A comparative description of the mesosomal musculature in Sphecidae and Ampulicidae (Hymenoptera, Apoidea) using 3D techniques

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Abstract

Conflicting hypotheses about the relationships among the major lineages of aculeate Hymenoptera clearly show the necessity of detailed comparative morphological studies. Using micro-computed tomography and 3D reconstructions, the skeletal musculature of the meso- and metathorax and the first and second abdominal segment in Apoidea are described. Females of Sceliphron destillatorium, Sphex (Fernaldina) lucae (both Sphecidae), and Ampulex compressa (Ampulicidae) were examined. The morphological terminology provided by the Hymenoptera Anatomy Ontology is used. Up to 42 muscles were found. The three species differ in certain numerical and structural aspects. Ampulicidae differs significantly from Sphecidae in the metathorax and the anterior abdomen. The metapleural apodeme and paracoxal ridge are weakly developed in Ampulicidae, which affect some muscular structures. Furthermore, the muscles that insert on the coxae and trochanters are broader and longer in Ampulicidae. A conspicuous characteristic of Sphecidae is the absence of the metaphragma. Overall, we identified four hitherto unrecognized muscles. Our work suggests additional investigations on structures discussed in this paper.

Key Words

Aculeata, anatomy, microCT, phylogeny, propodeum, thorax

Introduction

Hymenoptera form one of the largest insect orders and comprise more than 150,000 extant species (Aguiar et al. 2013). The group of interest examined in this paper constitutes a subclade of Hymenoptera, the Aculeata (stinging wasps, bees, and ants; Sharkey et al. 2012). Derived from the modified ovipositor, the stinger is a synapomorphy of aculeate Hymenoptera and a key innovation for their evolutionary success (Sharkey et al. 2012; Schmidt 2016). The nature of phylogenetic relationships within the monophyletic Aculeata is still contested (e.g., Königsmann 1978; Lomholdt 1982; Rasnitsyn 1988; Alexander 1992; Brothers and Carpenter 1993; Ronquist et al. 1999; Peters et al. 2011, 2017; Sharkey et al. 2012; Johnson et al. 2013; Branstetter et al. 2017). Traditionally, Aculeata is divided into three lineages: Chrysidoidae, Vespoidae, and Apoidea (O’Neill 2001; Branstetter et al. 2017).

About 10,000 species of digger wasps (also named apoid wasps) as part of the species-rich superfamily Apoidea are currently known (Pulawski 2020). The most obvious synapomorphy of Apoidea is the rounded pronotal lobe (Ohl and Engel 2007). Apoidea is divided into the monophyletic Anthophila (bees) and the paraphyletic apoid wasps. The latter comprises Ampulicidae, Crabronidae, Heterogynaidae, and Sphecidae (e.g., Branstetter et al. 2017). Recent phylogenomic and molecular analyses suggest Ampulicidae is the sister to the rest of the Apoidea (Debevec et al. 2012 [ribosomal 28S and protein-coding nuclear genes]; Sann et al. 2018 [target DNA enrichment and transcriptomic sequence data]). However, contradictory evidence on the phylogenetic rela-
tionships within the apoid wasps (e.g., Lohrmann et al. 2008; Ohl and Spahn 2010; Debevec et al. 2012; Sharkey et al. 2012; Branstetter et al. 2017) remains unresolved. Based upon different research methods, most results suggest, that Sphecidae and Ampulicidae are well-supported clades (Ohl and Spahn 2010 [morphological study]; Branstetter et al. 2017 [ultrasonic element phylogenomics]; Peters et al. 2017 [protein-coding genes]), whereas Crabronidae are likely to be paraphyletic (Lohrmann et al. 2008 [nuclear long-wavelength-opsin and mitochondrial cytochrome-c-oxidase]; Debevec et al. 2012; Branstetter et al. 2017; Peters et al. 2017). However, Sann et al. (2018) found Crabronidae to be polyphyletic. Another unresolved issue is the position of Heterogynaidae within Apoidea (Ohl and Bleidorn 2006). Debevec et al. (2012) obtained two different results: Heterogynaidae nested within Crabronidae (maximum likelihood tree) and as sister to a monophyletic group of Sphecidae sensu stricto, Crabronidae and Anthophila (Bayesian tree). The first result was already proposed by Ohl and Bleidorn (2006 [long-wavelength opsins]). Branstetter et al. (2017) found Heterogynaidae to be sister to a grouping of paraphyletic Crabroninae and Sphecidae.

Morphological characters are still one of the major sources of phylogenetic inference (e.g., Friedrich and Beutel 2010; Ohl and Spahn 2010; Vilhelmsen et al. 2010; Zimmermann and Vilhelmsen 2016; Liu et al. 2019). Nevertheless, internal mesosomal structures are insufficiently studied across Hymenoptera, as predicated by Vilhelmsen et al. (2010), who provided detailed information for many apocritan wasps and other Hymenoptera; especially the mesosomal musculature of Pison chilense (Crabronidae) and external mesosomal characters for Pison chilense, Stangeella cyaniventris (Sphecidae), and Ampulex compressa (Ampulicidae) are described. They demonstrated, that the mesosomal region reveals considerable information for phylogenetic research. Previously, indispensable work about the mesosomal musculature in Hymenoptera was presented by Maki (1938), Snodgrass (1942; in particular, for Apis), Heraty (1989), and Matsuda (1970), followed by Prentice (1998). Recent substantial work was accomplished by Mikó et al. (2007). They dissected the musculature of the head and mesosoma in a review of the parasitic wasp family Scelionidae. Furthermore, a reinterpretation of the delimitation of the metapostnotum in Chrysidioidea was presented by Kawada et al. (2015). Moreover, Porto et al. (2016) defined internal mesosomal characters of bees and evaluated the potential of these structures, concluding that they are of great value to phylogenetic investigations. Garcia et al. (2017) described several body parts of three new species of the rare ant genus Zaspilchins, resulting in a comparative character matrix for species-level taxonomy. Subsequently, Liu et al. (2019) provided insights on the mesosoma of an ant worker of Myrmecia for comparisons with other Aculeata and to gain new information about evolution and body function.

A state-of-the-art method for morphological analyses is the three-dimensional imaging, using micro-computed tomography (microCT). It is a highly powerful technique (Faulwetter et al. 2013 and references therein; Garcia et al. 2017; Liu et al. 2019), as it makes internal structures visible without destroying the specimen. Moreover, the digital 3D models can be created repeatedly to work on different goals and the data can easily be shared worldwide.

By using 3D imaging, we aim to expand the basic morphological knowledge for phylogenetic investigations within Aculeata. In this paper we present data of muscular structures in the mesosoma of Sceliphron distellatorium (Illiger, 1807), Sphex (Fernaldina) lucae de Saussure, 1867 (both Sphecidae), and Ampulex compressa (Fabricius, 1781) (Ampulicidae) (Fig. 1). These wasps are solitary and nest-provisioning predators with different lifestyles (e.g., Williams 1942; Bohart and Menke 1976; Fouad et al. 1994; Haspel and Libersat 2003; Libersat 2003; Ohl and Spahn 2010). Both families were selected for their large number of plesiomorphic characters within digger wasps (Ohl and Spahn 2010), which might help to reconstruct the ancestral apoid anatomy. Primarily, we illustrate and describe mesosomal conformations of the skeletal musculature, with focus on the mesothorax, metathorax, and the first abdominal segment (metasoma). We also describe muscles that originate in the mesosoma and insert in the second abdominal segment (metasoma) because of strong interrelations of these muscles in this transition zone between both tagmata. The wasp waist allows for increased movability of the abdomen and, therefore, is an important anatomical cluster for various physical activities requiring precise movements of the abdomen below the body. This includes, for instance, stinging prey or enemies for defence, laying eggs (Williams 1942; Bohart and Menke 1976), carrying prey between mid or hind legs and abdomen while in flight, dragging prey forwards or backwards (Bohart and Menke 1976), and increasing balance in flight (at least when the second abdominal segment is petiolate; Bohart and Menke 1976).

Material and methods

Specimens and body parts examined

Sphex and Ampulex were taken from the collection of the Museum für Naturkunde Berlin (MfN) and Sceliphron was collected in the field (Table 1). To examine and compare the muscle sets, specimens of the same sex (females) were selected. We analysed the musculature of the mesothorax, metathorax, and the first and second abdominal segments.

Preparation, microCT, and 3D reconstruction

The extremities of the specimens were removed to minimize the scan field for optimizing the resolution of the data sets. Furthermore, the tip of the gaster was removed to facilitate the infiltration of the iodine, which intensifies the visibility of the musculature in the scan. Following Metscher (2009) and Gignac et al. (2016), our specimens were contrasted in a 25% iodine solution in pure ethanol.
Figure 1. Portraits of the three specimens examined, lateral view. A. *Sceliphron destillatorium*, body size 20 mm; B. *Sphex (Fernaldina) lucae*, body size 18 mm; C. *Ampulex compressa*, body size 21 mm.

