating from the lateral areas of the mesepisternal region and from the pleural apophysis; its insertion is located on the lateral margin of the mesoscutellum.

IItpm7**, Mesanepisterno-axillaris* (Fig. 4a): flexors of the forewing (IItpm7a**, M. mesanepisterno-axillaris ventral*, ***IItpm7b**, M. mesanepisterno-axillaris medial* and ***IItpm7c**, M. mesanepisterno-axillaris dorsal*) [**VAR**: variation coded into character 8 (Fig. 10), below]: **IItpm7a** originates from the subalar apophysis cavity, while **IItpm7b** and **IItpm7c** originate laterally from the mesepisternum; all three branches insert, by a shared tendon, on the third mesoaxillary sclerite. In bees, the absence of separation between **IItpm7b** and **IItpm7c** by the mesepisternal ridge (Figs. 4a, 8c) is likely a synapomorphy, as among apoid wasps most species, except the Bembicidae species, have this separation (Fig. 8b).

***IIspm1**, M. mesopleura-sternalis* (Fig. 4a) [**VAR**: variation coded into character 9 (Fig. 10), below]: depressor of the mesobasalar sclerite. Muscle that originates from the anterior margin of the mesepisternum (Fig. 8c) and/or the subspiracular area (Figs. 4a, 8b) and inserts on the mesobasalar sclerite. The morphology of **IIspm1** observed in *A. mellifera*, in which this muscle origin is restricted to the anterior region of the mesepisternum, is interpreted as an autapomorphy of the species.

***IIspm2**, M. mesofurca-pleuralis* (Fig. 4c-d) [**INVAR**]: furco-pleural muscle with uncertain function. Muscle originates from the posterolateral area of the mesepisternum and inserts on the tip of the free distal portion of the mesofurcal arm.

***IIpcm4**, M. propleuro-coxalis posterior* (Fig. 4d) [**VAR**: variation coded into character 10 (Fig. 10), below]: lateral promotor of the mesocoxa. Muscle origin branched (Fig. 8e) or not (Fig. 4d), located laterally on the mesepisternum; insertion located laterally on the mesocoxal base. The variation of origin point of **IIpcm4** suggests multiple independent changes in Apoidea, further suggesting a complex evolution of this muscle. This variation was already described by Wille (1956), although not coded as character and character states.

***IIscm1**, M. mesofurca-coxalis anterior* (Fig. 4c) [**VAR**: variation coded into character 11 (Fig. 10), below]: medial promotor of the mesocoxa. Muscle that originates posteroventrally from the mesodiscrimenal lamella and inserts antero- (Fig. 4c) or mediolaterally (Fig. 8f) on the mesocoxal base. In bees, the muscle consistently inserts anterolaterally on the mesocoxa, a feature also observed in the bembicid species but not in the other two apoid wasps studied.

***IIdvm6**, M. mesocoxa-subalaris* (Figs. 4c-d) [**INVAR**]: lateral remotor of the mesocoxa. Slender muscle that originates posterolaterally from the mesocoxal base and inserts on the mesosubalar sclerite.

***IIscm2**, M. mesofurca-coxalis posterior* (Fig. 4c) [**INVAR**]: medial remotor of the mesocoxa. Muscle that originates anteroventrally from the mesodiscrimenal lamella of the mesofurca and inserts posteromedially on the base of the mesocoxa.

***IIscm6**, M. mesofurca-trochanteralis* (Fig. 4c) [**VAR**: variation coded into character 12 (Fig. 10), below]: thoracic depressor of the mesotrochanter. Broad muscle with origin restricted to the coalesced furcal arms (Fig. 4c) or also reaching free basal portion of mesofurcal arms (Fig. 8a). Insertion located on the depressor tendon of the trochanter. The morphology of IIscm6 observed in *M. quadrifasciata*, in which this muscle is restricted to the coalesced furcal arms, is interpreted as an autapomorphy of that species.

***IIIdlm1**, M. mesophragma-metaphragmalis* (Fig. 6a) [**INVAR**]: retractor of the mesophragma. Longitudinal muscle of the mesophragma that originates from the propodeal ridge and inserts on the posterior surface of the mesophragma.

***IIItpm5**, M. metanoto-pleuralis medialis* (Fig. 4a) [**INVAR**]: one of the hindwing elevators. Muscle that originates from the free distal portion of the metafurcal arm and dorsal metafurcal lamella and inserts on the dorsolateral metanotal area.

IIItpm6, *M. metanoto-pleuralis posterior* (Fig. 4a) [**INVAR**]: one of the hindwing elevators. Muscle that originates from the dorsal metafurcal lamella and inserts on the tip of the dorsolateral metanotal area.

IIIdvm1, *M. metanoto-sternalis* (Fig. 4a) [**INVAR**]: one of the hindwing elevators. Tergosternal muscle of the metathorax that originates from the dorsolateral metanotal area and inserts on the filamentous process of the free distal portion of the metafurcal arm.

IIItpm7, *M. metanepisterno-axillaris* (Fig. 4a, b) [**INVAR**]: flexor of the hindwing. Broad muscle with its origin located on the anterior inflection of the metepisternum and the insertion located on the third metaaxillary sclerite.

IIIspm1, *M. metapleura-sternalis* (Fig. 4b) [**INVAR**]: depressor of the metabasalar sclerite. Muscle arising from the ventral region of the metapectus and inserting on the metabasalar sclerite.

IIItpm11, *M. metapleura-subalaris* (Fig. 4b) [**INVAR**]: depressor of the metasubalar sclerite. Muscle arising from the anterior inflection of the metapectus and inserting on the metasubalar sclerite.

IIIpcm4, *M. metanepisterno-coxalis posterior* (Fig. 5c-d) [**INVAR**]: lateral promotor of the metacoxa. Muscle that originates broadly from the intercoxal lamella and the metapleural ridge and inserts anterolaterally on base of the metacoxa.

IIIdvm6, *M. metacoxa-subalaris* (Fig. 5a-c) [**INVAR**]: lateral remotor of the metacoxa. Muscle of the metathorax originating posterolaterally from the metacoxal base and inserting on the metasubalar sclerite.

IIIscm1, *M. metafurca-coxalis anterior* (Fig. 5b-d) [**VAR**: variation coded into character 13 (Fig. 10), below]: medial promotor of the metacoxa. Broad muscle that originates from the intercoxal lamella, reaching (Fig. 5b-d), or not (Fig. 9a), the free basal portion of the metafurcal arms and inserting anteriorly on the metacoxal base. The morphology of IIIscm1 reaching the free basal portion of the metafurcal arms is observed in bees and *Steniolia duplicata* (Bembicidae).

IIIscm2*, *M. metafurca-coxalis posterior (Fig. 5a) [**VAR**: variation coded into character 14 (Fig. 10), below]: posterior remotor of the metacoxa. Muscle that originates from the free basal portion of the metafurcal arm, sometimes extending to the coalesced furcal arms (Fig. 9b) or not (Fig. 5a), and inserts posteriorly on the metacoxal base. The morphology of **IIIscm2** observed in all members of Andrenidae, Colletidae, and Halictidae, in which this muscle origin extends to the coalesced furcal arms, is likely a synapomorphy of the clade formed by these three bee families.

IIIscm3*, *M. metafurca-coxalis medialis (Fig. 5c) [**VAR**: variation coded into character 15 (Fig. 10), below]: medial remotor of the metacoxa. Muscle that originates from the metadiscrimenal lamella, sometimes extending to the free basal portion of the metafurcal arms (Fig. 5c) or not (Fig. 9c), and inserts posteromedially on the metacoxal base. The morphology of **IIIscm3** reaching the free basal portion of the metafurcal arms observed in all bees and *T. lactitarse* (Crabronidae).

IIIscm6*, *M. metafurca-trochanteralis (Fig. 5a-b) [**INVAR**]: thoracic depressor of the metatrochanter. Muscle that originates from the posterior surface of the mesofurcal bridge and coalesced furcal arms and inserts on the depressor tendon of the metatrochanter.

IIIvomm*, *M. metafurca-abdominosternalis medialis (Fig. 6a) [**VAR**: variation coded into character 16 (Fig. 10), below]: medial depressor of the abdomen. Muscle that originates from the free basal portion of the metafurcal arm, sometimes extending to the coalesced furcal arms (Fig. 6a) or not (Fig. 9d), inserting medially on the first metasomal segment. While its insertion remains consistent across species, its origin varies in *T. fiebrigi*, *S. quadripunctata*, *A. mellifera*, and *H. carinata*, extending to the coalesced furcal arms, whereas in the remaining analyzed species, it is restricted to the free basal portion of the metafurcal arm. This variation was already described by Wille (1956), although not coded as character and character states.

