Comparative morphology of the musculature of the sting apparatus in *Ampulex compressa* (Hymenoptera, Ampulicidae) and *Sceliphron destillatorium* (Hymenoptera, Sphecidae)

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Abstract

The sting apparatus of aculeate Hymenoptera is derived from the ovipositor and is their most prominent apomorphy. In contrast to the frequently analysed sclerites of the sting apparatus, the associated musculature has largely been neglected. In this study, we use micro-computed tomography to present a detailed description of the musculature of the sting apparatus of *Ampulex compressa* (Ampulicidae) and *Sceliphron destillatorium* (Sphecidae). We found that 12 of 15 muscles corresponding to the sting apparatus are homologous between both species examined and 13 muscles in comparison with Hymenoptera described in the literature. All muscles identified as critical for the act of stinging were found in both species. Moreover, we found the ventral tergum 8-tergum 9 muscle and the tergum 8-tergum 8 muscles in *A. compressa* and the second valvifer-second valvifer muscle in *S. destillatorium*. For the first time, we describe the ventral tergum 8-tergum 9 muscle and the second valvifer-second valvifer muscle that interconnects both body sides, in Hymenoptera.

Key Words

abdomen, Aculeata, anatomy, microCT, stinger

Introduction

With over 150,000 described species, Hymenoptera is one of the largest insect orders (Aguiar et al. 2013). Approximately 70,000 of the species belong to the subclade Aculeata (Grimaldi and Engel 2005). The name-giving apomorphic character of the Aculeata (aculeatus: Latin for sharp, spiny) is the sting apparatus that evolved from the ovipositor (Smith 1970, Sharkey et al. 2012). The monophyletic Aculeata are divided into three superfamilies: Apoidea, Chrysidioidea, and Vespioidea (Aguiar et al. 1992, Branstetter et al. 2017). Apoidea comprises the monophyletic Anthophila (bees) and the paraphyletic “apoid wasps”, Sphecidae in the broad sense, or “digger wasps” (Ohl and Engel 2007, Branstetter et al. 2017).

Currently, about 10,000 species of apoid wasps are known (Pulawski 2020). Historically, they are divided into Crabronidae, Ampulicidae, Sphecidae, and Heterogynaidae (Branstetter et al. 2017). Recent studies suggest Ammoplanina to be the sister-group of Anthophila (Sann et al. 2018). While Sphecidae and Ampulicidae are thought to be well supported clades (Ohl and Spahn 2010, Peters et al. 2017, Sann et al. 2018), the monophyly of Crabronidae (Ohl and Bleidorn 2006, Lohrmann et al. 2008, Ohl and Spahn 2010, Peters et al. 2017) is questioned in several studies (e.g. Lohrmann et al. 2008, Sann et al. 2018). Heterogynaidae are found nested within Bembicini by Sann et al. (2018), while Branstetter et al. (2017) suggested Heterogynaidae to be the sister-group to a paraphyletic grouping of Crabroninae and Sphecidae. Furthermore, Sann et al. (2018) suggested Ammoplatinidae, Psenidae, Pemphredonidae, Philantidae, Bembicidae, Crabronidae, Mellinidae, Sphecidae, Astatidae and Ampulicidae to be monophyletic taxa.
Morphological studies are important sources for phylogenetic analyses (e.g. Ohl and Spahn 2010, Vilhelmsen et al. 2010, Da Silva et al. 2014, Zimmermann and Vilhelmsen 2016, Liu et al. 2019, Willsch et al. 2020, Barboza et al. 2021). Much has been written about the sclerites of the sting apparatus of Aculeata (e.g. Snodgrass 1933, Trojan 1935, Rietschel 1937, Kugler 1978, Hermann and Chao 2002, Packer 2003), including apoid wasps (Matushkina 2011, Mosel 2014, Gadallah and Assery 2015, Matushkina and Stetsun 2016). Apart from older studies (Snodgrass 1933, Trojan 1935, Rietschel 1937), examination of the musculature of the sting apparatus has been largely neglected. More recently, Kumpanenko and Gladun (2018) published a study on the musculature and sclerites of the sting apparatus of Cryptocheilus versicolor (Pompilidae). Barbosa et al. (2021) published the latest work on the sting apparatus of Chrysidoidea, which is the most basal taxon of Aculeata. Both recent publications of Kumpanenko and Gladun (2018) and Barbosa et al. (2021) do not describe the musculature connecting the sting apparatus to the cuticle, though.