Table 1. Basic information about the specimen collection, classification, preparation, and settings for microCT scanning.

| Specimens            | Sceliphron destillatorium | Sphex (Fernaldina) lucae | Ampulex compressa  |
|----------------------|----------------------------|--------------------------|--------------------|
| MfN collection number| MfN_Hym_Sph_I004239        | MfN_Hym_Sph_I000635      | MfN_Hym_Amp_I000029|
| Location/label data  | GRECE, Crete, Afrata [little road], 35°34'38.31"N, 23°4'2.3"E | USA, New Mexico, Hidalgo Co., Gray Ranch, 20.6 mi S Animas | GERMANY, Berlin, MfN breed, Oviposition 6 Aug. 2015, Eclosion 27 Sept. 2015 |
| Date of collection   | 24 June 2015               | 28 Aug. 2003             | 28 Sept. 2015      |
| Leg.                 | M. Willsch                | S. Schiller, L. Richert  | L. Kirschey        |
| Det.                 | M. Willsch 2015           | M. Ohl 2004              | L. Kirschey 2015   |
| Family               | Sphecidae                 | Sphecidae                | Ampulicidae        |
| Sex                  | Female                     | Female                   | Female             |
| Body size (mm)       | 20                         | 18                       | 21                 |
| Storage              | 96% ethanol                | 96% ethanol              | 96% ethanol        |
| Sample preparation   | 25% Iodine staining, critical point drying | 25% Iodine staining, critical point drying | 25% Iodine staining, critical point drying |
| Scanning medium      | Air                        | Air                      | Air                |
| Voltage (kV)         | 48                         | 48                       | 50                 |
| Current (µA)         | 250                        | 250                      | 275                |
| Number of images     | 1000                       | 1000                     | 1440               |
| Rotation steps       | 0.36                       | 0.36                     | 0.25               |
| Exposure time (ms)   | 1000                       | 1000                     | 1000               |
| Resolution (µm/pixel)| 3.40                       | 4.69                     | 5.00               |

(100%) for three days and washed out with pure ethanol for 30 seconds. The wasps were dried using a critical point dryer (Leica EM CPD300; Table 1). Afterwards, the three specimens were scanned at the Visualisation Laboratory of the MfN using a Phoenix nanotom X-ray|s tube (General Electric) at 48–50 kV and 250–275 µA. At 1 second per image 1000–1440 projections were generated per scan. The different kV- and projection-settings depended on the respective specimen size, which was also responsible for the range of the effective voxel size between 3.4–5 µm (Table 1). The cone beam reconstruction was performed using the CT reconstruction software PHOENIX(X-RAY DATOS)X version 2.0 (GE Sensing & Inspection Technologies GmbH).

3D segmentation and post-processing

The raw microCT image data were visualised and analysed by using a Wacom Cintiq 22HD interactive pen display and the software AMIRA ZIB EDITION 2020.02 and former versions (provided by the Zuse Institute Berlin). All muscles were segmented and labelled manually by using appropriate segmentation tools in AMIRA. Segmented materials were transformed into high-resolution surfaces using the Isosurface-Tool in AMIRA. The reconstruction was accomplished for one body side of the specimens, as no structural asymmetries were observed in this region. Therefore, the number of muscles given in the results refers to one-half of the body. For post-editing (e.g., picture artefacts, file size reduction, file converting, figure compilation) we exported TIF-files from AMIRA into ADOBE PHOTOSHOP CS6.

Terminology

Skeletal musculature was categorised based on insertion sites. The muscle terminology of the Hymenoptera Anatomy Ontology (HAO; http://portal.hymao.org/projects/32/public/ontology/) (Mikó et al. 2007; Vilhelmsen et al. 2010; Yoder et al. 2010; Seltmann et al. 2012) has been adopted here. In this connection, we provide a list of Universal Resource Identifiers (URI) for each muscular and cuticular term (Suppl. material 1: Table S1). It was created by using the “analyze” tool on the HAO website. Newly
detected muscles, not listed in the HAO so far or found in other literature, were also named in the HAO-scheme by the areas of origin and insertion with additional topographical orientation, if required (Table 2). The abbreviations used for the designation of muscles and sclerite structures are composed of the basic terms as follows:

Region of origin and insertion:

- **3ax2** third axillary sclerite of fore wing
- **3ax3** third axillary sclerite of hind wing
- **ba** basalar
- **cx** coxa
- **fu** furca
- **ism** intersegmental membrane
- **occ** occlusor
- **pe** pectus
- **ph** pteral
- **pl** pleuron
- **S** sternum
- **s** thoracic sternum
- **sa** subalar
- **sp** spiracle
- **T1** first abdominal tergite/propodeum
- **T2** second abdominal tergite
- **tr** trochanter

**Divided thorax:**

- **1** located on the prothorax
- **2** located on the mesothorax
- **3** located on the metathorax

**Positions:**

- **a** anterior
- **d** dorsal
- **l** lateral
- **m** medial
- **p** posterior
- **v** ventral

Order; mostly stated for functional groups of muscles:

- **a or 1** first
- **b or 2** second
- **c or 3** third

Descriptions, that involve the meso- and metafurca, are based on the terminology of Porto et al. (2016). The descriptions in the results were ordered by the point of insertion from mesosoma towards metasoma and by relevant functional groups, if possible (Table 2). In this comparative work, *Sceliphron destillatorium* serves as reference species (Fig. 2). In addition, a homologisation with the generalised nomenclature for the thoracic musculature of Neoptera following Friedrich and Beutel (2008) is presented in Table 2.

**Data availability**

The large image data sets accomplished for this study are available online as a data publication in conjunction with this paper. Thus, our images and raw data are freely accessible via the MfN data repository (Willsch 2019; https://doi.org/10.7479/dft0-yy6m). Moreover, images will be available on the HAO portal (http://portal.hymao.org).

**Results**

We found 42 muscle pairs within the analysed tagmata of the three species (Table 2). There are 37 muscles in *Sceliphron* (mesothorax 18, metathorax 14, first and second abdominal segments 5), 39 in *Ampulex* (mesothorax 19, metathorax 16, first and second abdominal segments 4), and 40 muscles in *Sphex* (mesothorax 20, metathorax 15, first and second abdominal segments 5). The following description of the skeletal musculature in *Sceliphron* serves as structural basis. Subsequently, comparative descriptions of differing muscles in *Sphex* and *Ampulex* are given. Each muscle absent in one or two of the compared species examined is mentioned below (see also Table 2):

**Sceliphron destillatorium** (Illiger, 1807)

**Mesothorax. Ventral mesofurco-profurcal muscle** (*pl2-fu1v*; Fig. 3A) arises ventromedially from the mesofurcal bridge, then runs horizontal and inserts ventrally on the base of the furcula. First **mesopleuro-mesobasalar muscle** (*ism1,2-ba2*; Fig. 3C) arises anterodorsally from the mesopleuron, fuses with ism1,2-ba2, and inserts on the mesobasalar. This vertical, fan-shaped muscle is situated proximally of the intersegmental membrane anteromedially to ism1,2-ba2, runs obliquely, and inserts posteriorly on the anterior thoracic spiracle. Externally, the spiracle is covered by the pronotal lobe. **Intersegmental membrane-mesobasalar muscle** (*ism1,2-ba2*; Fig. 3C) arises from both the intersegmental membrane between the pronotum and mesoscutus, and from the mesopleuron, and inserts on the mesobasalar after fusing with *pl2-ba2*. First **mesopleuro-third axillary sclerite of fore wing muscle** (*pl2-3ax2a*; Fig. 3D) arises anterodorsally from the mesopleuron and inserts on the third axillary sclerite of the fore wing; it is short and fan-shaped. **Second mesopleuro-third axillary sclerite of fore wing muscle** (*pl2-3ax2b*; Fig. 3D) arises anterolaterally from the mesopleuron. This vertical, fan-shaped muscle is situated ventral to *pl2-3ax2a* and inserts on the third axillary sclerite of the fore wing. **Third mesopleuro-third axillary sclerite of fore wing muscle** (*pl2-3ax2c*; Fig. 3D) arises...
Figure 2. Volume rendering of the mesosomal exoskeleton of *Sceliphron destillatorium*, anterior to the left. A. Dorsal surface view; B. Lateral surface view; C. Ventral surface view. Abbreviations: N1 – pronotal lobe, N3 – metanotum, cx1 – procoxa, cx2 – mesocoxa, cx3 – metacoxa, pl2 – mesopleuron, pl3 – metapleuron, tr2 – mesotrochanter, S1 – prosternum, S2 – mesosternum, S3 – metasternum, scl2 – mesoscutellum, T1 – propodeum, tg – tegula. Scale bars: 0.9 mm (A, B), 1 mm (C).