IIIvolm*, *M. metafurca-abdominosternalis lateralis (Fig. 6b) [**VAR**: variation coded into character 17 (Fig. 10), below]: ventral lateromotor of the abdomen. Muscle that originates from the metadiscrimenal lamella and inserts on the sternum of the first metasomal segment. While its insertion remains consistent across species, its origin varies: in Apidae and Oxaeinae, it is positioned anteriorly (Figs. 6b, 9e) on the metadiscrimenal lamella, whereas in all remaining species, it is located posteriorly (Fig. 9f).

***1domm**, M. tergo-tergalis orthomedialis* (Fig. 6c) [**INVAR**]: medial elevator of the abdomen. Muscle that originates broadly from the anterior wall of the propodeum and inserts medially on the constricted margin of the first metasomal tergum.

***1dolm**, M. tergo-tergalis ortholateralis* (Fig. 6d) [**INVAR**]: dorsal lateromotor of the abdomen. Muscle that originates from the lateral wall of the propodeum and inserts laterally on the anterior margin of the first metasomal tergum.

***IAspim1**, M. spiracularis I superior* (Fig. 6c) [**INVAR**]: occlusor of the propodeal spiracle. Muscle of the propodeal spiracle, originating from the sclerotized area above the propodeal spiracle and inserting on the sclerotized area below the propodeal spiracle.

***IAspim2**, M. spiracularis I posterior* (Fig. 6c) [**INVAR**]: dilator of the propodeal spiracle. Muscle of the propodeal spiracle originating from the small metapleural coxal process and inserting on the sclerotized area below the propodeal spiracle.

Character statements

Below are described the 17 morphological characters and their respective states derived from the extrinsic mesosomal musculature of bees, which were scored for the 13 species of Apoidea (ten bee and three wasp species) (Table S2). These characters summarize the overall morphological variation described in the previous section and are interpreted in a phylogenetic context being optimized onto a phylogenetic hypothesis for bees.

01. Origin of **Idlm1**: (0) dorsolaterally at the prophragma (Fig. 7a); (1) dorsomedially at the prophragma (Fig. 2a-b).

02. Origin of **Ivlm3**: (0) ventrally at the posterior profurcal branch (Fig. 7b); (1) dorsally at the posterior profurcal branch (Fig. 2c).

03. Origin of **Ivlm7**: (0) broadly at the mesofurcal bridge (Figs. 3d, 4c, 8d); (1) narrowly, restricted to the ventral margin of the mesofurcal bridge (Fig. 7c).

04. Insertion of **Ivlm7**: (0) single, at the posterior face of the profurcal arm (Fig. 7e); (1) two, at the posterior face of the profurcal arm (Fig. 7d).

05. Origin of **Idvm18**: (0) laterally at the pronotum (Fig. 7a); (1) dorsolaterally at the pronotum (Fig. 2a).

06. Origin of **Iscm5**: (0) restricted to the horizontal plate of the meso-metafurca (Figs. 4d, 7c, 8d); (1) reaching the free basal portion of the mesofurcal arms (Fig. 8a).

07. Insertion of **IIIdlm3**: (0) medially at the anterior margin of the internal metanotal ridge (Fig. 7f); (1) laterally at the anterior margin of the internal metanotal ridge (Fig. 3b).

08. Origin of **IItpm7b** and **IItpm7c**: (0) separated by the mesepisternal ridge (Fig. 8b); (1) not separated by the mesepisternal ridge (Figs. 4a, 8c).

09. Origin of **IIspm1**: (0) at the anterior margin of the mesopectus (Fig. 8c); (1) at the anterior margin of the mesopectus and at the subalar area (Figs. 4a, 8b).

10. Origin of **IIpcm4**: (0) branched (Fig. 8e); (1) not branched (Fig. 4d).

11. Insertion of **IIscm1**: (0) anterolateral (Fig. 4c); (1) mediolateral (Fig. 8f).

12. Origin of **IIscm6**: (0) restricted to the coalesced furcal arms (Fig. 4c); (1) reaching the free basal portion of the mesofurcal arms (Fig. 8a).

13. Origin of **IIIscm1**: (0) reaching the free basal portion of metafurcal arms (Fig. 5b-d); (1) restricted to the metadiscrimenal lamella (Fig. 9a).

14. Origin of **IIIscm2**: (0) reaching the coalesced furcal arms (Fig. 9b); (1) restricted to the free basal portion of the metafurcal arms (Fig. 5a).

15. Origin of **IIIscm3**: (0) reaching the free basal portion of metafurcal arms (Fig. 5c); (1) restricted to the metadiscrimenal lamella (Fig. 9c).

16. Origin of **IIIvomm**: (0) broadly at the free distal portion of metafurcal arm (Fig. 9d); (1) at the free distal portion of metafurcal arms and coalesced furcal arms (Fig. 6a).

17. Origin of **IIIvolm**: (0) anteriorly at the metadiscrimenal lamella (Figs. 6b, 9e); (1) posteriorly at the metadiscrimenal lamella (Fig. 9f).

DISCUSSION

The mesosoma, a critical ~~thoracic~~ region in bees, exhibits remarkable structural conservation across this diverse insect group, as first highlighted by Wille (1956). ~~In his~~ seminal work, ~~Wille (1956)~~ explored the evolutionary trends of ten key thoracic muscles, providing insights into their morphological consistency and variation. These muscles include the lateral promotor of the middle coxa (herein **IIpcm4**, M. propleuro-coxalis posterior; coded as character 10), the pleurotergal muscle of the mesothorax (herein **IItpm5**, M. mesonoto-pleuralis medialis; showing no significant variation in this study), the median depressor of the abdomen (herein **IIIvomm**, M. metafurca-abdominosternalis medialis; coded as character 16), the lateral promotor of the hind coxa (herein **IIIpcm4**, M. metanepisterno-coxalis posterior; lacking notable variation here) and the mesal remotor of the hind coxa (herein **IIIscm2**, M. metafurca-coxalis posterior, and **IIIscm3**, M. metafurca-coxalis medialis; where variation aligns with broader patterns of Hymenoptera, following Meira et al. [2024]). This study builds on Wille's (1956) findings, revealing that only 16 of 58 mesosomal muscle groups exhibit substantial variation across 10 bee and three apoid wasp taxa, reflecting a balance between phylogenetic conservatism and functional adaptation.

The limited variation, primarily in muscle origin points, underscores the mesosoma's evolutionary stability, with insertion points remaining highly conserved. This pattern suggests that origin points are less functionally constrained, allowing evolutionary flexibility without compromising muscle function, as the insertion point primarily dictates movement mechanics (Snodgrass, 1935). One notable pattern of variation is observed in the muscle **Idlm1**. In bees, the muscle origin is either dorsomedially or dorsolaterally positioned on the prophragma, while apoid wasps consistently exhibit a dorsolateral origin. Among the examined stingless bee species, *M. quadrifasciata*, *T. fiebrigi*, and *S. quadripunctata* (Apidae: Meliponini), the dorsomedial origin appears to be a shared derived feature, suggesting a synapomorphy (char: 1, state: 1; Fig. 2a-b, 10) for the tribe. Conversely, other bee taxa have a lateral origin (char: 1, state: 0; Fig. 7a, 10), indicating independent evolutionary shifts in **Idlm1**. Aibekova et al. (2022) noted that variations in thoracic muscle origins are likely linked to specific functional demands, such as movements of the head and the mesosomal appendages, further supporting the idea that the observed differences in **Idlm1** may reflect both phylogenetic history and biomechanical adaptations. These evolutionary morphological changes also support the findings of Vilhelmsen et al. (2010), when noting that mesosomal musculature can indicate both evolutionary history and functional adaptations.

Similarly, the variation in the muscle group **Ivlm3** suggests significant phylogenetic implications. While most bees exhibit a dorsal origin (char: 2, state: 1; Fig. 2c, 10), both andrenid species, as well as *O. divaricatus* [Halictidae] and *H. carinata* [Melittidae] retain a ventral origin (char: 2, state: 0; Fig. 7b, 10). This distribution suggests that the dorsal attachment may represent an ancestral condition preserved in specific lineages, whereas the ventral origin likely evolved independently in other groups. The ventral origin likely represents a derived condition, as interpreted by Vilhelmsen (2000b), potentially facilitating stronger head depression movements, such as those associated with certain foraging or ground-nesting behaviors. The dorsal origin, conversely, may enhance head flexibility as well as biomechanical demands for varied head positioning (Dudley, 2000).