We present a comparative description of the musculature of two representatives of apoid wasps: Ampulex compressa (Fabricius, 1781) (Amphulicidae) and Sceliphron destillatorium (Illiger, 1807) (Sphecidae). Aposid wasps use their stinger primarily as a tool for hunting prey for the offspring (Grimaldi and Engel 2005). The adult female is usually a solitary, vegetarian flower visitor, while the larva feed on parasitized arthropods deposited in the nest (Grimaldi and Engel 2005, Ohl and Engel 2007). S. destillatorium builds a mud nest and provides multiple cells with parasylid spiders (Fateryga and Kobylyuk 2014). The spiders are carried with the mandibles (Evans 1962). After provisioning, the cells are sealed with mud and either additional cells or a new nest is constructed (Bohart and Menke 1976, Fateryga and Kovblyuk 2014). A. compressa preys upon cockroaches and its unusual stinging behaviour is well documented (Haspel et al. 2003, Libersat 2003, Catania 2018): The adult female grasps the lateral margin of the cockroach’s pronotum, then bends her metasoma downwards to sting the cockroach for paralyzing. Two stings accurately hit the ganglia of the head and thorax. The venom inhibits the escape behaviour of the cockroach while the locomotion remains unaffected (Liberas 2003). This allows A. compressa to lead her prey into the nest by grasping one antenna. A single egg is laid on the cockroach before the wasp finally seals the nest entrance with substrate (Bohart and Menke 1976, Haspel et al. 2003, Libersat 2003, Catania 2018).

The aim of this study is to provide detailed morphological descriptions of the sting apparatus in order to contribute to the understanding of morphological adaptations and the phylogeny of the paraphyletic apoid wasps. A. compressa and S. destillatorium where chosen as otherwise well-studied species within the apoid wasps (see above), with a high number of plesiomorphic characters (Ohl and Spahn 2010) and a basal position within the Apoidea (Branstetter et al. 2017, Sann et al. 2018). In addition to evaluating the phylogenetic significance of morphological characters, this study discusses functional aspects of the sting apparatus in apoid wasps.

Methods

Specimens examined

We compared the musculature of the sting apparatus of the females of A. compressa (Amphulicidae) and S. destillatorium (Sphecidae). Muscles directly connected to the sting apparatus were described. The specimens belong to the Hymenoptera collection of the Museum für Naturkunde Berlin (MfN). In addition to the µCT scans of one specimen per species, dissections of four additional specimens of A. compressa and three additional specimens of S. destillatorium were conducted to confirm the spatial position and shape of the sclerites and muscles. See Suppl. material 1: Table S1 for a detailed list of specimens and their collection/rearing data.

3D imaging

The specimens used for 3D imaging were stored in 96% ethanol. They were stained with 25% iodine in pure ethanol (100%), to improve contrast in µCT scans (Metscher 2009, Gignac et al. 2016, Willsch et al. 2020). Before staining, the extremities and the anterior half of the thorax were removed to avoid obstructions during the scan and to ensure a uniform staining result. After three days of staining, the specimens were washed in 100% ethanol. They were stained with 25% iodine in pure ethanol (100%), to improve contrast in µCT scans (Metscher 2009, Gignac et al. 2016, Willsch et al. 2020). Before staining, the extremities and the anterior half of the thorax were removed to avoid obstructions during the scan and to ensure a uniform staining result. After three days of staining, the specimens were washed in 100% ethanol for 30 seconds to remove excess iodine. To prevent fissures and ripped muscles, the specimens were dried carefully using a critical point dryer (Leica EM CPD300).

The specimens were imaged using a µCT (Phoenix nanotom X-ray s) at 50 kV and 150 µA. 1440 images per scan were taken, each with an exposure time of 1 second. For S. destillatorium, a resolution of 4 µm/pixel was achieved. The resolution for A. compressa was set at 3.6 µm/pixel. The size of the specimens required a multi-scan consisting of three single scans. The stacking and the cone beam reconstruction was accomplished with the software PHOENIX X-RAY DATOS | X 2.0 (GE Sensing & Inspection Technologies GmbH). We manually segmented the musculature and sclerites using AMIRA ZIB Edition 6.4.0 and former versions (provided by the Zuse Institute Berlin). Only the left body side was segmented, based on the bilateral symmetry.

Dissections

The dissected specimens were preserved in 96% or 70% ethanol. The dissections were conducted under a Leica S8APO Binocular with forceps, scissors and preparation needles. The gaster was opened by destructive removal.
of the fourth abdominal segment. Consecutive terga and sternae could easily be removed as a whole with forceps. Only the seventh tergite and sternite had to be removed with great care, to not rip the muscles of T8. Once the sting apparatus was exposed, muscles and sclerites were removed one by one from the outside, or scissors were used to cut a sclerite with all muscles loose from the rest of the sting apparatus.

The illustrations were drawn in Adobe Illustrator CS5 and labelled as well as resized in Photoshop CS5. The remaining sclerites are stored in separate tubes with the specimens in the alcohol collection of the MN.