Figure 3. *Sceliphron destillatorium*, volume rendering, mesosomal musculature, A–C: medial view, anterior to the right, D: lateral view, anterior to the left. A. Muscles discernible from the centre; B. Muscles positioned sublateral; C. Muscles located sublateral and lateral; D. Laterally positioned muscles. Abbreviations: fu2-fu1v – ventral mesofurco-profurcal; pl2-t2a – first mesopleuro-mesonotal; pl2-ba2 – mesopleuro-mesobasalar; sp1occ – anterior thoracic spiracle occlusor; ism1,2-ba2 – intersegmental membrane-mesobasalar; pl2-3ax2a – first mesopleuro-third axillary sclerite of fore wing; pl2-3ax2b – second mesopleuro-third axillary sclerite of fore wing; pl2-3ax2c – third mesopleuro-third axillary sclerite of fore wing; pl2-t2b – second mesopleuro-mesonotal; cx2-sa2 – mesocoxo-mesobasalar; fu2a-ph2 – anterior mesofurco-mesolathroprophragmal; pl2a-fu2 – anterior mesopleuro-mesofurcal; pl2-cx2 – mesopleuro-mesocoxal; s2-cx2 – mesosterno-mesocoxal; fu2-cx2 – mesofurco-mesocoxal; fu2l-tr2 – lateral mesofurco-mesotrochanteral; fu2m-tr2 – median mesofurco-mesotrochanteral; ph1-ph2 – prophragmo-mesophragmal; pl3a-ba3 – anterior metapleuro-metasubalar; t2p-t3 – posterior mesonoto-metanotal; pl3a-t3 – anterolateral metapleuro-metanotal; pl3d-3ax3 – dorsal metapleuro-third axillary sclerite of hind wing; pl3-sa3 – metapleuro-metatasubalar; cx3-sa3 – metacoxo-metatasubalar; pl3m-cx3 – median metapleuro-metacoxal; fu3l-cx3 – lateral metapleuro-metacoxal; fu3m-cx3 – median metapleuro-metacoxal; pl3l-cx3 – lateral metapleuro-metacoxal; fu3-tr3 – metapleuro-metatrochanteral; pl3-tr3 – metapleuro-metatrochanteral; ph2m-ph3 – median mesofurco-metaphragmal; ph3-T2 – metaphragmo-second abdominal tergal; T1-S2 – propodeo-second abdominal sternal; fu3-S2 – metapleuro-second abdominal sternal; s3-S2 – metasterno-second abdominal sternal. Scale bars: 0.8 mm (A–C), 0.9 mm (D).
Table 2. Terminology of the thoracic and abdominal musculature of all specimens examined. Origination and insertion are described on the basis of *Sceliphron*. If a muscle is absent in *Sceliphron*, the description refers to *Sphex* or *Ampulex*, respectively, if absent in *Sphex*. The list is sorted caudad (from thorax towards abdomen) by insertions of the muscles and by assumed functional groups. X = muscle present; - = muscle absent; ? = uncertain homology. A homologisation with the generalised nomenclature for neopteran thoracic muscles of Friedrich and Beutel (2008) is presented.

| Abbreviation | Name of muscle | Origin | Insertion | *Sceliphron* distillatorium | *Sphex* lucae | *Ampulex* compressa | Neoptera terminology |
|--------------|---------------|--------|-----------|----------------------------|---------------|-------------------|-------------------|
| Mesothorax   |               |        |           |                            |               |                   |                   |
| fi2-fu1v     | ventral mesofurco-profurcal | mesofurcal bridge | profurca | X | X | X | ? | lv1lm7 |
| pi2-t2a      | first mesopleuro-mesonotal | mesopleuru | mesoscutum | X | X | X | ldmv1 |
| pi2-ba2      | mesopleuro-mesobasalar | mesopleuron | mesobasalar | X | X | X | lispn1 |
| sp1occ       | anterior thoracic spiracle occlusor | intersegmental membrane | anterior thoracic spiracle | X | X | - |
| ism1,2-ba2   | intersegmental membrane-mesobasalar | intersegmental membrane, mesopleuron | mesobasalar | X | X | X | lppm2 |
| pi2-3ax2a    | first mesopleuro-third axillary sclerite of fore wing | mesopleuron | third axillary sclerite of fore wing | X | X | X | lppm7 |
| pi2-3ax2b    | second mesopleuro-third axillary sclerite of fore wing | mesopleuron | third axillary sclerite of fore wing | X | X | X | lppm9 |
| pi2-3ax2c    | third mesopleuro-third axillary sclerite of fore wing | mesopleuron | third axillary sclerite of fore wing | X | X | X | lppm9 |
| pi2-t2b      | second mesopleuro-mesonotal | mesopleuron | lateral axillar area of mesonotum | X | X | X | lppm5 |
| cx2-sa2      | mesocoixo-mesosubalar | mesocoixo | mesosubalar | X | X | X | ldmv6 |
| fi2a-ph2     | anterior mesofurco-mesolateral | mesofurcal arm | mesolaterophragma | X | X | X | ldmv8 |
| sp3occ       | posterior thoracic spiracle occlusor | mesofurcal arm | posterior thoracic spiracle | X | X | - |  |
| pi2a-fi2     | anterior mesopleuro-mesofurcal | mesopleuron, mesopimental ridge | mesofurcal arm | X | X | X | lispn2 |
| pi2-cx2      | mesopleuro-mesocoxal | mesopleuron | mesocoxal (anterolateral) | X | X | X | lpcm4 |
| pi2-cx2b*    | second mesopleuro-mesocoxal | mesopleure, mesopleural spiral apode | mesocoxa (dorsolateral) | - | X | X² | lpcm4? |
| s2-cx2       | mesosterno-mesocoxal | mesodiscrimenal lamella, mesopectus | mesocoxa (anterolateral) | X | X | X | lscm3 |
| fu2-cx2      | mesofurco-mesocoxal | mesodiscrimenal lamella | mesocoxa (anteromedial) | X | X | X | lscm2 |
| fu2-tr2      | lateral mesofurco-mesotrochanteral | mesopleuron, mesofurcal arm | mesotrochanteral apode (lateral) | X | X | X | lscm6 |
| fu2m-tr2     | median mesofurco-mesotrochanteral | mesopleuron, mesofurcal arm | mesotrochanteral apode (lateral) | X | X | X³ | lscm6 |
| ph1-ph2      | prophragmo-mesosphragmal | prophragma | mesophragma | X | X | X | ldlm1 |

Number of mesothoracic muscles (max. 20): 18 20 19

Metathorax

| Abbreviation | Name of muscle | Origin | Insertion | *Sceliphron* distillatorium | *Sphex* lucae | *Ampulex* compressa | Neoptera terminology |
|--------------|---------------|--------|-----------|----------------------------|---------------|-------------------|-------------------|
| pi3a-ba2     | anterior metapleuro-metabasalar | metapleuron, paracoxal ridge | metabasalar | X | X | X² | llspn1 |
| l2p-t3       | posterior mesonoto-metanotal | mesoscutellum | mesophragmal spine in metanotum | X | X | X² | ldlm3 |
| pi3la-t3     | anterolateral metapleuro-metanotal | metapleural apode, metafurcal arm | metanotal apode | X | X | X² | ltlpm5 |
| pi3lp-t3     | posterolateral metapleuro-metanotal | metapleural apode, metafurcal arm | metanotal apode | X | X | X² | ltlpm5 |
| pi3v-3ax3    | ventril metapleuro-third axillary sclerite of hind wing | metapleuron, mesopimental ridge | third axillary sclerite of hind wing | X | X | X² | ltlpm9 |
| pi3d-3ax3    | dorsal metapleuro-third axillary sclerite of hind wing | mesopimental ridge | third axillary sclerite of hind wing | X | X | X | ltlpm7 |
| pi3-sa3      | metapleuro-metasubalar | metapleuron, metapleural apode | metasubalar | X | X | X² | lltpm11 |
| cx3-sa3      | metacoxo-metasubalar | metacoxa (sublateral) | metasubalar | X | X | X | ldlm6 |
| pc3-fu3*     | lateral metapecto-metafurcal | metapectus | paracoxal ridge | - | - | X | - |
| fu3-cx3*     | metafurco-metacoxal | mesofurcal arm | metacoxa (medial) | - | - | X | llscm3? |
laterally from the mesopleuron, positioned farther ventrally and posterior to pl2-3ax2b, and inserts on the third axillary sclerite of the fore wing. It is the most extended and fan-shaped of the three fore wing muscles. Second mesopleuro-mesotrochanteral muscle (pl2-t2; Fig. 3C) arises, somewhat dorsal to pl2-3ax2c, from the mesopleuron, is fan-shaped and inserts on the ventral surface of the lateral axillary area of the mesonotum. Mesocoxo-mesosubalar muscle (cx2-sa2; Fig. 3D) arises from the mesocoxal apophysis, which corresponds with the cuticular pit and the paracoxal ridge. This muscle is slim and elongated and inserts on the mesosubalar. Anterior mesofurco-mesotrochanteral muscle (fu2a-ph2; Fig. 3B) arises from the anterior dorsal surface of the mesofurcal arm and inserts on the mesolateral margin. This horizontal, beam-shaped muscle is the largest in all species examined. Mesofurco-mesocoxal muscle (pl2-cx2; Fig. 3A) arises from the prophragma and inserts on the mesocoxa. Second mesopleuro-mesocoxal muscle (pl2-cx2b) is absent. Mesosterno-mesocoxal muscle (s2-cx2; Fig. 3B) arises mainly from the mesodiscriminal lamella and partly from the mesepimeron; it is located ventrally of pl2-cx2 and inserts anterolaterally on the mesocoxa. Metathorax. Anterior metapleuro-metabasalar muscle (pl3a-ba3; Fig. 3C) arises from both the meta-