Based on our results, apoid wasps and bees differ in the morphology of the **Ivlm7**. The extent of variation in Apoidea remains unexplored, but we sampled representatives of three taxa that represent at least a fraction of the diversity of apoid wasps. While most taxa exhibit a broad attachment to the mesofurcal bridge (char: 3, state: 0; Figs. 3d, 4c, 8d, 10), the species *A. mellifera* and *T. fiebrigi* display a restricted ventral attachment (char: 3, state: 1; Fig. 7c, 10). The restricted ventral attachment could be linked to an enhancement of the thoracic rigidity, supporting the high-frequency wing beats (Dickinson, 2006). Additionally, **Ivlm7** can have one (char: 4, state: 0; Fig. 7e, 10) or two (char: 4, state: 1; Fig. 7d, 10) insertions in bees, suggesting biomechanical adaptations for enhanced propectus retraction, potentially aiding in precise leg movements during nesting or pollen collection (Michener, 2007).

The metathoracic muscle **IIIscm2** (M. metafurca-coxalis posterior) exhibits a phylogenetically informative pattern, with an extended origin to the coalesced furcal arms in Andrenidae, Colletidae, and Halictidae (char: 14, state: 0; Fig. 9b, 10), which supports their closer evolutionary relationship (Almeida et al., 2023). This configuration likely enhances coxal stability, which is critical for the rapid, agile flight of these families, as they often forage in open habitats (Roubik, 1989). In contrast, the restricted origin in other bees and apoid wasps (char: 14, state: 1; Fig. 5a, 10) may reflect adaptations for less dynamic flight patterns, as seen in Megachilidae, which prioritize load-carrying for nest provisioning (Michener, 2007). Similarly, **IIIvolm** (M. metafurca-abdominosternalis lateralis) shows an anterior origin in Apidae and *O. flavescens* (char: 17, state: 0; Figs. 6b, 9e, 10), potentially improving metasomal flexibility for pollen transport or nest manipulation, contrasting with the posterior origin in other taxa (char: 17, state: 1; Fig. 9f, 10).

The muscle **IIpcm4** (M. propleuro-coxalis posterior) exhibits a branched origin in some lineages (char: 10, state: 0; Fig. 8e, 10), transitioning from fan-shaped to V-shaped forms, as noted by Wille (1956). This branching likely increases muscle force distribution, supporting the powerful leg movements required for digging in ground-nesting bees, like Andrenidae or Halictidae (Michener, 2007). The not-branched form (char: 10, state: 1; Fig. 4d, 10) in other lineages may favor streamlined leg motion (Dickinson, 2006), suitable for hovering or precise landing. Changes of **IIpcm4** in unrelated bee lineages suggest that biomechanical demands may drive morphological variation even more than the degree of phylogenetic proximity.

The absence of separation between **IItpm7b** and **IItpm7c** by the mesepisternal ridge in bees (char: 8, state: 1; Figs. 4a, 8c, 10) is a potential synapomorphy, distinguishing them from most apoid wasps. This fused configuration may enhance wing flexor efficiency, supporting sustained flight (Dudley, 2000), as observed in behaviors as hovering and foraging on flowers. In contrast, the separated configuration in apoid wasps (char: 8, state: 0; Fig. 8b, 10) likely may reflects their predatory lifestyle, requiring rapid and powerful wing movements (Chapman, 2013).

Several autapomorphies have been identified within specific taxa. In *A. mellifera*, unique features include the lateral origin of the muscle group **Idvm18** (char: 5, state: 0; Fig. 7a, 10), the restricted origin of the muscle group **IIspm1** to the anterior area of the mesepisternum (char: 9, state: 0; Fig. 8c, 10) and the medial insertion of **IIIdlm3** (char: 7, state: 0; Fig. 7e, 10). In *L. huberi*, the extension of the origin of **Iscm5** to the free basal portion of the mesofurcal arms (char: 6, state: 1; Fig. 8a, 10) highlight species-specific adaptations. The restricted origin point of **IIscm6** (char: 12, state: 0; Fig. 4c, 10) in *M. quadrifasciata* is suggested to be another autapomorphy. Such autapomorphies underscore the importance of detailed analyses of the skeletomusculature for understanding evolutionary differentiation, as emphasized by Vilhelmsen et al. (2010).

These morpho-functional patterns suggest that mesosomal musculature variations are not randomly distributed, but may have been shaped by ecological and biomechanical demands. For example, muscle configurations supporting head mobility (**Idlm1**, **Ivlm3**) may enhance foraging efficiency in diverse habitats, while those affecting coxal and metasomal movement (**IIIscm2**, **IIIvolm**) likely to enhance flight and nest building behaviors. Future studies integrating biomechanical modeling and kinematic analyses could further elucidate how these variations influence flight dynamics, pollen collection, or nest construction.

CONCLUSION

This study elucidates the phylogenetic significance of mesosomal musculature in bees by examining 10 bee species and three apoid wasp taxa, revealing distinct patterns of variation within a largely conserved anatomical framework. Of the 58 muscle groups analyzed, only 16 exhibit significant variation, primarily in origin points, with the prothoracic (e.g., **Idlm1**, **Ivlm3**) and metathoracic (e.g., **IIIscm2**, **IIIvolm**) muscles proving most informative. Some synapomorphies, such as the dorsomedial **Idlm1** in Meliponini and the extended **IIIscm2** in Andrenidae, Colletidae, and Halictidae, underscore close phylogenetic relationships within these lineages, while the fused **IItpm7b** and **IItpm7c** of bees distinguish them from their close relatives in the Apoidea. These variations suggest biomechanical adaptations for flight, foraging, and nesting, as required by ground-nesting bees, highlighting functional specialization. The detailed character systems established here provide a foundation for phylogenetic interpretations of the variation in the mesosomal musculature of bees. Expanding taxonomic sampling in the future will likely enhance our understanding of mesosomal evolution and its impact on the diverse ecological strategies that contribute to the evolutionary success of bees and apoid wasps. Moreover, future research employing advanced techniques, such as micro-computed tomography, will undoubtedly help improve our knowledge of muscular variation in Apoidea.

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Figure 1

Morphological diversity of Apoidea

Morphological diversity of Apoidea represented by a sample of 13 species analyzed in this comparative research of the mesosomal skeletomuscular morphology. Bees were the main focus of the investigation and were represented by 10 species: (a) *Oxaea flavescens*, female and (b) *Psaenythia bergii*, female [Andrenidae]; (c) *Apis mellifera*, female worker (d) *Melipona quadrifasciata*, female worker, (e) *Schwarziana quadripunctata*, female worker, and (f) *Tetragonisca fiebrigi*, female worker [Apidae]; (g) *Tetraglossula anthracina*, female [Colletidae]; (h) *Oragapostemon divaricatus*, female [Halictidae]; (i) *Hesperapis carinata*, male [Melittidae], (j) *Lithurgus huberi*, female [Megachilidae]; complemented by three apoid wasps: (k) *Steniolia duplicata*, female [Bembicidae], (l) *Trachypus boharti*, female [Philantidae], and (m) *Trypoxylon lactitarse*, female [Crabronidae]. Scale bar = 5 mm.


a
b
c
d
f
e
h
g
j
i
k
l
m

Figure 2

Prothoracic skeletomusculature

Prothoracic skeletomusculature of *Melipona quadrifasciata*: (a) muscles associated with the profurca, pronotum, and propleura—posterolateral view of the head; (b) muscles associated with the head and propectus—dorsal view of propectus; (c) muscles associated with the propectus—dorsal view of propectus; (d) muscles associated with the propleura—dorsal view of propleura; (e) muscles associated with propectus—dorsal view of propectus; (f) muscles associated with propectus and procoxa—posterior view of propectus. Muscle labels are displayed in bold and larger font to differentiate them from labels applied to sclerites and body regions; photomicrographs not to scale. Stars indicate structure orientation according to three axes: anterior–posterior, dorsal–ventral, and left–right.