Terminology

Based on Vilhelmsen (2000a, b), Vilhelmsen et al. (2001) and the Hymenoptera Anatomy Ontology (HAO, Yoder et al. 2010, http://portal.hymao.org/projects/32/public/ontology/), muscle terminology is composed of the points of origin and insertion. The fan-like part of a muscle is the origin, whereas the tendon is located at the insertion. If possible, the insertion and origin of muscles already described in the literature were adopted to ensure comparability, e.g. when the muscle has tendons on both ends. To differentiate muscles with origin or insertion on the same sclerite, suffixes (for abbreviations see below) are used (summed up in Mikó et al. 2007). Sclerite terminology is based on the HAO and Vilhelmsen (2001). The names of muscles were adopted from the HAO if possible; new names were assigned according to the HAO conventions, if necessary. Comparisons to illustrations by Kumpanenko and Gladun (2018, fig. 6c) as well as Matushkina (2011, fig. 1A) confirm that the scanned S. destillatorium was preserved with an extended stinger. To maintain comparability between both specimens, the following descriptions of muscle insertions and origins assume the positions of the sclerites with retracted stinger in S. destillatorium. The abbreviations used are the following:

Sclerites:

1vf first valvifer;
2vf second valvifer;
(1–3) vv first to third valvula, respectively;
fu furcula;
fr first ramus;
2r second ramus;
T8 tergum 8;
T9 tergum 9;
T7 abdominal tergum 7;
S7 abdominal sternum 7;
sp spiracle;
spa sensillar patch.

Suffixes:

d dorsal;
v ventral;
l lateral;
m medial;
a anterior;
p posterior.

Results

The metasomal apex

In A. compressa, the metasoma is laterally compressed and acuminate. Both the ventral and dorsal metasomal margins are bent towards the tip in a nearly identical way. The dorsal margin of the metasomal apex in S. destillatorium is straight and the ventral metasomal margin bends up towards the tip with the first, second and third valvulae. The stinger is noticeably farther extended in our scan of S. destillatorium, compared to A. compressa (Fig. 1).

Sclerites

Dissections under the binocular confirm that the sting apparatus of both species consists of the same skeletal structures with similar spatial relation as in previously studied Hymenoptera: T8 and T9, a pair of first and second valvifers with their respective first and second rami, the furcula, the first and two second valvulae forming the sting and a pair of third valvulae (Rietschel 1937, Kugler 1978, Matushkina 2011, Da Silva et al. 2014, Mosel 2014, Kumpanenko and Gladun 2018) (Fig. 1). T8 of A. compressa is arch-shaped and nearly rectangular in lateral view. The arch is sclerotised anteriorly and consists of a round, membranous area posteriorly; left and right half of T8, delimiting the membranous area, do not connect posteriorly (Fig. 1A). In contrast, the sclerotised parts of T8 of S. destillatorium show a complex, question mark-like shape in lateral view, the sclerotised dorsal arch is thin. A membranous area, limited posteriorly by a lightly sclerotised arch, gives T8 of S. destillatorium an outline similar to T8 of A. compressa (Fig. 1B, the posterior arch is indicated by a single yellow outline). In both species T8 forms a lateral lamella ventrally, which muscles attach to. The T9 of S. destillatorium shows a heavily sclerotised, dorsal anal arch with anterdorsal apodems on each side that connects the oval halves of the sclerite (Fig. 1B). In A. compressa the anal arch is thin and positioned on the anterior edge of T9, thereby not overarching the second valvifer. The second valvifers bear strong interspecific differences (Figs 1, 5). In A. compressa the second valvifer is roughly triangular with the dorsal and ventral edge connecting posteriorly without a conspicuous posterior edge. In S. destillatorium the second valvifer is approximately trapezoidal, with an anterior edge between dorsal and ventral edge. The third valvula is not connected to the posterior edge. The articular process of the second valvifer is very pronounced and elongate.
Both species possess sensillar patches on the second valvifer (Vilhelmsen 2001c, Matushkina 2011), ventral to the first valvifer-second valvifer articulation. The first valvifer are of comparable form, with a convex and heavily sclerotised posterior edge between the dorsal articulation to T9 and the ventral articulation to the second valvifer. It is longer, stretched anteriorly, in *S. destillatorium* Mosel (2014) provides detailed descriptions and illustrations of the sclerites of *A. compressa*.

The position of the sclerites differs among the specimens in our scans (Fig. 1D). In *S. destillatorium*, the second valvifer, T9, and the first valvifer are rotated roughly 90° posteroventrally in relation to the stinger and the T8. The second valvifer and T9 maintain their relative position to each other. The first valvifer, articulating to the T9, is located ventrally in *S. destillatorium* and dorsally in *A. compressa*. The third valvula of *S. destillatorium* is bent ventrally in a sharp angle from the second valvifer and the stinger is extended far beyond the apex of the metasoma (Fig. 1C, D).