| Abbreviation | Name of muscle | Origin | Insertion | Scoliephon destillatorium X | X | X | Neoptera terminology |
|--------------|---------------|--------|-----------|---------------------------|---|---|---------------------|
| pl3m-cx3     | median metapleuro-metacoxal | metapleurus, mesodiscriminal lamella | metacoxa (ventrolateral) | X | X | X | IIIscm1 |
| fu3l-cx3     | lateral metafurco-metacoxal | paracoxal ridge, mesodiscriminal lamella | metacoxa (lateral) | X | X | X\(\d\) | IIIscm2 |
| fu3m-cx3     | median metafurco-metacoxal | metafurca, mesodiscriminal lamella | metacoxa (medial) | X | X | X\(\d\) | IIIscm2 |
| s3-cx3\*     | metasterno-metacoxal | mesodiscriminal lamella | metacoxa (medial) | – | X | – | IIIscm1? |
| pl3l-cx3     | lateral metapleuro-metacoxal | metapleuron, paracoxal ridge | metacoxa (dorsolateral) | X | X | X | IIIpcm4 |
| fu3-t3       | metafurco-metatrochanteral | metatropical arm | metatrochanteral apodeme (central) | X | X | X | IIIpcm6 |
| pl3-t3       | metapleuro-metatrochanteral | metapleuron, metatropical apodeme | metatrochanteral apodeme (central) | X? | X? | X? | IIIpcm6 |
|              | Number of metathoracic muscles (max. 17): | | | 14 | 15 | 16 | |
| First and second abdominal segment | | | | | | | |
| ph2m-ph3     | median mesosphragmo-metaphragmal | mesospargma | median process | X | X | – | IIIdm1 |
| ph3-t2       | mesosphragmo-second abdominal tergal | propodeum | second abdominal sternite | X | X | X | – |
| T1-S2        | propodeo-second abdominal sternite | propodeum | second abdominal sternite (lateral) | X | X | X | – |
| fu3-S2       | metafurco-second abdominal sternite | metafurcal arm | second abdominal sternite (ventro-submedial) | X | X | X\(\d\) | IIIvlm2 |
| s3-S2        | metasterno-second abdominal sternite | mesodiscriminal lamella, metasternum | second abdominal sternite (lateral) | X | X | X\(\d\) | – |
|              | Number of first and second abdominal segment muscles (max. 5): | | | 5 | 5 | 4 | |
| Total number of muscles (max. 42): | | | | 37 | 40 | 39 | |

\* = newly identified; d = difference in structure or position, amplified in chapter Results

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pleuron and from the anterior surface of the paracoxal ridge and inserts on the metabasalare. This longitudinal, lateral muscle extends between the mesopleural and paracoxal ridge. **Posterior mesonoto-metanotal muscle** (t2p-t3; Fig. 3C) arises from the mesoscutellum and inserts laterally on a spine-shaped apodeme, which is located dorsally on the mesoscutum at the transition of the meso- and metascutellum; it is fan-like. **Anterolateral metapleuro-metanotal muscle** (pl3la-t3; Fig. 3B) arises anterolaterally from the metapleural apodeme and metatropical arm and inserts laterally on the metanotal apodeme. It is located dorsally and is fan-like. Adjacent muscles are fu3-S2 and fu3-tr3, which arise posterior to the metatropical arm. **Posteriorlateral metapleuro-metanotal muscle** (pl3lp-t3; Fig. 3D) arises from the metapleuron and inserts on the metanotum by fusing with pl3la-t3, which lies ventral to the small pl3lp-t3. **Ventral metapleuro-third axillary sclerite of hind wing muscle** (pl3v-3ax3; Fig. 3D) arises from the posterior surface of the mesepimeral ridge and the metapleuron. The muscle is located lateral to pl3d-3ax3 and fuses with it, then both insert on the third axillary sclerite of the hind wing. **Dorsal metapleuro-third axillary sclerite of hind wing muscle** (pl3d-3ax3; Fig. 3C) arises dorso-submedial of pl3v-3ax3 from the posterior surface of the mesepimeral ridge, fuses with pl3v-3ax3 along half its length, and inserts on the third axillary sclerite of the hind wing; it is small and compact. **Metapleuro-metasubalar muscle** (pl3-sa3; Fig. 3C) arises from the metapleuron and partly from the metapleural apodeme and inserts on the metasubalar, ventral to the hind wing. **Metacoxo-metasubalar muscle** (cx3-sa3; Fig. 3C) arises from the sublateral margin of the metacoxa and inserts on the metasubalar by fusing with pl3-sa3; it is long and slim. **Lateral metapecto-metapleural muscle** (pc3l-fu3) and **metanofuro-metacoxal muscle** (fu3-cx3) are absent. **Median metapleuro-metacoxal muscle** (pl3m-cx3; Fig. 3B) arises ventromedially from the metapostnotum and the metadiscal lamella, inserts ventrolaterally on the metacoxa. **Lateral metafurco-metacoxal muscle** (fu3l-cx3; Fig. 3C) arises sublaterally from the posterior surface of the paracoxal ridge and the metadiscal lamella and inserts laterally on the metacoxa. **Median metafurco-metacoxal muscle** (fu3m-cx3; Fig. 3A, B) arises posteroventromedially from both the metafurca and the metadiscal lamella and inserts medially on the metacoxa. **Metasterno-metacoxal muscle** (s3-cx3) is absent (see *Sphex*). **Lateral metafurco-metacoxal muscle** (pl3l-cx3; Fig. 3D) arises laterally from the metapleurax and posteriorly from the paracoxal ridge and inserts on the dorsal lateral margin of the metacoxa. The muscle is located anteriorly on the metapleural ridge. **Metafurco-metatrochanteral muscle** (fu3-tr3; Fig. 3C) arises posteriorly of the metatrochanteral arm, inserts centrally on the metatrochanteral apodeme by fusing with pl3-tr3. **Metapleuro-metatrochanteral muscle** (pl3-tr3; Fig. 3D) arises from the metapleuran and partly from the metapleural apodeme, then fuses with fu3-tr3, and inserts centrally on the metatrochanteral apode-}

**First and second abdominal segment. Median mesophragmo-metaphragmal muscle** (ph2m-ph3; Fig. 3A) arises posteroventromedially from the mesophragma and inserts anterior to the median process of the propodeum; it is short and square. **Metaphragmo-second abdominal tergal muscle** (ph3-T2; Fig. 3A–D) arises dorsolaterally from the propodeum, inserts dorsally on the second abdominal tergite; it is a large muscle. **Propodeo-second abdominal sternumal muscle** (T1-S2; Fig. 3B, D) arises dorsolaterally from the propodeum, right above pl3l-cx3 and laterally of ph3-T2; it is large and inserts on the lateral margin of the second abdominal sternite. **Metafurco-second abdominal sternumal muscle** (fu3-S2; Fig. 3B) arises posteriorly from the submedial metatropical arm, located dorsally of fu3-tr3, and inserts ventro-submedially on the second abdominal sternite; it is elongate and slightly fan-shaped. **Metasterno-second abdominal sternumal muscle** (s3-S2; Fig. 3A) arises from the metadiscional lamella and metasternum, inserts on the lateral margin of the second abdominal sternite, and is fan-shaped and bent.

**Sphex (Fernaldina) lucae de Saussure, 1867**

**Mesothorax. Posterior thoracic spiracle occlusor muscle** (sp3occ; Fig. 4A–D) arises medial on the mesepimeral ridge, anterior to the mesofurcal arm, inserts on the posterior thoracic spiracle (sp2), which additionally is surrounded by pl2-t2b (anterodorsal), pl2a-fu2 (posterodorsal), and pl2-cx2b (ventral). The small tracheal occlusor muscle sp3occ is located submedial of pl2a-fu2. **Second mesopleuro-mesocoxal muscle** (pl2-cx2b; first description; Fig. 4A, C, D) arises from the mesopleuron and partly from the mesopleural spiracle apodeme, fuses with pl2-cx2 and inserts dorsolaterally on the mesocoxa; it lies anterior to the mesepimeral ridge. **Lateral mesofuro-mesophragmal muscle** (fu2l-tr2; Fig. 4A, D) arises from the anterior surface of the mesofugal arm, runs lateral to fu2m-tr2 and fuses with the same, then both insert laterally on the mesotrochanteral apode-}

**Metathorax. Lateral metapleuto-metatropical muscle** (pc3l-fu3) and **metanofuro-metacoxal muscle** (fu3-cx3) are absent. **Metasterno-metacoxal muscle** (s3-cx3; first description; Fig. 4B) arises from the metadiscal lamella and inserts medially on the metacoxa.