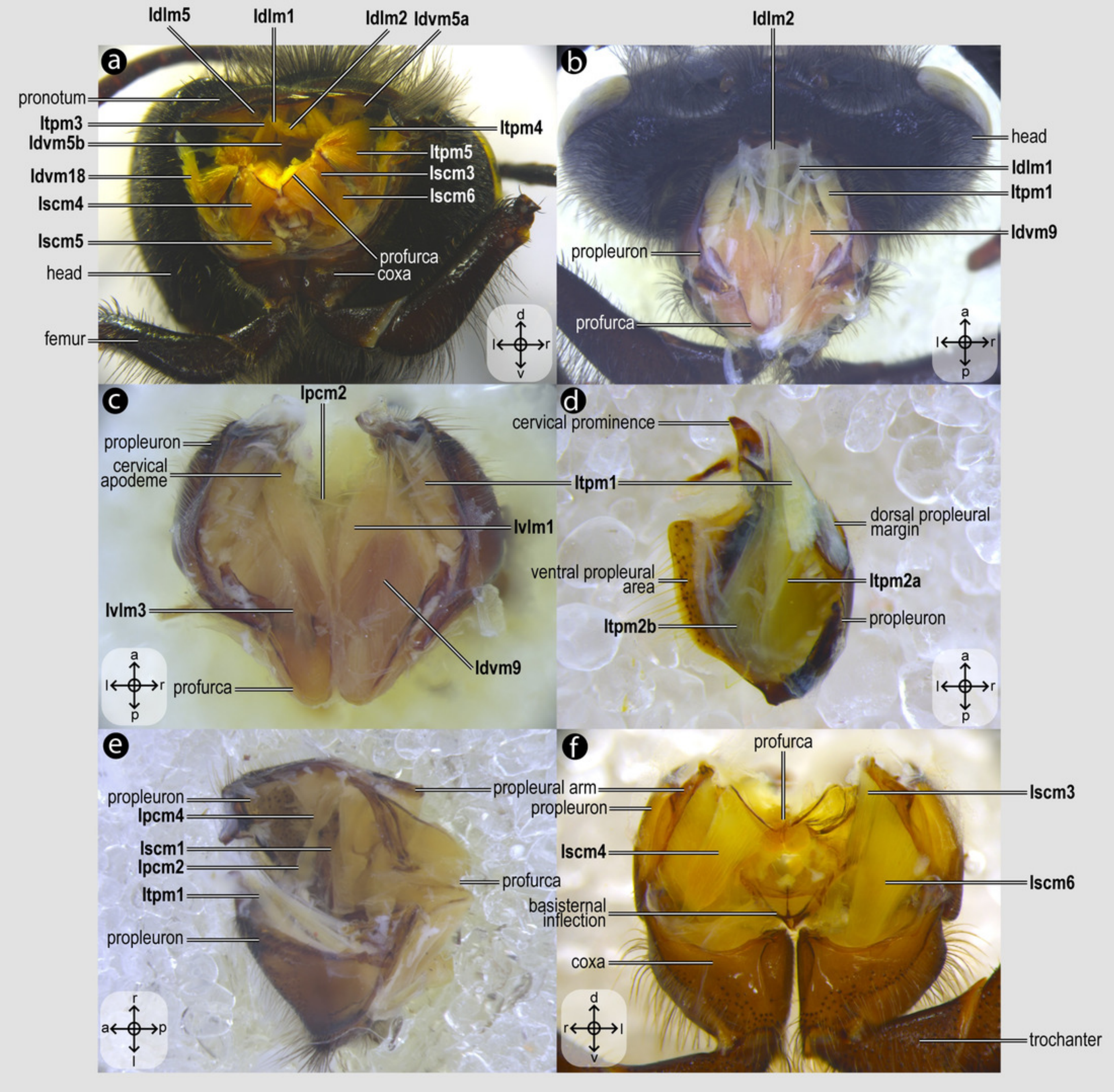

a
Idlm5
Idlm1
Idlm2
Idvm5a
pronotum
Itpm3
Idvm5b
Idvm18
Iscm4
Iscm5
head
femur
Itpm4
Itpm5
Iscm3
Iscm6
profurca
coxa
d
l
r
v
b
Idlm2
head
Idlm1
Itpm1
Idvm9
propleuron
profurca
a
l
r
p
c
Ipcm2
propleuron
cervical apodeme
Ivlm1
Ivlm3
Idvm9
profurca
a
l
r
p
d
cervical prominence
Itpm1
dorsal propleural margin
ventral propleural area
Itpm2a
propleuron
Itpm2b
a
l
r
p
e
propleuron
Ipcm4
Iscm1
Ipcm2
Itpm1
propleuron
propleural arm
profurca
r
a
p
l
f
profurca
propleural arm
propleuron
Iscm3
Iscm4
Iscm6
basisternal inflection
coxa
trochanter
d
r
l
v

Figure 3

Mesothoracic skeletomusculature

Mesothoracic skeletomusculature of *Melipona quadrifasciata*: (a) indirect flight muscles—dorsal view of mesosoma; (b) scutoscutellar muscles—ventral view of mesoscutellum; (c) first spiracular muscles—anterior view of mesepisternum; (d) muscles associated with the meso/metafurca—anterior view of the meso/metafurca. Muscle labels are displayed in bold and larger font to differentiate them from labels applied to sclerites and body regions; photomicrographs not to scale Stars indicate structure orientation according to three axes: anterior–posterior, dorsal–ventral, and left–right.

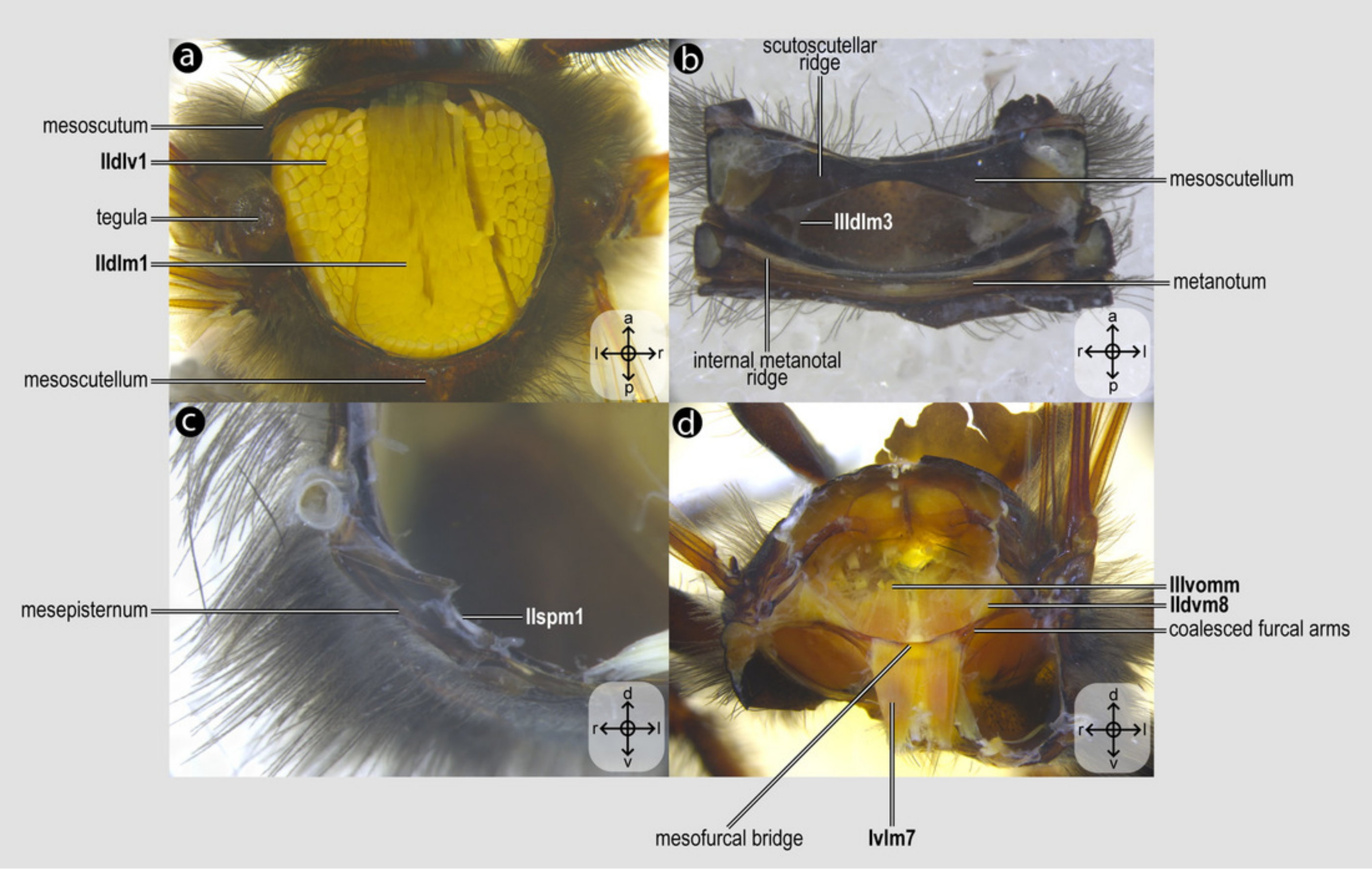


Figure 4

Direct flight muscles and mesocoxal skeletomusculature

Direct flight muscles and mesocoxal skeletomusculature of *Melipona quadrifasciata* : (a) muscles associated with the mesoaxillary sclerites—mesal view of the mesepisternum; b) muscles associated with the metaaxillary sclerites—mesal view of the metapectus; c) mesocoxal muscles associated with the meso/metafurca—anterior view of the metafurca; d) pleural muscles associated with the meso/metafurca—anterior view of the meso/metafurca. Muscle labels are displayed in bold and larger font to differentiate them from labels applied to sclerites and body regions; photomicrographs not to scale. The star depicted in (a) indicates structure orientation according to three axes: anterior–posterior, dorsal–ventral, and left–right. All figures oriented according to the star depicted in (a).