**Ampulex compressa**

Abdominal tergum and abdominal sternum 7 to tergum 8 (Fig. 2A)

**Dorsal tergum 7-tergum 8 muscle (dT7-T8)** arises anteriorly on the apodeme of T7 and inserts ventrally on the sclerotised arch between both halves of T8. **Ventral tergum 7-tergum 8 muscle (vT7-T8)** arises on the apodeme of T7 and inserts on the anterior edge of the T8, ventral of dT7-T8. The muscle is not bipartite in *A. compressa*. **Posterior tergum 7-tergum 8 muscle (pT7-T8)** arises anterodorsally on T7 and inserts close to the anterior edge of T8, dorsal of the lateral lamella, where T8 is most sclerotised. **Sternum 7-tergum 8 muscle (S7-T8)** arises anteriorly on the apodeme of S7 and inserts anteroventral on the medial flank of the lateral lamella of T8.

**Muscles**

We found 15 muscles (Table 1) that interconnect the sclerites of the sting apparatus or connect the sting apparatus as a whole to the cuticle. Fourteen of them appear in *A. compressa* and 13 in *S. destillatorium*. There are 12 muscles homologous between both species. In the following, the musculature of *A. compressa* is described in detail. Additional and differing muscles of *S. destillatorium* are specified subsequently.
Table 1. Muscles with origin and insertion. 1 = muscle present, 0 = muscle absent.

| Abbr. | Name                                      | Origin                                      | Insertion                                      | Ampulex compressa | Sceliphron destillatorium |
|-------|-------------------------------------------|---------------------------------------------|------------------------------------------------|-------------------|---------------------------|
|       |                                            |                                             |                                                |                   |                           |
|       |                                            |                                              |                                                | 1                 | 1                         |
|       |                                            |                                              |                                                | 1                 | 1                         |
|       |                                            |                                              |                                                |                   |                           |
|       |                                            |                                              |                                                |                   |                           |
|       |                                            |                                              |                                                |                   |                           |
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|       |                                            |                                              |                                                |                   |                           |
|       |                                            |                                              |                                                |                   |                           |

Figure 2. The musculature connecting T8 to the cuticula. Lateral view, anterior to the right, line drawing from dissections. A. Ampulex compressa; B. Sceliphron destillatorium. dT7-T8 – dorsal tergum 7-tergum 8 muscle; vT7-T8 (a & b) – ventral tergum 7-tergum 8 muscle (portion a and b respectively); pT7-T8 – posterior tergum 7-tergum 8 muscle; S7-T8 – sternum 7-tergum 8 muscle.

**Tergum 8 (Fig. 3A)**

**Tergum 8-tergum 8 muscle (T8-T8)** arises on the medial flank near the anterior edge of T8 and inserts on the ventral medial flank of T8 posteroventrally on the sclerotised rim of the spiracle.

**Tergum 8 to tergum 9 and first valvifer (Fig. 3A)**

**Dorsal tergum 8-tergum 9 muscle (dT8-T9)** arises on the anterodorsal edge of T8 and inserts laterally on the posterior edge of T9, dorsal of the anal arch (Fig. 3D). **Lateral tergum 8-tergum 9 muscle (dT8-T9)** arises dorsally, along the connection of both halves of T8 on the medial flank, dorsal of dT8-T9. It inserts centrally on the lateral flank of T9. The **dT8-T9** and **dT8-T9** arise close together and run in a roughly perpendicular angle from each other. The **Ventral tergum 8-tergum 9 muscle (vT8-T9)** arises anteroventrally on the medial flank of T8...
and inserts centrally on the lateral flank of T9, near the posteroventral edge. The muscle is small and rod-like.

Tergum 9 to second valvifer (Fig. 4A)

**Dorsal tergum 9-second valvifer muscle a (dT9-2vf a)** arises from the lateral flank of T9, dorsal of the anal arc. The muscle inserts at the posterior edge of the articular process of the second valvifer. **Dorsal tergum 9-second valvifer muscle b (dT9-2vf b)** arises posterodorsally from the medial flank of T9 anterior of the anal arch. It inserts on the posterior edge of the articular process of the second valvifer, ventral to dT9-2vf a. The anal arch of T9 is not heavily sclerotised, thus dT9-2vf a and b arise on the flank of the sclerite. They run parallel and only differ in their origin on the medial or lateral flank of T9. **Ventral tergum 9-second valvifer muscle (vT9-2vf)** arises from the medial flank of T9 along the anterior and ventral edge, inserts laterally along the very short posterior edge of the second valvifer. **Posterior tergum 9-second valvifer muscle (pT9-2vf)** arises anterodorsally on the anal arch and inserts posteriorly on the dorsal edge of the second valvifer, close to m2vf-fu and vT9-2vf.