**Ampulex compressa** (Fabricius, 1781)

**Mesothorax. Second mesopleuro-mesocoxal muscle** (pl2-cx2b; Fig. 5C, D, E, G, H) is thinner than in *Sphex*. It arises from the mesopleural spiracle apodeme, fuses with pl2-cx3 (Fig. 5A, C, D, G, H) and inserts dorsolaterally on the mesocoxa. **Lateral mesofuro-mesotrochanteral muscle** (fu2l-tr2) is absent. **Median mesofur-
Figure 4. The mesosomal musculature of *Sphex (Fernaldina) lucae* divergent to *S. destillatorium*; volume rendering, transparent exoskeleton. **A.** Lateral view, anterior to the left; **B.** Medial view, anterior to the right; **C.** Anterior view on the posterior thoracic spiracle occlusor; **D.** Dorsomedial view, anterior top right. Abbreviations: \textit{sp3occ} – posterior thoracic spiracle occlusor; \textit{sp2} – posterior spiracle; \textit{pl2-cx2} – mesopleuro-mesocoxal; \textit{pl2-cx2b} – second mesopleuro-mesocoxal; \textit{fu2l-tr2} – lateral mesofurco-mesotrochanteral; \textit{fu2m-tr2} – median mesofurco-mesotrochanteral; \textit{s3-cx3} – metasterno-metacoxal; \textit{pl3m-cx3} – median metapleuro metacoxal; \textit{fu3m-cx3} – lateral metafurco metacoxal; \textit{fu3l-cx3} – median metafurco metacoxal; \textit{pl3l-cx3} – lateral metafurco metacoxal; \textit{mepr} – mesepimeral ridge. Scale bars: 0.7 mm (A), 0.6 mm (B), 0.3 mm (C), 0.5 mm (D).

**co-mesotrochanteral muscle** (\textit{fu2m-tr2}; Fig. 5B, D, E) is larger than in Sphecidae, arises from the ventral surface of the mesofurcal arm, and inserts medially on the mesotrochanteral apodeme.

**Metathorax.** **Anterior metapleuro-metabasalar muscle** (\textit{pl3a-ba3}; Fig. 5C, E) arises from the metapleuron, posterior to the mesepimeral ridge, and inserts on the metabasalare. This muscle is shorter than in *Sceliphron*, as it originates farther up. The paracoxal ridge is not very distinct. **Posterior mesonoto-metanotal muscle** (\textit{t2p-t3}; Fig. 5A–D, F) arises from the upper sclerite of the mesoscutellum and inserts on the lower surface of the mesoscutellum; rectangular. There is no filament connecting it to another structure. **Anterolateral metapleuro-metanotal muscle** (\textit{pl3a-t3}; Fig. 5B, E, F) mainly arises anterolaterally from the metapleural arm (touching \textit{pl3-tr3} and partly \textit{fu3-tr3}, which originate on the posterior surface of the metafurcal arm) and partly from the metapleuron and inserts on the metanotum. **Posterolateral metapleuro-metanotal muscle** (\textit{pl3lp-t3}; Fig. 5C, D) arises from the metapleuron fuses with \textit{pl3la-t3}, which is covered dorsally by \textit{pl3lp-t3}, and inserts on the metanotum. It is larger than in *Sceliphron* and *Sphex* and fan-shaped. **Ventral metapleuro-third axillary sclerite of hind wing muscle** (\textit{pl3v-3ax3}; Fig. 5A, E) arises from the posterior surface of the mesepimeral ridge. This slim muscle is fused with \textit{pl3d-3ax3} and inserts on the third axillary sclerite of the hind wing. **Metapleuro-metasubalar muscle** (\textit{pl3sa3}; Fig. 5C, D) arises from the metapleuron at the posterior face of the mesepimeral ridge, and inserts on the metasubalare. **Lateral metapleco-metfurcal muscle** (\textit{pc3l-fu3}; first description; Fig. 5C, G, H) the slender muscle arises anterior to the metacoxa laterally from the metapectus, and inserts on the posterior surface of the paracoxal ridge. **Metafurco-metacoxal muscle** (\textit{fu3-cx3}; first description; Fig. 5C, G, H) arises medi"
Figure 5. The mesosomal musculature of *Ampulex compressa* divergent to Sphecidae; volume rendering, transparent exoskeleton. A–C anterior to the right; D–H anterior to the left. A. Medial view, all relevant muscles visible from the centre; B. Medial view on submedial muscles; C. Medial view, further lateral located muscles; D. All relevant muscles discernible from lateral view; E. Muscles located sublateral, lateral view; F. Muscles located further medial, lateral view; G. All newly identified muscles (plus pl2-cx2), lateral view; H. Dorsolateral view on all newly identified muscles (plus pl2-cx2). Abbreviations: sp1occ – anterior thoracic spiracle occlusor; ism1,2-ba2 – intersegmental membrane-mesobasalar; pl2-t2b – second mesopleuro-mesobasalar; pl2-cx2 – mesopleuro-mesocoxal; pl2-cx2b – second mesopleuro-mesocoxal; pl2-ex2b – second mesopleuro-mesocoxal; fu2-cx2 – mesofurco-mesocoxal; fu2m-tr2 – median mesofurco-mesotrochanteral; pl3a-ba3 – anterior metapleuro-metabasalar; t2p-t3 – posterior mesonoto-metanotal; pl3a-t3 – anterolateral metapleuro-metanotal; pl3lp-t3 – posterolateral metapleuro-metanotal; pl3v-3ax3 – ventral metapleuro-third axillary sclerite of hind wing; pl3-sa3 – metapleuro-metasubalar; pc3-fu3 – lateral metapecto-metafurcal; fu3-cx3 – metafurco-metacoxal; fu3l-cx3 – lateral metafurco-metacoxal; fu3m-cx3 – median metafurco-metacoxal; pl3l-cx3 – lateral metafurco-metacoxal; ph3-T2 – metathoraco-second abdominal tergal; T1-S2 – propodeo-second abdominal sternal; fu3-S2 – metafurco-second abdominal sternal; fu3m-cx3 – metafurco-metatrochanteral; fu3l-cx3 – lateral metafurco-metatrochanteral; fu3m-cx3 – metafurco-metatrochanteral; pl3-tr3 – metafurco-metatrochanteral; ph3-T2 – metathoraco-second abdominal tergal; T1-S2 – propodeo-second abdominal sternal; fu3-S2 – metafurco-second abdominal sternal; s3-S2 – metasterno-second abdominal sternal. Scale bars: 0.7 mm (A–C), 0.8 mm (D, E), 0.6 mm (F–H).
from the metafurcal arm, fuses with fu3l-cx3, and inserts medially on the metacoxa; it is slender and flattened. The median metafurco-metacoxal muscle (fu3m-cx3; Fig. 5A, F) arises posteromedially from the metafurca and from the metadiscrimenal lamella and inserts medially on the metacoxa. The lower metafurcal area runs further cranial and offers more posterior space filled by this muscle. Lateral metafurco-metacoxal muscle (fu3l-cx3; Fig. 5B, D) arises from the metapectus and inserts dorsolateral on the metacoxa. **Metasterno-metacoxal muscle** (s3-cx3) is absent. Metapleuro-metatrochanteral muscle (pl3-tr3; Fig. 5D, E) arises posteriorly from the metafurcal arm, which merges into a spiracle at that position. The muscle is positioned laterally of fu3-tr3, fuses with it and inserts on the metatrochanteral apodeme. **Metapleural apodeme** and **paracoxal ridge** weakly developed (Fig. 6C, D); **metapleural apodeme** fused with lateral metafurcal arms (Fig. 6D).

**Second abdominal segment.** Median mesophragmo-metaphragmal muscle (ph2m-ph3) is absent. The mesophragma in *Ampulex* is rectangular like the outer cuticle and lacks a posterior notch for the insertion of a muscle. **Metaphragmo-second abdominal tergal muscle** (ph3-T2; Fig. 5A–C, F) arises from the metaphragma and propodeum, inserts dorsally on the second abdominal tergite; broad, large muscle extended to the posterior region. **Metafurco-second abdominal sternal muscle** (fu3-S2; Fig. 5A) arises posteriorly from the metafurcal arm, positioned posteromedial to fu3-tr3, inserts anteromedially on the second abdominal sternite. In length and width distinctly more gracile than in *Sceliphron*. **Metasterno-second abdominal sternal muscle** (s3-S2; Fig. 5A) arises from the metadiscrimenal lamella and inserts on the anterolateral margin of the second abdominal sternite. It is noticeably smaller and neither fan-like nor bent, as in *Sceliphron*.