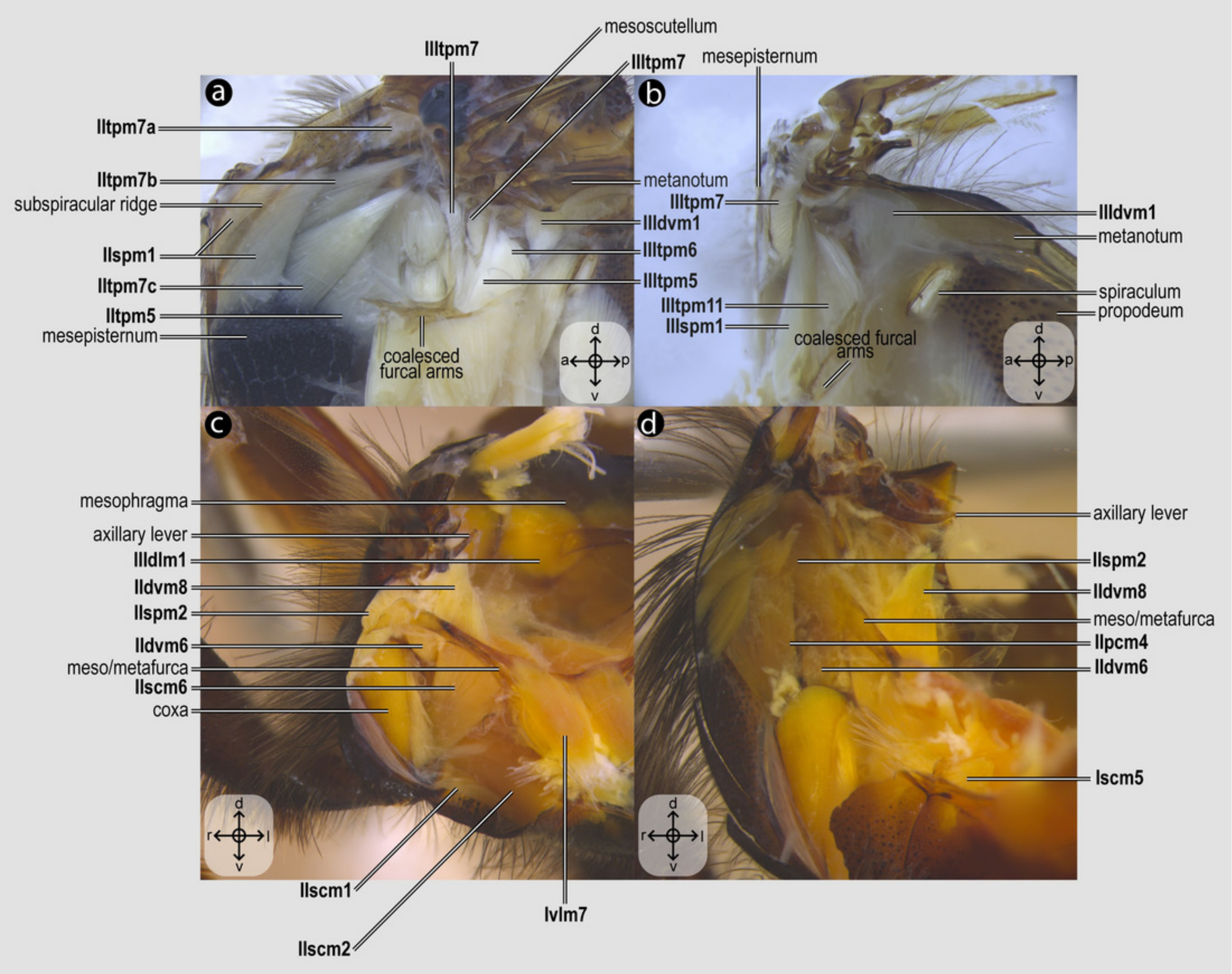

mesoscutellum
IIItpm7
IIItpm7
mesepisternum
a
b
IItpm7a
IItpm7b
subspiracular ridge
IIspm1
IItpm7c
IItpm5
mesepisternum
coalesced furcal arms
metanotum
IIIdvm1
IIItpm6
IIItpm5
d
a
p
v
IIItpm7
IIIdvm1
metanotum
spiraculum
propodeum
IIItpm11
IIIspm1
coalesced furcal arms
c
d
mesophragma
axillary lever
IIIdlm1
IIdvm8
IIspm2
IIdvm6
meso/metafurca
IIscm6
coxa
r
l
IIscm1
IIscm2
Ivlm7
axillary lever
IIspm2
IIdvm8
meso/metafurca
IIpcm4
IIdvm6
Iscm5

Figure 5

Metacoxal muscles

Metacoxal muscles of *Melipona quadrifasciata* : (a), (b), (c), (d) metacoxal muscles associated with the meso/metafurca—dorsal view of the metafurca. Muscle labels are displayed in bold and larger font to differentiate them from labels applied to sclerites and body regions; photomicrographs not to scale. The star depicted in (a) indicates structure orientation according to two axes: anterior–posterior and left–right.

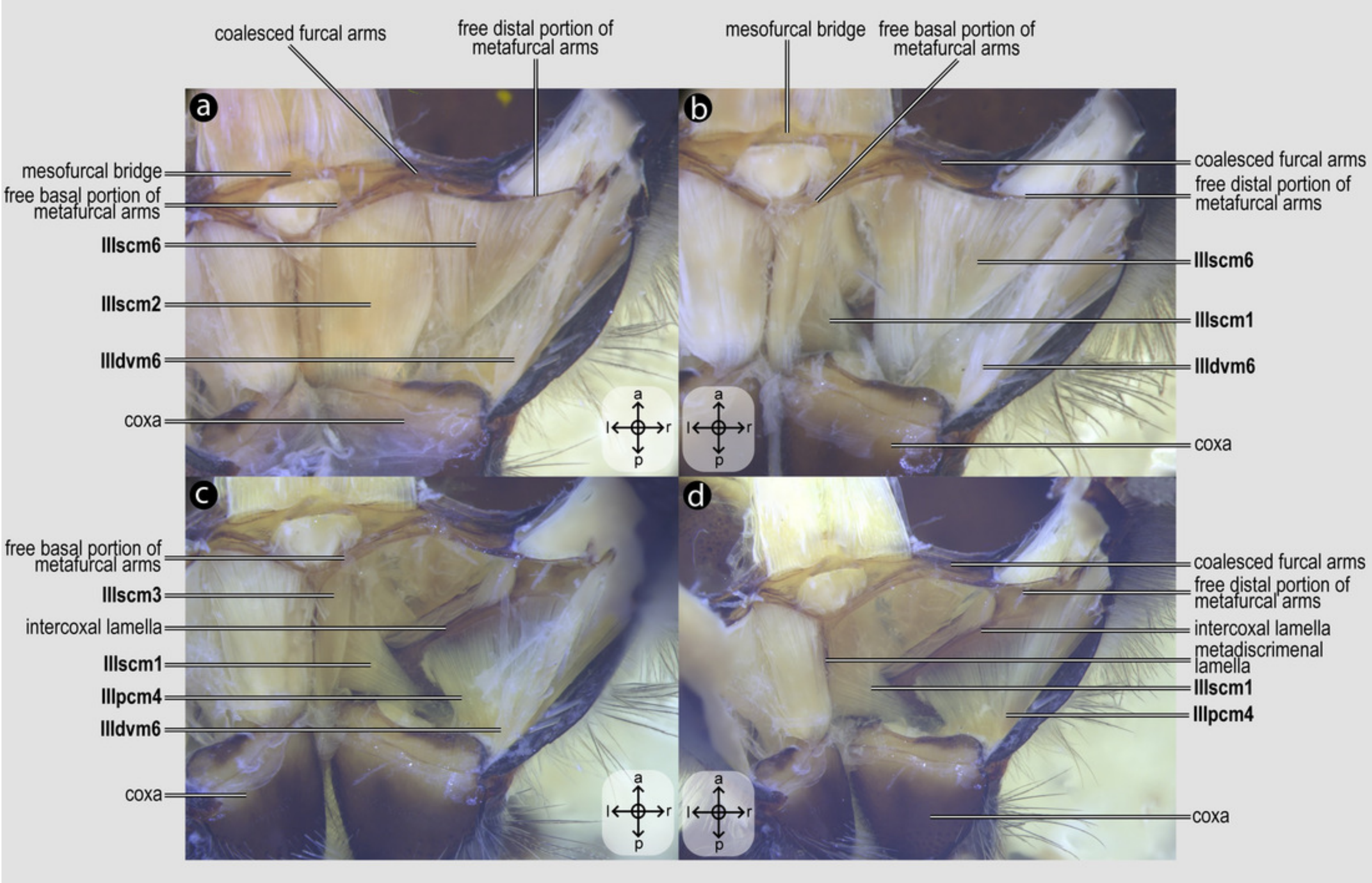


Figure 6

Propodeal muscles

Propodeal muscles of *Melipona quadrifasciata* : (-a) propodeal muscles associated with the metafurca—dorsal view of metafurca; (b) propodeal muscles associated with metafurca—dorsolateral view of metafurca; (c) propodeal and spiracular muscles associated with the propodeum—medial view of the propodeum; (d) propodeal muscles—dorsal view of the metafurca. Muscle labels are displayed in bold and larger font to differentiate them from labels applied to sclerites and body regions; photomicrographs not to scale. Stars indicate structure orientation according to three axes: anterior–posterior, dorsal–ventral, and left–right.