Second valvifer to furcula (Fig. 5A)

**Lateral second valvifer-furcula muscle (l2vf-fu)** arises anterodorsally on the medial flank of the second valvifer, runs lateral of m2vf-fu and inserts on the dorsal arm of the furcula. **Medial second valvifer-furcula muscle (m2vf-fu)** arises posteroventrally on the second valvifer, close to m2vf-fu and vT9-2vf, runs medial of l2vf-fu and inserts on the lateral arm of the furcula.

**Sceliphron destillatorium**

Abdominal tergum and abdominal sternum 7 to tergum 8 (Fig. 2B)

**Dorsal tergum 7-tergum 8 muscle (dT7-T8)** arises on the anterior apodeme of T7 and inserts dorsally on the sclerotised arch between both halves of T8. **Ventral tergum 7-tergum 8 muscle a (vT7-T8 a)** arises on the apodeme of T7 and inserts dorsal on the anterior edge of T8, ventral of dT7-T8. **Posterior tergum 7-tergum 8 muscle (pT7-T8)** arises anterodorsally on T7 and inserts close to the ventral edge of T8, anterodorsal of the lateral lamella, close to the sclerotised edge. **Ventral tergum 7-tergum 8 muscle b (vT7-T8 b)** arises on the apodeme of T7 and inserts dorsally on the anterior edge of T8, ventral to the insert of vT7-T8 a.

Tergum 9 to second valvifer (Fig. 4B)

**Dorsal tergum 9-second valvifer muscle a (dT9-2vf a)** arises from the anterior edge of the anal arch, ventral of the apodeme. It inserts at the posterior edge of the articular process of the second valvifer, ventral to dT9-2vf a. **Posterior tergum 9-second valvifer muscle (pT9-2vf)** arises posteriorly on the ventral edge of the anal arch of tergum 9 and inserts posteriorly on the dorsal edge of the second valvifer, close to m2vf-fu and vT9-2vf.
Second valvifer to furcula (Fig. 5B)

Second valvifer-second valvifer muscle (2vf-2vf) arises on the dorsal edge of the medial flank of the second valvifer, ventral of the insertion of dT9-2vf, and inserts on the equivalent second valvifer of the opposite body side.

Discussion

The sclerites of the sting apparatus derived from abdominal terga and sterna (Snodgrass 1933, Rietschel 1937). Concealed cuticular segments formed the sclerites T8 and T9 of the sting apparatus. These sclerites are modifications of the respective abdominal tergum 8 and 9. Likewise, the abdominal segment 8 formed the first valvula, first valvifer and first ramus. The abdominal segment 9 formed the second valvifer, second ramus, as well as the second and third valvula (Snodgrass 1933, Trojan 1935, Rietschel 1937, Scudder 1961). All sclerites typically found in the sting apparatus (Matushkina and Stetsun 2016, Kumpanenko and Gladun 2018) were identified in both specimens (Fig. 1). The iodine staining was not intensive enough to distinguish the tiniest sclerotised structures. Therefore, we identified the rami and furcula in *A. compressa* only by dissection.

In comparison with dissected specimens, *S. destillatorium* shows a rotation and ventral shift of the stinger in the CT scan. Comparisons of the stinger and sclerites (Fig. 1B, D) to the illustrations of Kumpanenko and Gladun (2018, fig. 6c) as well as Matushkina (2011, fig. 1A) indicate that our specimen was preserved with a fully extended stinger. In relation to T8, T9 is rotated and shifted anteriorly. The posterior edge of the second valvifer is lifted and the resulting rotation pushes the stinger forward. This results in the sharp angle between second valvifer and third valvula (Fig. 1D). A high density of chemo- and mechanoreceptors on the posterior tip of the third valvula suggests that the posterior third remains on the stinger while being extended, presumably to guide the stinger (Matushkina 2011, Kumpanenko and Gladun 2018). While an extended stinger is of little consequence for the comparison of muscles by origin and insertion, it does allow for an insight into the process of stinging. Nevertheless, the muscles in *S. destillatorium* were described assuming the sclerite position with retracted stinger to maintain comparability.