**Discussion**

The cladistic analyses by Vilhelmsen et al. (2010) inferred Crabronidae (*Pison*) as being the closest relative of Sphecidae (*Stangeella*) and Ampulicidae (*Ampulex*) and all three taxa constitute a monophyletic Apoidea. However, many anatomic structures of Ampulicidae and Sphecidae we studied differ significantly from each other, whereas
the two species within Sphecidae show many similarities. Especially, the metathoracic musculature varies remarkably between the families. The muscles that insert on the notum, coxae, and trochanters show distinct structural divergences. Furthermore, the number and origin of muscles varies, due to the less distinct metapleural apodeme and paraxial ridge in Ampulex (additional muscles inserting on the coxae in Ampulex: *pl2-cx2b*, *fu3-cx3*) in Sphex: *pl2-cx2b*, *s3-cx3*; absent muscle in Ampulex: *fu2l-tr2*; origin different in Ampulex: *t2p-t3*, *pl3l-t3*, *pl3-cr3* (Fig. 3–6; Table 2). In addition, some of the meso- and metacoxal muscles, as well as a mesotrochanteral and a metanotal muscle of *Ampulex* tend to be larger compared to Sphecidae (*pl2-cx2*, *fu2-cx2*, *fu2m-tr2*, *pl3p-t3*, *fu3-cx3*, *pl3l-cx3*). The *pl3l-cx3* is also larger in Sphex (Fig. 4A) compared to *Sceliphron* (Fig. 3D). Strong levators and depressors attaching on the coxae might be needed for backwards dragging of large prey and speaks for an adaptation to this conspicuous hunting behaviour (Williams 1942). On the contrary, *pl2-cx2b* in *Ampulex* (Fig. 5C, D, E, G, H) is narrower than in *Sphex* (Fig. 4A, C, D); *fu2l-tr2* in *Sphex* is smaller than in *Sceliphron* (Figs 3C, 4A, D). However, muscles supposedly involved in the movement of the notum, coxae, and trochanters should be checked carefully in subsequent studies.

**Mesothorax.** The mesopleural pit in *Sceliphron* presumably developed by muscle and spiracle reduction. According to Vilhelmsen et al. (2010), the occurrence of the mesopleural pit shows high variances within and amongst superfamilies. Spiracle reduction likely occurred independently in different groups. Snodgrass (1942), for instance, found the posterior thoracic spiracle in honeybee workers without a closing apparatus. Each of the other spiracles is equipped with an occlusor muscle (Snodgrass 1942). Vilhelmsen et al. (2010) documented the absence of the posterior thoracic spiracle in Stephanidae and Pteromalidae, while they evidenced its presence (without *sp3occ*) in the apoid family Crabronidae, as well as in Rhopalosomatidae (*Vespoidae*), and the non-aculeate families Cynipidae, Evanidae, and Trigonalidae. Hence, not only Apoidea but also Sphexiformes *sensu lato* bear a high variance of the development of this spiracle-muscle-complex. Duncan (1939) presented an illustration of the closing mechanism of the posterior thoracic spiracle in *Vespula*. The occlusor muscles we found in *Sphex* and *Ampulex* (Figs 4A–D, 5F–H) show wider attachment points than the fan-shaped muscle described in Duncan’s work. In the neopteran representatives, like *Zoroptypus*, examined by Friedrich and Beutel (2008; Table 2), *sp3occ* was not revealed. Concluding, other related specimens should be examined to exclude all doubts about the homologisation of the posterior thoracic spiracle and *sp3occ* and to gain further insights into the different formations.

In all species examined, *pl2-cx2* is located as described by the HAO, with origin on the mesoepulum and anterolateral insertion on the mesocoxa (Figs 3D, 4A, D, 5A, C, D, G, H). However, it is larger and extending farther anteriorly in *Ampulex* (Fig. 5A, C, D, G, H). *Ampulex* distinct-ly shows the additional and slender mesocoxal muscle *pl2-cx2b*, which we describe here for the first time (Fig. 5C–E, G, H). In *Sphex* it is broader and closely adjacent to *pl2-cx2* (Fig. 4A, C, D). It is absent in *Sceliphron*. Consequently, the development of *pl2-cx2b* should be examined in other species to clarify the phylogenetic relevance.

The muscles *fu2l-tr2* and *fu2m-tr2* in *Ampulex*, which insert on the mesotrochanter, seem to have been coalesced completely, making a separation impossible (compare Fig. 7A, B). Because of the insertion and the rather medial position, we reasonably homologized the structure with *fu2m-tr2* by excluding *fu2l-tr2* for *Ampulex*. The unambiguous identification of both muscles in Sphecidae appears to indicate an autopomorphic feature of Apoideae. However, Vilhelmsen et al. (2010; see also references therein) stated that both muscles were found in Evanidae, Platygastroidea, most Proctotrupoidea, *Plumarius*, and Apoidea, which might include all genera they examined (i.e., *Ampulex*, *Apis*, Bombus, *Pison*, *Stangeella*). However, the authors noted the absence of *fu2l-tr2* in *Orthogonalys* (Trigonaloidea) and of *fu2m-tr2* in Ceraphroidea, Chalcidoidea, and Stephanoidea. Nevertheless, they explained that a secondary subdivision of *fu2m-tr2* may have led to the development of *fu2l-tr2*. In summary, the contrariness refering to *fu2l-tr2* needs to be clarified by additional studies on *Ampulex*, in particular.

In addition, *fu2l-tr2* fills the mesopleural area in *Sceliphron* (Fig. 3C), whereas this muscle is smaller in *Sphex* (Fig. 4A, D). In contrast, *pl2-cx2b* extends over the mesopleural region in *Sphex* and *Ampulex* (Figs 4A, C, D, 5C–E, G, H). In *Ampulex*, the origin of this muscle is the same spiracle apodeme as that from which *sp3occ* arises (Fig. 5E–H); in *Sphex* it partly originates from the posterior thoracic spiracle and partly from the mesopleuron (Fig. 4A, C, D). However, we recommend a closer look at these different formations in other species before drawing phylogenetic conclusions.

**Metathorax.** The different constructions of the metathoracic muscles mainly depend on variations of the skeletal structures. The slight difference in the metapleural origin of *pl3a-ba3* in *Ampulex* (Fig. 5C, E) is a consequence of the less distinct development of the paraxial ridge (Fig. 6). As shown by Vilhelmsen et al. (2010), the paraxial ridge is weakly developed in Ampulicidae and non-apocritan Hymenoptera, whereas it is highly variable within apocritan groups. *Orthogonalys* (Trigonaloidea), which serves as reference species in the paper of Vilhelmsen et al. (2010), has a weakly developed paraxial ridge, except for the ventralmost part. As no other information about the structure in *Pison* (Crabronidae) is available, it should be identical. We confirm the differences noted by Vilhelmsen et al. (2010), as the paraxial ridge is weakly developed in Ampulicidae and well-marked in Sphecidae (Fig. 6). Additionally, Vilhelmsen et al. (2010) described a distinct paraxial ridge in Chrysidoidae, Evanioidea, and Stephanoidea.

The muscle *t2p-t3* inserts laterally on a spine, which is located dorsoally on the mesoepulum in Sphecidae (Fig. 6).
Figure 7. Comparison of fu2m-tr2 – median mesofurcro-mesotrochanteral muscle and fu2l-tr2 – lateral mesofurcro-mesotrochanteral muscle, anterolateral view. A. Sceliphron destillatorium; B. Ampulex compressa. Scale bars: 0.4 mm (A), 0.5 mm (B).

Figure 8. Comparison of t2p-t3 – posterior mesonoto-metanotal muscle, posteromedial view. A. Sceliphron destillatorium; B. Ampulex compressa. Scale bars: 0.2 mm.

Vilhelmsen et al. (2010) revealed in Apoidea and Vespoidea a typical lateral insertion on the metanotum, which is not yet observed in other groups; this might indicate that this feature is synapomorphic in both superfamilies. Although we found the mesoscutellum to be of similar shape in all analysed species, t2p-t3 in Ampulex is instead located entirely between the upper and lower mesoscutellar sclerite (Fig. 8B). So far, this modification seems to be unique. To verify this, further representatives of Ampulicidae should be examined.