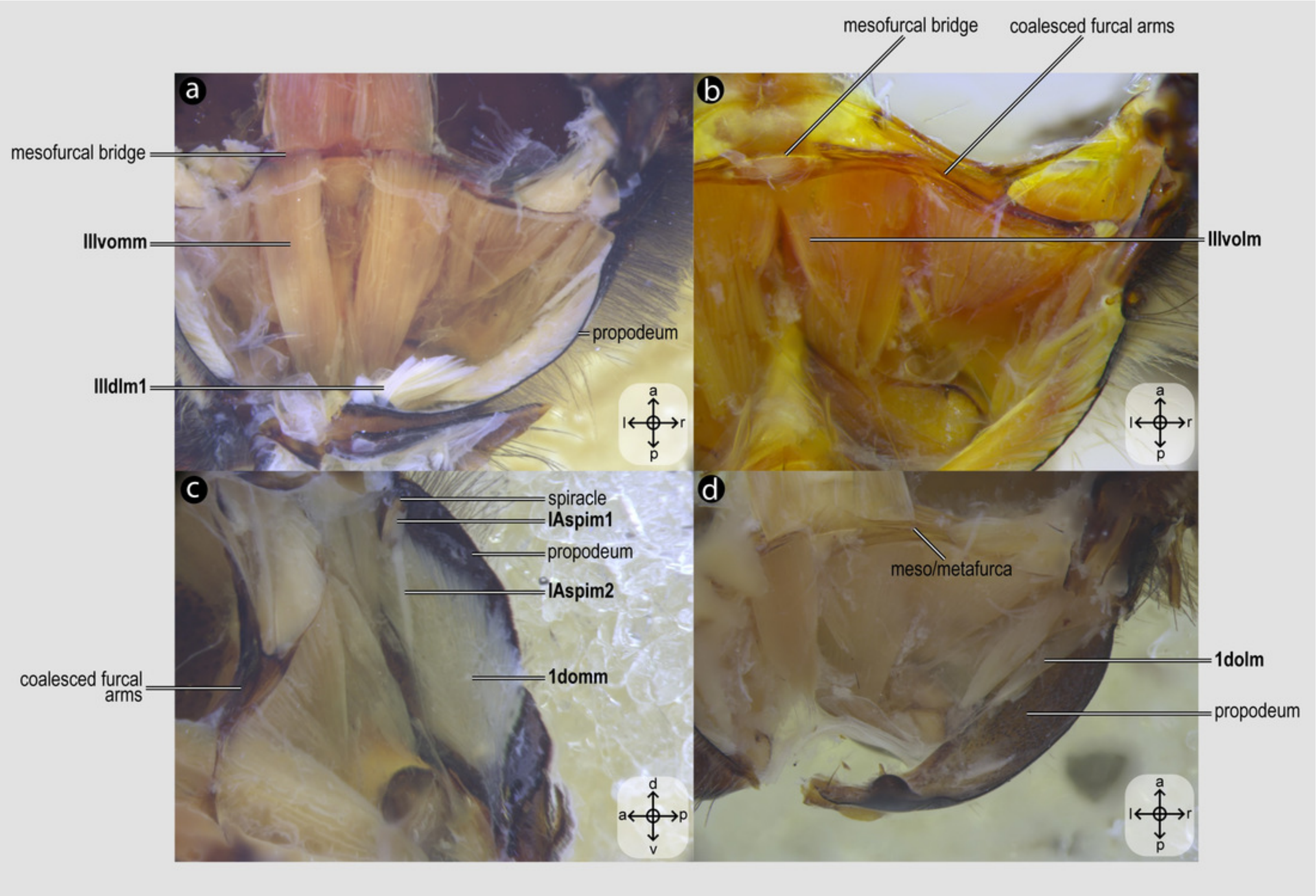


Figure 7

Variation of thoracic skeletomusculature: Part 1

Photomicrographs of dissections of female representatives of Apoidea: (a) *Apis mellifera*, anterior view of the propectus; (b) *Oxaea* flavescens, dorsal view of the propectus; (c) *Apis* mellifera, anterior view of the mesosoma; (d) *Lithurgus huberi*, lateral view of the profurca; (e) *Trypoxylon lactitarse*, posterolateral view of the prospectus; (f) *Apis mellifera*, ventral view of mesoscutellum. Arrowheads indicate morphological conditions coded as character states. Stars indicate structure orientation according to three axes: anterior–posterior, dorsal–ventral, and left–right.