All four muscles, the *dorsal tergum 7-tergum 8 muscle (dT7-T8)*, the *ventral tergum 7-tergum 8 muscle (vT7-T8)*, the *posterior tergum 7-tergum 8 muscle (pT7-T8)* and the *sternum 7-tergum 8 muscle (S7-T8)*, connecting T8 to T7 and S7, can be found in both examined species (Fig. 2). We propose dT7-T8 and S7-T8 to be homologous to Rietschels (1937) muscle 1 and muscle 8, respectively. He described a wide interspecific variety in sclerite and muscle morphology of T8 in *Vespa, Prosopis, Bombus* and *Apis mellifera* queen and worker. Despite
this high variability, dT7-T8 and S7-T8 were found in all specimens Rietschel (1937) described. The muscles have a similar position, origin and insertion (compare Fig. 2 to Rietschel 1937, figs 7, 9, 11). The S7-T8 is the only muscle in all described specimens connecting to a sternum. Rietschel (1937) found dT7-T8 to be bipartite in some species (e.g. of Bombus and Vespa), but undivided in others (e.g. of Apis queen). Neither S. destillatorium nor A. compressa show a division of dT7-T8. As the muscle is rod-like, or at least equally fan-shaped at both sides, we adopted origin and insertion described by Rietschel (1937) for the designation. In S. destillatorium vt7-T8 is bipartite (Fig. 2B). The insertion of vt7-T8 a and b in S. destillatorium and vt7-T8 in A. compressa on the anterior edge of the T8, as well as the origin ventral to dT7-T8 matches Rietschels (1937) description of his muscle 2. The fourth muscle, pt7-T8 (Fig. 2A), is homologue to muscle 3 of Rietschel (1937). We were not able to certainly identify this muscle in the T8, as it is the only muscle connecting the T8 to the cuticula that is directed posteriorly and might as such enhance the manoeuvrability of the T8, and thereby the sting apparatus as a whole. All four muscles (ventral, dorsal and posterior T7-T8 as well as S7-T8) are not described in the HAO.

Muscles described in the HAO and by Vilhelmsen et al. (2001, Orussidae), Vilhelmsen (2000c, basal Hymenoptera) and Ernst et al. (2013, Ceraphronoidea) were studied in species with an ovipositor. The evolution of the ovipositor into the sting apparatus is evident in the high number of homologous muscles explained below. The homologies between the sting apparatus of Chrysidioidea (Barbosa et al. 2021) and the species described herein can be tracked comparing both to the pompilid Cryptocheilus versicolor (Kumpanenko and Gladun 2018). Chrysidioidea is the sister group to the monophyletic taxon containing Pompilioidea and Apoidea, within which Pompilioidea occupies a relatively basal position.

The tergum 8-first valvifer muscle (T8-1vf) is the only muscle associated with the first valvifer (Fig. 3). It is therefore easy to homologize it with muscle 14 in Rietschel (1937), M4 in Kumpanenko and Gladun (2018) and with the muscle described on the HAO.

The HAO describes the dorsal tergum 8-tergum 9 muscle (dT8-T9) (Fig. 3) arising on the anteromedian margin of T8 and inserting on the anteromedian margin of T9. This description may be misleading, because the only way to connect T8 and T9 is, that dT8-T9 arises from T8, facing the centre of the body (medial), and inserts on the flank of T9, facing the cuticle (lateral). In our case, we described the muscle as originating medially on T8 and inserting laterally on T9. The homology of dT8-T9 to the described muscle can be deduced from their comparable situation and course (compare Fig. 3 to Ernst et al. 2013, figs 1B, 2A, D), as well as from their insertion.

As the anal arch in S. destillatorium is well pronounced, we can clearly identify the insertion of dT8-T9 on the anal arch (Fig. 3B). In A. compressa, with a thin and less sclerotised anal arch, dT8-T9 appears to insert on the posterodorsal edge of T9, dorsal of the anal arch (Fig. 3A). The lesser sclerotisation might not provide the necessary stability for strong muscles to arise from the anal arch. In return it may allow for the halves of T9 to be moved more independently, enhancing stinger manoeuvrability.

The dT8-T9 and the lateral tergum 8-tergum 9 muscles (iT8-T9) originate close together and run in roughly a perpendicular angle from each other. This muscle group, including T8-1vf, can be observed in both of our species (Fig. 3) as well as in those described by Ernst et al. (2013, figs 1B, 2A, D), Kumpanenko and Gladun (2018, fig. 5A, muscle 2 and 3) and Rietschel (1937, figs 9b, 10b, 11b, muscle 10 and 11).

The tergum 8-tergum 8 muscle (T8-T8) is the only one interconnecting T8 and was only found in A. compressa (Fig. 3A). However, Rietschel (1937) identified two muscles in every specimen illustrated (Bombus, Vespa, Prosopis, Apis mellifera queen and worker), which interconnect the T8. As T8-T8 arises on the ventral rim
of the spiral, it matches the description of Rietschel (1937) muscle 13. Muscle 12 is supposed to run across the spiral, which is not the case for T8-T9. Kumpankenko and Gladun (2018) neither described any muscles interconnecting the same female tergum, nor did they mention their absence. The HAO does not provide any information on such muscles.

Moreover, the ventral tergum 8-tergum 9 muscle (vT8-T9) is only present in A. compressa (Fig. 3B). So far, we did not find any information about this muscle in the literature. In contrast to the scanning result, the dissections of A. compressa could not verify the existence of vT8-T9, which might be due to its small size. On the scan it is clearly identifiable on both body sides.