The metanotal muscle pl3la-t3 in Ampulex differs from that in Sphecidae because of the weakly developed metapleural apodeme, which leads to a rather more lateral than submedial position on the thorax (Fig. 5B, F). We found a fusion of the lateral metafurcal arms with the metapleural apodeme in Ampulex (Fig. 6D), as already observed by Vilhelmsen et al. (2010) in the same species, other apoid taxa (Stangeella, Apis, Bombus, Pison), and in Vespoidea. Vilhelmsen et al. (2010) stated that most apocritan Hymenoptera have a metapleural apodeme that is often fused with the lateral metafurcal arms. In non-apocritan Hymenoptera, the metapleural apodeme shows high morphological diversity. In many cases, this may not be easy to recognize (Vilhelmsen et al. 2010). Studies on more species from both families are necessary to determine if the structures found in the present study are family-specific. Sphecidae has a well-developed metapleural apodeme, similar to Cynipoidea (Vilhelmsen et al. 2010), which is an important characteristic. Our results corroborate the conclusion by Vilhelmsen et al. (2010), that the development of the metapleural apodeme is highly variable within Apocrita and, moreover, even within Apoidea.

Additionally, the weakly developed metapleural apodeme in Ampulex influenced the origin of pl3-sa3, which only originates from the metapleuron and inserts on the metasubalar (Figs 5C, D, 9B). The origin of the metatrochanteral muscle pl3-tr3 is also affected in Ampulex (Figs 5D, E, 6C, D). This muscle originates from a delicate sclerite, which provides a narrow surface of origin. This sclerite arose from the fusion of the metafurcal arm and metapleural apodeme and is equal to the medial margin of the metapleural apodeme and metafurcal arm.
Figure 9. Comparison of pl3-sa3 – metapleuro-metasubalar muscle, dorsolateral view, anterior to the left. A. Sceliphron destillatorium; B. Ampulex compressa. Further abbreviations: mpa – mesopleural apodeme; mtpa – metapleural apodeme; pl3 – metapleuron; sa3 – metasubalar; sp2 – posterior thoracic spiracle. Scale bars: 0.2 mm (A), 0.4 mm (B).

Figure 10. Illustration of the metaphragma (ph3) in the propodeum (T1) of Ampulex compressa. A. Medial view, anterior to the right; B. Anteromedial view on ph3-T2 – metaphragmo-second abdominal tergal muscle; C. Anteromedial view on ph3; D. Posterior view on the vertical part of propodeum. Scale bars: 0.6 mm (A), 0.3 mm (B, C), 0.9 mm (D).

The homology of the metanotal muscle, which we tentatively assign to pl3lp-t3, according to the HAO terminology, cannot be assured. In the HAO, it is described as fan-shaped and posterolaterally originating from the metapleuron. However, size, structure, and position of pl3lp-t3 are different among the species examined (Figs 3D, 5C, D). In Ampulex, pl3lp-t3 shows great similarity to the description of it by the HAO (wide, fan-shaped, and arises laterally from the metapleuron), whereas in Sphecidae, pl3lp-t3 is very small and compact but still fan-shaped and located sublaterally. It appears to originate from the metanotum and to insert on the metapleu-
The metaphragma we observed in Sphex (Fig. 4B). It is located ventrally to the first abdominal segment and might serve to strengthen the metanotum function from the lower centre of the body. From Sphex cereus and Sphex cereus cereus, the metaphragma was found to be absent in honeybees by Snodgrass (1942). The HA0 describes the metaphragma as the site of origin of the mesophragmo-metaphragmal and metaphragmo-second abdominal tergal muscles. Although the third phragma was found to be absent in honeybees by Snodgrass (1942), it is absent in bees and other taxa. Nevertheless, ph3m-ph3 (Fig. 3A) and ph3m-T2 (Fig. 3A–D) in Sphecidae are homologue muscles. The metaphragma is usually located between the metanotum and the first abdominal segment (Snodgrass 1942). The HAO describes the metaphragma as the site of origin of the mesophragmo-metaphragmal and metaphragmo-second abdominal tergal muscles. Although the third phragma was found to be absent in honeybees by Snodgrass (1942), we cannot confirm this specific character of Sphex, but not of the family Sphecidae.

First and second abdominal segment. The metaphragma is conspicuously absent in Sphecidae among all studied taxa. Nevertheless, ph2m-ph3 (Fig. 3A) and ph3-T2 (Fig. 3A–D) in Sphecidae are homologue muscles. The metaphragma is usually located between the metanotum and the first abdominal segment (Snodgrass 1942). The HAO describes the metaphragma as the site of origin of the mesophragmo-metaphragmal and metaphragmo-second abdominal tergal muscles. Although the third phragma was found to be absent in honeybees by Snodgrass (1942), we cannot confirm this specific character of Sphex, but not of the family Sphecidae.

Conclusions

We recommend additional investigations of the structures and features presented in this paper. It would be of great value to analyse the tagmata and other characteristics in the family Heterogynaidae and additional species of Crabronidae, Ampulicidae, and Sphecidae. Due to the unresolved phylogenetic position of Heterogynaidae and the paraphyly of the Crabronidae, the study of more species from these taxa might be desirable. Structural investigations of more species of Vespoidea and Chrysidoidea would be helpful for clarifying controversial assumptions about phylogenetic relationships within Aculeata. Structures of phylogenetic significance were mainly found in the metathorax, i.e., the metapleural apodeme, paracoxal ridge, metaphragma, and the origin and insertion of associated muscles. Future studies should also focus on: the muscles that insert into the legs, the posterior thoracic spiracle as well as the occlusor muscle in closely related species, and the four muscles described here for the first time in Sphecidae and Ampulicidae.

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References

Aguiar AP, Deans AR, Engel MS, Forshage M, Huber JT, Jennings JT, Johnson NF, Lelej AS, Longino JT, Lohrmann V, Miki L, Ohl M, Rasmussen C, Taeger A, Sikk Ki JU D (2013) Order Hymenoptera. In: Zhang Z-Q (Ed.) Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness (addenda 2013). ZooKeys 3703: 31–82. https://doi.org/10.11646/zootaxa.3703.1.12

Alexander BA (1992) A cladistic analysis of the subfamily Philanthinae (Hymenoptera: Sphecidae). Systematic Entomology 17(2): 91–108. https://doi.org/10.1111/j.1365-3113.1992.tb00324.x.

Bohart RM, Menke AS (1976) Sphecid wasps of the world. A generic revision. University of California Press, Berkeley, Los Angeles, London, 695 pp. https://archive.org/details/bub_gb_FExMjuRhjPLC/page/n27

Branstetter MG, Danforth BN, Pitts JP, Faircloth BC, Ward PS, Bunting ML, Gates MW, Kula RR, Brady SG (2017) Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. Current Biology 27(7): 1019–1025. https://doi.org/10.1016/j.cub.2017.03.027

Brothers DJ, Carpenter JM (1993) Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera). Journal of Hymenoptera Research 2(1): 227–304.

Debevec AH, Cardinal S, Danforth BN (2012) Identifying the sister group to the bees: a molecular phylogeny of Aculeata with an emphasis on the superfamil Apoidea. Zoologica Scripta 41(5): 527–535. https://doi.org/10.1111/j.1463-6409.2012.00549.x.

Duncan CD (1939) A contribution to the biology of North American vespine wasps. Stanford University Publication, University Series Biology and Science 8: 1–272.

Faulwetter S, Vasileiadou A, Kouratoras M, Daillianis T, Arvanitidis C (2013) Micro-computed tomography: Introducing new dimensions to taxonomy. ZooKeys 263: 1–45. https://doi.org/10.3897/zookays.263.4261

Fouda K, Libersat F, Rathmayer W (1994) The venom of the cockroach-hunting wasp Ampulex compressa changes motor thresholds: a novel tool for studying the neural control of arousal. Zoology 98: 23–34.

Friedrich F, Beutel RG (2008) The thorax of Zorotypus (Hexapoda, Zoraptera) and a new nomenclature for the musculature of Neoptera. Arthropod Structure & Development 37: 29–54. https://doi.org/10.1016/j.asd.2007.04.003

dez.pensoft.net
Friedrich F, Beutel RG (2010) Goodbye Halteria? The thoracic morphology of Endopterygota (Insecta) and its phylogenetic implications. Cladistics 26(6): 579–612. https://doi.org/10.1111/j.1096-0031.2010.00305.x

Garcia FH, Fischer G, Liu C, Audioso TL, Economo EP (2017) Next-generation morphological character discovery and evaluation: an X-ray micro-CT enhanced revision of the ant genus Zasphinctus Wheeler (Hymenoptera, Formicidae, Dorylinae) in the Afrotropics. ZooKeys 693: 33–93. https://doi.org/10.3897/zookeys.693.13012

Gignac PM, Kley NJ, Clarke JA, Colbert MW, Morhardt AC, Cerio D, Garcia FH, Fischer G, Liu C, Audioso TL, Economo EP (2017) Goodbye Halteria? The thoracic morphology of the mesosoma of Halteria (Hymenoptera: Formicidae). Cladistics 33(4): 666–669. https://doi.org/10.1111/clo.12449

Haspel G, Libersat F (2003) Wasp venom blocks central cholinergic synapses to induce transient paralysis in the cockroach prey. Developmental Neurobiology 54(4): 628–637. https://doi.org/10.1002/neu.10195