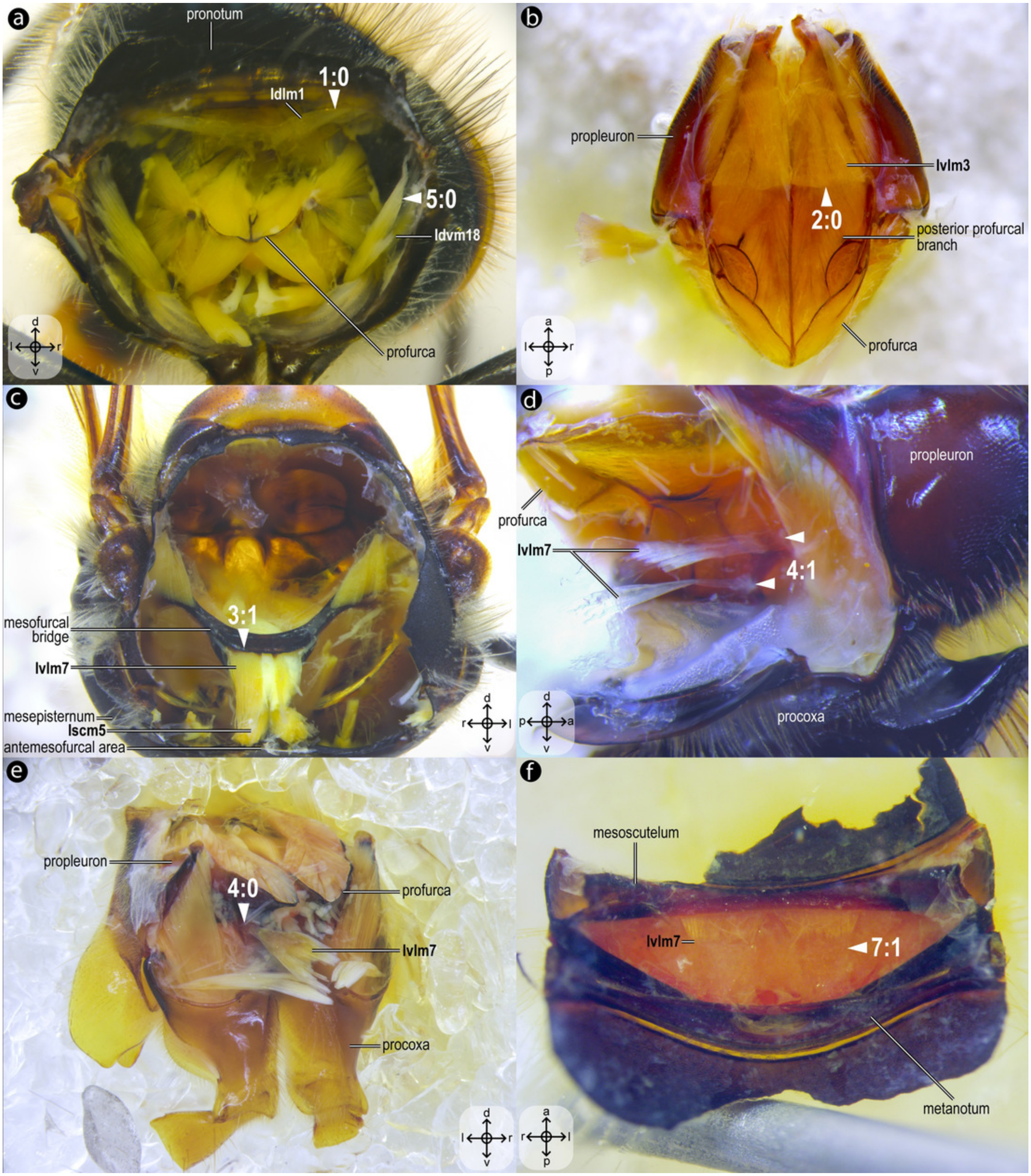

a
pronotum
1:0
ldlm1
5:0
ldvm18
profurca
d
l
r
v
b
propleuron
lvlm3
2:0
posterior profurcal branch
profurca
a
l
r
p
c
mesofurcal bridge
3:1
lvlm7
mesepisternum
lscm5
antemesofurcal area
d
r
l
v
d
propleuron
profurca
lvlm7
4:1
procoxa
d
p
a
v
e
propleuron
4:0
profurca
lvlm7
procoxa
d
l
r
v
f
mesoscutelum
lvlm7
7:1
metanotum
a
r
l
p

Figure 8

Variation of thoracic skeletomusculature: Part 2

Dissections of female representatives of Apoidea: (a) *Lithurgus huberi*, anterolateral view of the meso/metafurca; (b) *Trypoxylon lactitarse*, mesal view of the mesepisternum; (c) *Apis mellifera*, mesal view of the mesepisternum; (d), *Hesperapis carinata*, anterior view of the mesosoma; (e) *Psaenythia bergii*, mesal view of the mesepisternum; (f) *Trachypus boharti*, anterolateral view of meso/metafurca. Arrowheads indicate morphological conditions coded as character states. Stars indicate structure orientation according to three axes: anterior–posterior, dorsal–ventral, and left–right.

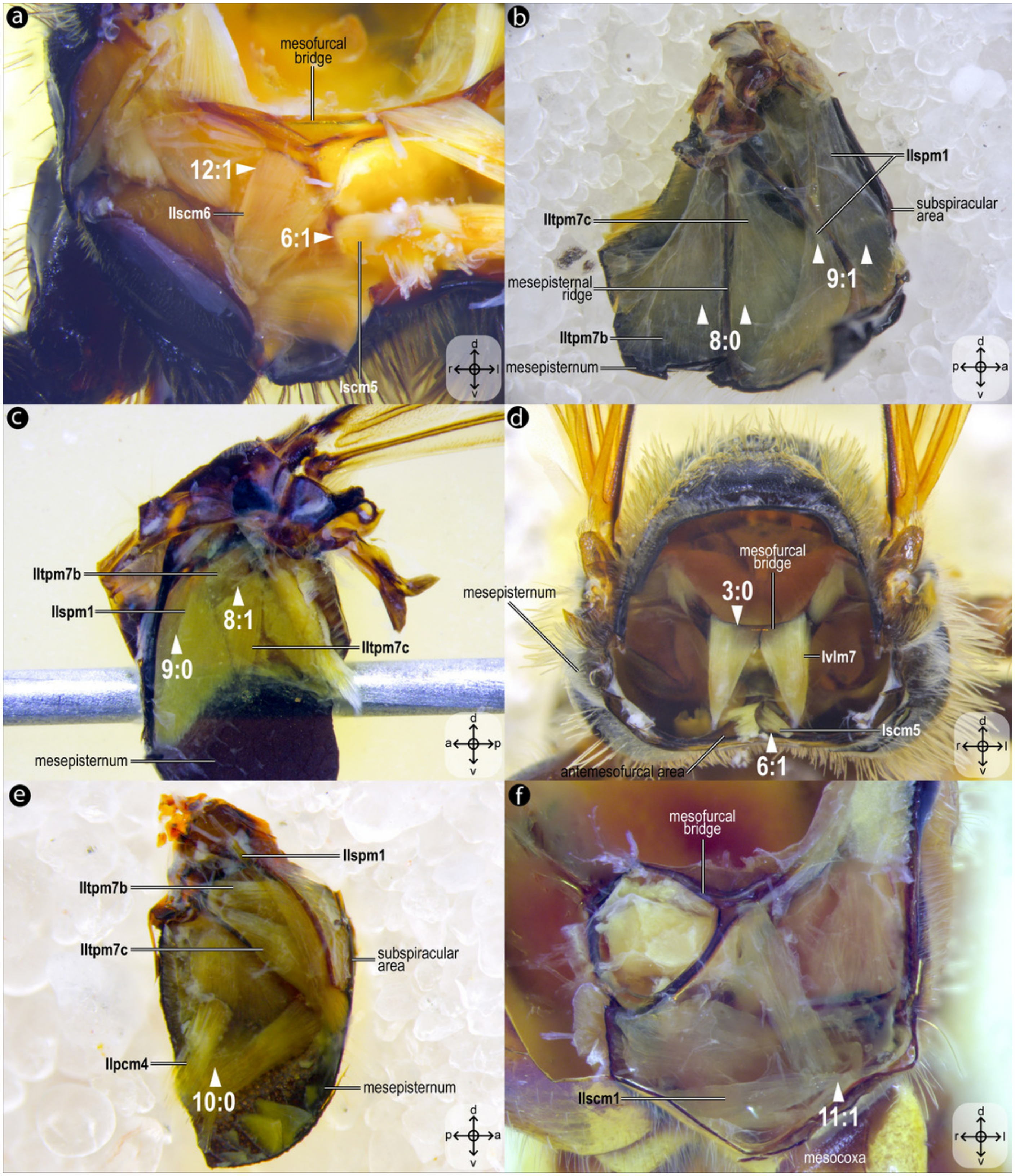

a
mesofurcal bridge
12:1
IIscm6
6:1
Iscm5
d
r
l
v
b
IIspm1
IItpm7c
subspiracular area
9:1
mesepisternal ridge
8:0
IItpm7b
mesepisternum
d
p
a
v
c
IItpm7b
IIspm1
8:1
IItpm7c
9:0
mesepisternum
d
a
p
v
d
mesofurcal bridge
mesepisternum
3:0
Ivlm7
Iscm5
antemesofurcal area
6:1
d
r
l
v
e
IIspm1
IItpm7b
IItpm7c
subspiracular area
IIpcm4
10:0
mesepisternum
d
p
a
v
f
mesofurcal bridge
IIscm1
11:1
mesocoxa
d
r
l
v

Figure 9

Variation of thoracic skeletomusculature: Part 3

Dissections of female representatives of Apoidea: (a) *Trachypus boharti*, dorsolateral view of the propodeum; (b) *Psaenythia bergii*, dorsal view of the propodeum; (c) *Trachypus boharti*, dorsal view of the propodeum; (d) *Apis mellifera* dorsal view of the propodeum; (e) *Apis mellifera*, dorsal view of the propodeum; (f) *Trachypus boharti*, dorsal view of the propodeum. Arrowheads indicate morphological conditions coded as character states. Stars indicate structure orientation according to two axes: anterior–posterior and left–right.