The dorsal tergum 9-second valvifer muscle (dT9-2vf) a and b and the ventral tergum 9-second valvifer muscle (vT9-2vf) form a group of muscles consistently found in our specimens (Fig. 4). They were also described by Rietschel (1937, muscles 17a/b and 18) and Kumpankenko and Gladun (2018, M5 a/b and 6). Furthermore, this group was described for other specimens with an ovipositor by the HAO, based on Ernst et al. (2013, fig. 4A–F) and Vilhelmsen et al. (2001, fig. 9A). The dT9-2vf is always split into two portions which originate on T9 and insert on the articular process of the second valvifer. In S. distillatorium, portion a and b of dT9-2vf originate from the anal arch. In A. compressa they originate on the lateral and medial flank of T9 respectively. The vT9-2vf is supposed to be the antagonist of dT9-2vf; the alternating contractions of both muscles extend and retract the first valvula (Rietschel 1937, Vilhelmsen 2000c, Kumpankenko and Gladun 2018). This interplay allows non-aculeate Hymenoptera to drill their ovipositor into the substrate and subsequently transport the egg into the cavity (Vilhelmsen 2000c). In apoid wasps these muscles, through the alternate movement of the first valvulae, pump the venom down the stinger (Kumpankenko and Gladun 2018). In the described position and function, vT9-2vf is homologe to Rietschels (1937) muscle 18 and the muscle described in the HAO. Kumpankenko and Gladun (2018) designate their M6 as arising from T9 and inserting on the second valvifer, as described in other literature. Contrarily, their figures (Kumpankenko and Gladun 2018, fig. 5B) show the muscle to be fan-shaped at the connection to the 2vf. Based on the similar situation and expected function, the muscle is homologue to our vT9-2vf.

The posterior T9-second valvifer muscle (pT9-2vf) was found in both specimens and clearly is homologous to the one described by the HAO, Vilhelmsen (2000b), Ernst et al. (2013), and Kumpankenko and Gladun (2018, M7) (Fig. 4). Due to the weakly sclerotised anal arch in A. compressa, we expected pT9-2vf to arise posteriorly on the dorsal edge of T9, comparable to dorsal and ventral T9-2vf, which arise on the dorsal flank of the T9. Instead the origin of pT9-2vf is found posteroverental on the anal arch in A. compressa (Fig. 4A). We support the assumption of Kumpankenko and Gladun (2018, on the basis of M7), that the pT9-2vf is responsible for transferring the rotation of T9 to the second valvifer.

A muscle connecting the second ramus and the base of the stinger, second ramus-second valvula muscle (2r-2vv), might be missing completely in A. compressa and S. distillatorium. It would be homologues to M10 of Kumpankenko and Gladun (2018) and to muscle 20 of Rietschel (1937) where it was only attributed a supporting role in stinger retraction. Only in the µCT scan of S. distillatorium indications of such a muscle (Fig. 6A) could be found, but dissections suggest that the reconstructed volume (Fig. 6B) is made up of parts of the rami and surrounding soft tissue. No indication of 2r-2vv could be found in any dissected specimen.

The medial second valvifer-furcula muscle (m2vf-fu) and the lateral second valvifer-furcula muscle (l2vf-fu) (Fig. 5) connect the second valvifer to the furcula. Both muscles run very close together in A. compressa and were difficult to separate. Hermann and Chao (2002) described, in contrast to Snodgrass (1933), two muscles connecting to the furcula. This was also observed by Rietschel (1937), who described two parts of muscle 19. His description and illustrations of part A matches M9 of Kumpankenko and Gladun (2018) and m2vf-fu; part B was described as arising near the articular process. Our observations confirm l2vf-fu arising on the ventral edge of the 2vf as described by Kumpankenko and Gladun (2018). The terminology used by Hermann and Chao (2002) appointed m2vf-fu as depressor and l2vf-fu as rotator of the stinger. The HAO has not listed any muscles interconnecting the second valvifer and the furcula. The second valvifer-second valvula muscle (2vf-2vf) (Fig. 5B) interconnects the second valvifer of both body sides in S. distillatorium; it is one continuous, traverse muscle. No comparable muscle was found in A. compressa. Vilhelmsen (2000c) described a muscle, the 2vf-genital membrane muscle, arising dorsally on the first valvifer and inserting on the genital membrane, thus close to its counterpart from the other body side. However, we could neither identify a membrane in-between 2vf-2vf nor any tissue posterior or anterior to the muscle.