Heraty JM (1989) Morphology of the mesosoma of Kapula (Hymenoptera: Eucharitidae) with emphasis on its phylogenetic implications. Canadian Journal of Zoology 67(1): 115–125. https://doi.org/10.1139/z89-018

Johnson BR, Borowicz ML, Chiu JC, Lee EK, Atallah J, Ward PS (2013) Phylogenomics resolves evolutionary relationships among ants, bees, and wasps. Current Biology 23: 2058–2062. https://doi.org/10.1016/j.cub.2013.08.050

Kawada R, Lanes GO, Azevedo CO (2015) Evolution of Metapos thorax in Flat Wasps (Hymenoptera, Bethylidae): Implications for Homology Assessments in Chrysidoidea. PLoS ONE 10(10): e0140051. https://doi.org/10.1371/journal.pone.0140051

Königsmann E (1978) Das phylogenetische System der Hymenoptera. Teil 4: Auculeata (Unterordnung Apocrita). Deutsche Entomologische Zeitschrift, Neue Folge 25: 365–435. https://doi.org/10.1002/mmnd.19780250408

Liberstat F (2003) Wasp venom cocktail to manipulate the behavior of its cockroach prey. Journal of Comparative Physiology A 189(7): 497–508. https://doi.org/10.1007/s00359-003-0432-0

Liu S-P, Richter A, Stoeassel A, Beutel RG (2019) The mesosomal anatomy of Myrmecia nigriceps workers and evolutionary transformations in Formicidae (Hymenoptera). Arthropod Systematics & Phylogeny 77(1): 1–19. https://doi.org/10.26049/ASP77-1-2019-01

Lohrmann V, Ohl M, Spahn P (2010) A cladistic analysis of the cockroach wasps of its cockroach prey. Journal of Comparative Physiology A 189(7): 2058–2062. https://doi.org/10.1002/jpc.21308

Metscher BD (2009) MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. BMC Physiology 9: 11. https://doi.org/10.1186/1472-6793-9-11

Mikó I, Vilhelmsen L, Johnson NF, Masner L, Pénzes Z (2007) Skeletomusculature of Scelionidae (Hymenoptera: Platygastroidea): head and mesosoma. Zootaxa 1571: 1–78. https://doi.org/10.11646/zootaxa.1571.1.1

N’Ceill KM (2001) Solitary wasps: natural history and behavior. Cornell University Press, Ithaca, NY, 406 pp.

Ohl M, Bleidorn C (2006) The phylogenetic position of the enigmatic wasp family Heterogynaidae based on molecular data, with description of a new, nocturnal species (Hymenoptera: Apoidea). Systematic Entomology 31(2): 321–337. https://doi.org/10.1111/j.1365-3113.2005.00313.x

Ohl M, Engel MS (2007) Die Fossilgeschichte der Bienen und ihrer nächsten Verwandten (Hymenoptera: Apoidea). Denisia 20: 687–700.

Ohl M, Spahn P (2010) A cladistic analysis of the cockroach wasps based on morphological data (Hymenoptera: Ampulicidae). Cladistics 26: 49–61. https://doi.org/10.1111/j.1096-0031.2009.00275.x

Peters RS, Meyer B, Krogmann L, Borner J, Meusemann K, Schütte K, Niehuis O, Misof B (2011) The taming of an impossible child: a standardized all-in approach to the phylogeny of Hymenoptera using public database sequences. BMC Biology 9: 55. https://doi.org/10.1186/1741-7007-9-55

Peters RS, Krogmann L, Mayer C, Donath A, Gunzel S, Meusemann K, Kozlov A, Podsidiadowski L, Petersen M, Lanef L, Diez PA, Heraty J, Kjer KM, Klopfstein S, Meier R, Polidori C, Schmitt T, Liu S, Zhou X, Wappler T, Rust J, Misof B, Niehuis O (2017) Evolutionary history of the Hymenoptera. Current Biology 27(7): 1013–1018. https://doi.org/10.1016/j.cub.2017.01.027

Porto DS, Almeida EAB, Vilhelmsen L (2016) Comparative morphological analysis of internal structures of the mesosoma of bees with an emphasis on the corbiculate clade (Apidae: Apini). Zoological Journal of the Linnean Society 179: 303–337. https://doi.org/10.1111/zoj.12466

Prentice MA (1998) The comparative morphology and phylogeny of the apoid wasps (Hymenoptera: Apoidea). Ph.D. Dissertation, University of California, Berkeley, 1439 pp.

Puławski WJ (2020) Catalog of Sphecidae (see “Number of Species” via https://www.calacademy.org/scientists/projects/catalog-of-sphicidae)

Rasnitsyn AP (1988) An Outline of Evolution of the Hymenopterous Insects (Order Vespida). Oriental Insects 22: 115–145. https://doi.org/10.1007/BF00351698.1988.11835485

Ronquist F, Rasnitsyn AP, Roy A, Eriksson K, Lindgren M (1999) Phylogeny of the Hymenoptera: A cladistic reanalysis of Rasnitsyn’s (1988) data. Zoologica Scripta 28: 13–50. https://doi.org/10.1046/j.1463-6409.1999.00023.x

Sann M, Niehuis O, Peters DS, Almeida EAB, Vilhelmsen L (2016) Comparative morphological analysis of the apoid wasps (Hymenoptera: Apoidea). Ph.D. Dissertation, University of California, Berkeley, 1439 pp.

Schmitt JD (2016) The sting of the wild. Johns Hopkins University Press, Ithaca, NY, 406 pp.

Seltenmann KC, Yoder MJ, Mikó I, Forshage M, Bertone MA, Agosti D, Austin AD, Balhoff JP, Borowicz ML, Brady SG, Broad GR, Brothers DJ, Burks RA, Buffington ML, Campbell HM, Dew KJ, Ernst AF, Fernández-Triana JL, Gates MW, Gibson GAP, Jennings JT, Johnson NF, Karlsson D, Kawada R, Krogmann L, Kula RR, Mullins...
PL, Ohl M, Rasmussen C, Ronquist F, Schulmeister S, Sharkey MJ, Talamas E, Tucker E, Vilhelmsen L, Ward PS, Wharton RA, Deans AR (2012) A hymenopterists’ guide to the Hymenoptera anatomy ontology: utility, clarification, and future directions. Journal of Hymenoptera Research 27: 67–88. https://doi.org/10.3897/jhr.27.2961

Sharkey MJ, Carpenter JM, Vilhelmsen L, Heraty J, Liljeblad J, Dowling APG, Schulmeister S, Murray D, Deans AR, Ronquist F, Krogmann L, Wheeler WC (2012) Phylogenetic relationships among superfamilies of Hymenoptera. Cladistics 28(1): 80–112. https://doi.org/10.1111/j.1096-0031.2011.00366.x

Snodgrass RE (1942) The skeletal-muscular mechanisms of the honey bee. Smithsonian Miscellaneous Collections 103(2): 1–120. http://hdl.handle.net/10088/22771

Vilhelmsen L, Mikó I, Krogmann L (2010) Beyond the wasp-waist: structural diversity and phylogenetic significance of the mesosoma in apocritan wasps (Insecta: Hymenoptera). Zoological Journal of the Linnean Society 159(1): 22–294. https://doi.org/10.1111/j.1096-3642.2009.00576.x

Williams FX (1942) *Amphulex compressa* (Fabr.), A cockroach-hunting wasp introduced from New Caledonia into Hawaii. Proceedings of the Hawaiian Entomological Society 11(2): 221–233. http://hdl.handle.net/10125/16067

Willsch M (2019) Micro-computed tomography scanning of the mesosomal musculature in Apoidea (aculeate Hymenoptera) – segmented structures and raw data. [Dataset]. Data Publisher: Museum für Naturkunde Berlin (MfN) – Leibniz Institute for Research on Evolution and Biodiversity. https://doi.org/10.7479/dff10-yy6m

Yoder MJ, Mikó I, Seltmann KC, Bertone MA, Deans AR (2010) A Gross Anatomy Ontology for Hymenoptera, PLOS ONE 5(12): e15991. https://doi.org/10.1371/journal.pone.0015991

Zimmermann D, Vilhelmsen L (2016) The sister group of Aculeata (Hymenoptera) – evidence from internal head anatomy, with emphasis on the tentorium. Arthropod Systematics & Phylogeny 74(2): 195–218. https://curis.ku.dk/portal/files/171386094/05_asp_74_2_zimmermann_195_218.pdf

**Supplementary material 1**

**Table S1**

| Authors                      | Data type   | Explanation note                                                                 |
|------------------------------|-------------|-----------------------------------------------------------------------------------|
| Maraike Willsch, Frank Friedrich, Daniel Baum, Ivo Jurisch, Michael Ohl | Excel file  | Provision of Universal Resource Identifiers (URIs) referring to structures mentioned in the paper. Automatic creation by using the Hymenoptera Anatomy Ontology "analyzer" (http://api.hymao.org/projects/32/public/ontology/analyze). |

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