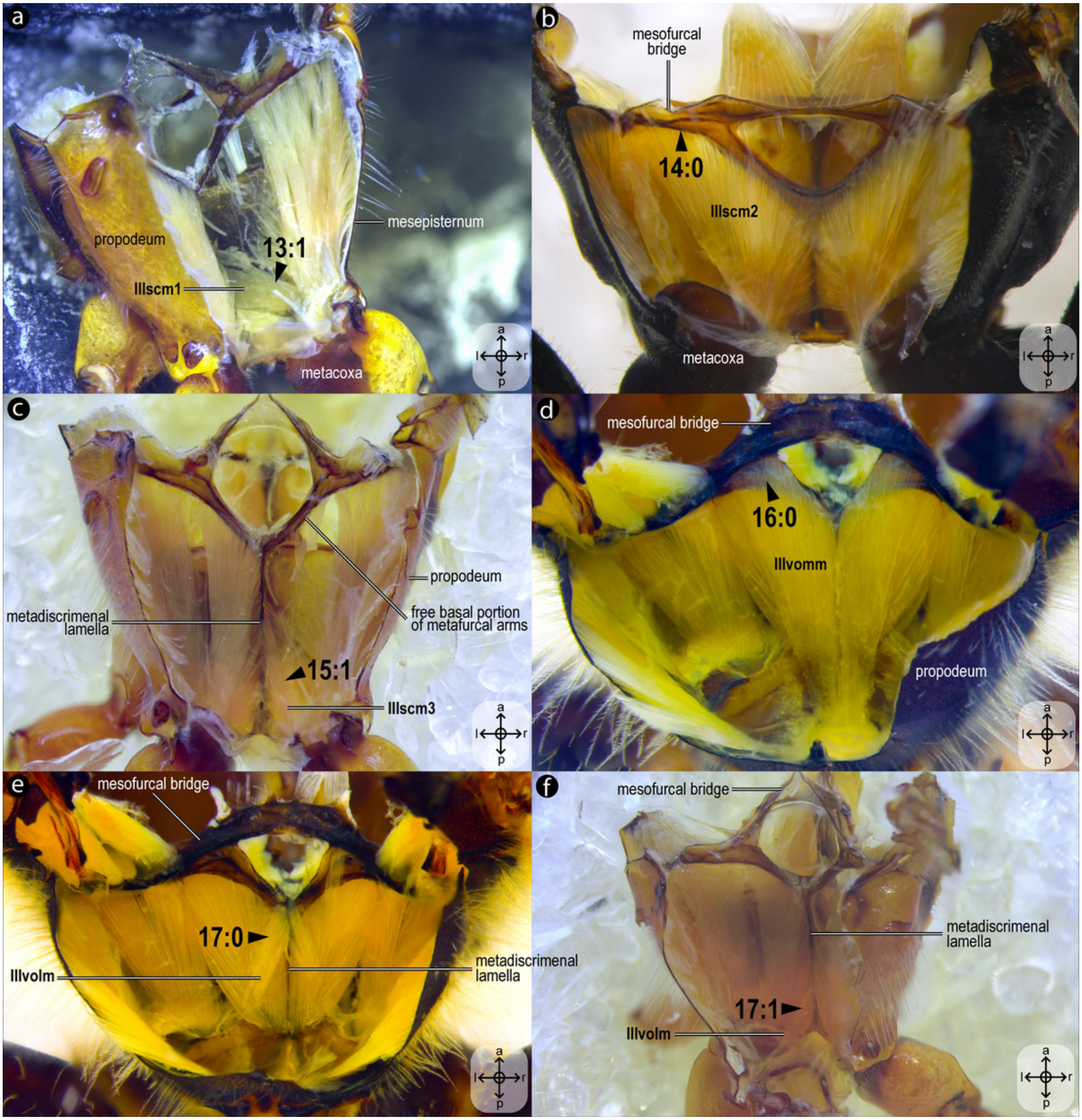
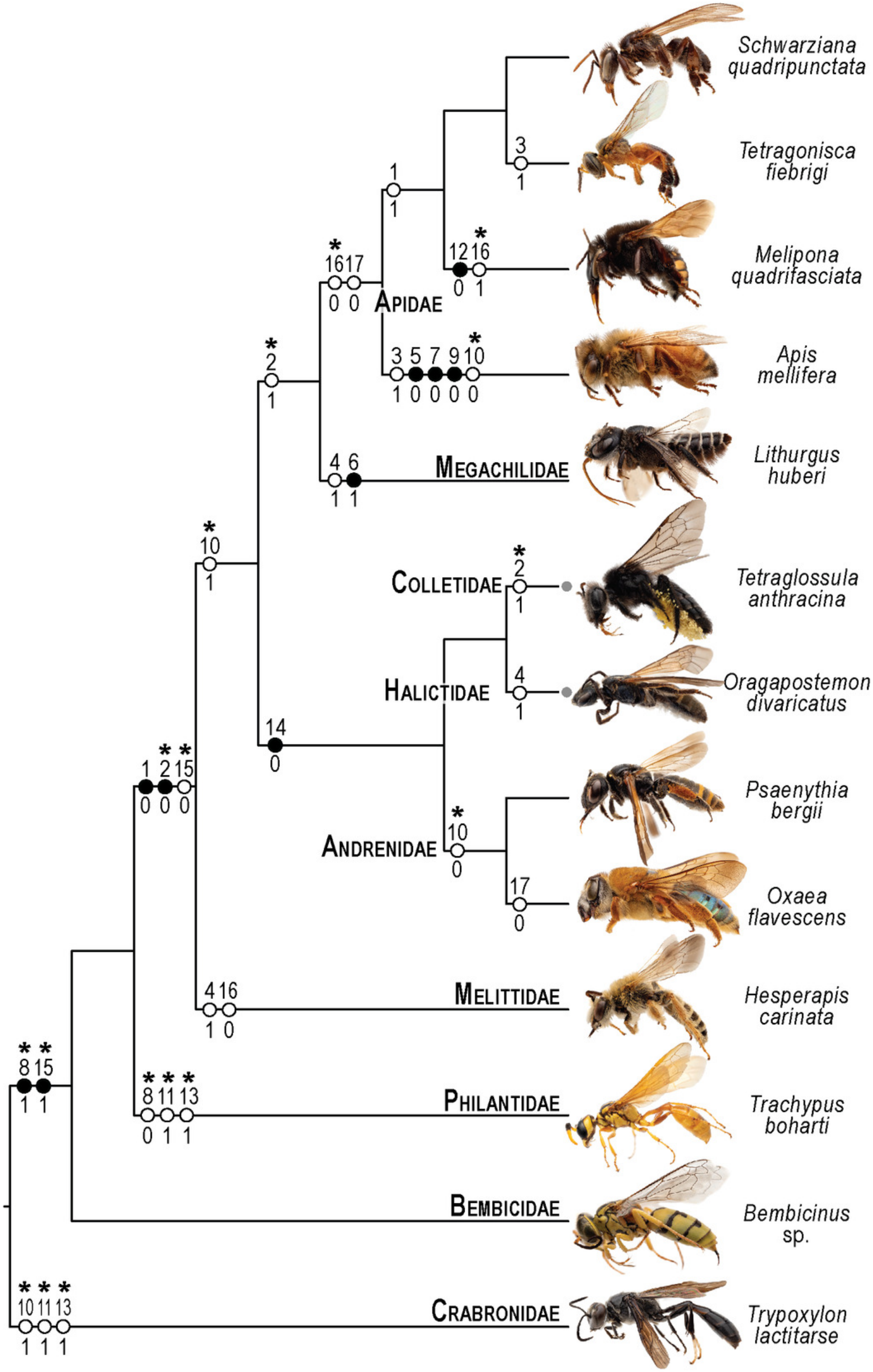

a
propodeum
IIIscm1
13:1
mesepisternum
metacoxa
a l r p
b
mesofurcal bridge
14:0
IIIscm2
metacoxa
a l r p
c
metadiscrimenal lamella
propodeum
free basal portion of metafurcal arms
15:1
IIIscm3
a l r p
d
mesofurcal bridge
16:0
IIIvomm
propodeum
a l r p
e
mesofurcal bridge
17:0
IIIvolm
metadiscrimenal lamella
a l r p
f
mesofurcal bridge
metadiscrimenal lamella
17:1
IIIvolm
a l r p

Figure 10

Phylogenetic interpretation of variation in the skeletomusculature of the mesosoma

Phylogenetic relationships among the 13 species of Apoidea investigated in this research follow Almeida et al. (2023) for relationships among bee taxa and Sann et al. (2018) for the three apoid wasp lineages. Evolutionary changes of 13 characters encoding variation in the skeletomusculature of the mesosoma were phylogenetically optimized onto the tree. Filled circles indicate unique transformations, while empty circles signify non-unique transformations; numbers above the circles denote character numbers, and those below indicate their apomorphic character states; ambiguous changes only recovered with accelerated transformation (ACCTRAN) are indicated by stars above their character numbers. Photographs on the left are not to scale and serve to illustrate the general appearance of each taxon (see Figure 1 for more details).


Schwarziana quadripunctata
3 1
Tetragonisca fiebrigi
1 1
12 16 * 0 1
Melipona quadrifasciata
* 16 17 0 0
APIDAE
3 5 7 9 10 * 1 0 0 0 0
Apis mellifera
* 2 1
4 6 1 1
MEGACHILIDAE
Lithurgus huberi
* 10 1
COLLETIDAE
* 2 1
Tetraglossula anthracina
HALICTIDAE
4 1
Oragapostemon divaricatus
14 0
Psaenythia bergii
ANDRENIDAE
* 10 0
17 0
Oxaea flavescens
1 2 15 * * 0 0 0
4 16 1 0
MELITTIDAE
Hesperapis carinata
* * 8 15 1 1
* * * 8 11 13 0 1 1
PHILANTIDAE
Trachypus boharti
BEMBICIDAE
Bembicinus sp.
* * * 10 11 13 1 1 1
CRABRONIDAE
Trypoxylon lactitarse