Additionally, we found two muscles connecting the sting apparatus to a membrane in both specimens. As they do not fulfil a function in stinger extension, we mention them only for completeness according to the region of interest. One muscle arises near the anteromedial edge of the T8, the second one arises postero-dorsally on the medial flank of T9 below the anal arch. The muscles insert on different round membranes, positioned centrally in between the sclerites of the sting apparatus. Most likely these are the gut and venom bladder. Possibly the one arising from the T9 is homologue to the T9-genital membrane muscle identified by Vilhelmsen (2000c). The presence of the muscles originating from T9 in A. compressa and the presence of the muscle originating from the T8 in S. destilla-
torium could be verified during dissections. The additional connections to the membranes might also be non-muscular tissue. Their presence would contradict the hypothesis of Barbosa et al. (2021), that the loss of muscles connecting to the genital membrane is an autapomorphy of Aculeata.

On the physiological act of stinging

Snodgrass (1933) and Rietschel (1937) showed that rising pressure in the metasoma is not responsible for the extension of the stinger. Based on Snodgrass (1933), Rietschel (1937) proposed m2vf-fu to be responsible for the extension. Abdominal sternum 7 supposedly works as a counter bearing to convert the downward movement of the stinger induced by m2vf-fu into a backward movement. Snodgrass (1933) remarked that the extension of the stinger is accompanied by a dorsal and anterior shift of the whole sting apparatus. But Rietschel (1937) entirely dismissed the proposition of Trojan (1935) that the extension of the stinger is caused by the musculature associated to T8 and T9. He discarded the explanation as too complex and the muscles as too weak to allow this movement. Kumpanenko and Gladun (2018) strongly suggested that the musculature of T8 and T9 indeed rotate T9 for extending the stinger. This rotation is supported by m2vf-fu, pushing the stinger farther posterior to pierce the integument of the prey (Kumpanenko and Gladun 2018).

Like Barbosa et al. (2021) in Chrysidoidea, we found several indications supporting the theory of Kumpanenko and Gladun (2018). The main evidence is the anterodorsal rotation of T9 and second valvifer. This rotation results in the ventral shift of the first valvifer, connecting the second valvifer and T9 (Fig. 1B, D). The DT8-T9 and T8-1vf most likely rotate T9, as described by Kumpanenko and Gladun (2018). It is difficult to assess whether vT8-T9, only present in A. compressa, supports the rotation of T9. Together with IT8-T9 it might as well act as additional antagonist to DT8-T9 and T8-1vf.

The rotation of T9 is transferred to the second valvifer via the elastic rami. Further rotation is provided by pT9-2vf, which was found in both specimens, opposed to Rietschel (1937), who did not find this muscle. The insertion of m2vf-fu in S. destillatorium with extended stinger (Fig. 1D) is found farther posterodorsal compared to the insertion in A. compressa (Fig. 1C), where it is located medial to l2vf-fu. This indicates that the furcula is rotated by m2vf-fu; the dorsal arm is now pulled posterodorsally, leveraging the force of l2vf-fu to maximally extend the stinger. This is in line with the anterior and posterior or second valvifer-second valvula muscles (Vilhelmsen 2000c), homologue to lateral and medial 2vf-fu muscles, serving as rotators to extend the first and second valvulae from in-between the ovipositor sheaths in non-aculeate Hymenoptera. The flexible rami act as antagonists of the lateral and dorsal 2vf-fu.

Conclusion

We provided descriptions and illustrations allowing for the comparison of the musculature of the sting apparatus in situ. We showed that this musculature is almost identical among A. compressa and S. destillatorium. The high number of identified homologies of our species with those described in other literature suggests that our collective understanding of the basal musculature continues to increase. See Suppl. material 2: Table S2 for a homologisation of the terminology used by different authors. As this is the first detailed study on the musculature of the sting apparatus in digger wasps, further research is needed to identify muscles of phylogenetic significance. Rietschel (1937) apparently dissected Ammophila and Cerceris, but did not specify which muscles beside S7-T8 he found. In addition to contemporary functional analyses of the sting apparatus, morphological studies should also deal with the musculature connecting to the cuticle, as it bears significant interspecific differences in the available literature (Snodgrass 1933, Rietschel 1937). Rietschels (1937) suggestion that the presence of dorsal, ventral and posterior 17-T8 is the plesiomorphic condition, seems to be supported by the presence of all three muscles in A. compressa and S. destillatorium.

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Supplementary material 1

Table S1. Detailed list of specimens and their collection/rearing data

Authors: Stefan Graf, Maraike Willsch, Michael Ohl
Data type: specimens, label data
Explanation note: Detailed list of specimens including MfN collection ID and all available label data.
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Link: https://doi.org/10.3897/dez.68.58217.suppl1

Supplementary material 2

Table S2. Overview of all muscles with HAO URIs and the proposed homologies

Authors: Stefan Graf, Maraike Willsch, Michael Ohl
Data type: morphological, homologies, URIs
Explanation note: All described muscles with additional HAO Universal Resource Identifiers (URI) and the proposed homologies with Rietschel (1937) and Kumpanenko and Gladun (2018).
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Link: https://doi.org/10.3897/dez.68.58217.suppl2