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Functional morphology and evolution of the sting sheaths in Aculeata (Hymenoptera)

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Abstract. The sting of the Aculeata or stinging wasps is a modified ovipositor; its function (killing or paralyzing prey, defense against predators) and the associated anatomical changes are apomorphic for Aculeata. The change in the purpose of the ovipositor/sting from being primarily an egg laying device to being primarily a weapon has resulted in modification of its handling that is supported by specific morphological adaptations. Here, we focus on the sheaths of the sting (3rd valvulae = gonoplacs) in Aculeata, which do not penetrate and envenom the prey but are responsible for cleaning the ovipositor proper and protecting it from damage, identification of the substrate for stinging, and, in some taxa, contain glands that produce alarm pheromones. The3rd valvulae may be divided into proximal and distal parts. No muscles insert on the 3rd valvulae, and in the process of stinging the movements of the 3rd valvulae are determined by the morphological features of the entire sting apparatus, e.g., the elastic cuticle between the 2nd valvifers and 3rd valvulae and also between the sclerites of the 3rd valvulae. The return of the 3rd valvulae to their resting position is facilitated by the presence of resilin-like proteins in these junctions. The structure and movements of the 3rd valvulae are discussed in the context of the sting function in various groups of Aculeata. The evolution of the 3rd valvulae is discussed; a secondary simplification of the 3rd valvulae structure is observed in representatives of Vespidae, Formicidae, Colletidae, Apidae, Melittidae.

Key words. Hymenoptera, phylogeny, resilin, sting apparatus, third valvulae.

1. Introduction

Aculeata is a monophyletic group that comprises, among others, Vespidae, Formicidae, Apoidea (including Anthophila), Chrysididae, Mutillidae and Pompilidae (Sharkey 2007). The sting is a slightly modified ovipositor; its function and the associated anatomical changes are considered apomorphic for Aculeata; it is secondarily lost by some members of this group, see below. Whereas other Hymenoptera (and other insects, which have an ovipositor) use their ovipositors primarily to lay eggs in various substrates, in Aculeata the sting functions exclusively as a weapon (Sharkey et al. 2012). Its main functions are killing or paralyzing prey as well as defense against predators (Macalintal & Starr 1996). The changes in the primary purpose of the ovipositor/sting have resulted in modification of its handling that is supported by specific morphological adaptations (Snodgrass 1910; Packer 2003). One consequence of the 'weaponisation' of the ovipositor in Aculeata is that, as a sting, it is comparatively short, i.e., less than the length of the abdomen/metasoma. In many parasitoid wasps the ovipositor length may be several times the length of the remaining body, in particular in those that target hosts concealed deep inside wood (Vilhelmsen 2003; Vilhelmsen & Turrisi 2011).

Like in other Hymenoptera the ovipositor apparatus of Aculeata consists of the unpaired tergum 8 and tergum 9 (subdivided in Aculeata), the paired 1st and 2nd valvifers (= gonangulum and 9th gonocoxite respectively), the 3rd valvulae (= gonoplacs), and the paired 1st and 2nd valvulae (= 8th and 9th gonapophyses) (Oeser 1961; Vilhelmsen 2000; alternative terminology by Klass 2003). The sting shaft or ovipositor proper, i.e., the parts which actually enter the substrate, is formed by the 1st and 2nd valvulae.

In all Aculeata with a well-developed sting, a furcula is present in its proximal region. The furcula is a forked

sclerite that ventrally has a strong but flexible dicondylic attachment to the anterior most region of the sting bulb, the venom reservoir (true 9th segmental accessory gland) opening medially at the ventral base of the 2nd valvulae (Oeser 1961; Hermann & Chao 1983). The presence of a furcula and the muscles that attach to it (arising from the 2nd valvifers) increases the speed, amplitude, and precision of the sting (Hermann & Chao 1983; Kumpanenko & Gladun 2017). The furcula is absent in "Symphyta" (Vilhelmsen 2000); among the non-aculeate Apocrita it is described only in *Microplitis croceipes* (Braconidae) (Hermann & Chao 1983). The presence of resilin-like proteins in the articulations of the sting apparatus probably also increases the efficiency of its movements (Hermann & Willer 1986; Kumpanenko & Gladun 2017).

For representatives of Aculeata which use the sting to paralyze prey (e.g., Pompilidae, some Sphecidae and Crabronidae), precision of stinging is very important; they have to deliver the venom close to the ganglia of the host through narrow areas of the arthrodial membrane (Rathmayer 1978). Precision is facilitated by mechanoreceptors located on the tip of the 3rd valvulae. Identification of the substrate for stinging/drilling is also a major function of the 3rd valvulae in other groups of parasitoid Hymenoptera (Le Ralec et al. 1996; Goubault et al. 2011). On the other hand, Hymenoptera that use the sting primarily for defense (e.g., stinging bees and ants) do not need to be nearly as precise when inserting their sting shaft in the target. The sting apparatus, including the 3rd valvulae, is mostly reduced in some subfamilies of ants, e.g., Formicinae (Hermann & Blum 1968) as well as in some representatives of Andrenidae, Apidae, and Megachilidae (Packer 2003). In addition to the receptor function, the 3rd valvulae are responsible for cleaning the sting shaft and protecting it from damage (Hermann & Blum 1968). They may also contain glands that produce an alarm pheromone (Cassier et al. 1994).

Information about the 3rd valvulae in representatives of various aculeate families is found scattered in papers devoted to the morphology of the sting apparatus (Hermann 1975; Kugler 1978; Vilhelmsen 2003; Packer 2003; Gadallah & Assery 2004; Matushkina 2011; da Silva et al. 2014; Matushkina & Stetsun 2016). There are several publications about receptors on the 3rd valvulae (Matushkina 2011; Gal et al. 2014; Matushkina & Stetsun 2016) and the glands in them (Cassier et al. 1994; Billen et al. 2013).

We are only aware of two detailed studies of the external morphology of the 3rd valvulae in Aculeata. Poore (1974) studied the valvulae in representatives of several aculeate families and identified three basic morphological types: the "constricted", the "two-segmented", i.e., subdivided, and the "unsegmented" (undivided). The subdivided type was further separated by Poore into three subtypes: with the basal sclerite (termed "segment" by Poore) twice the length of the second sclerite, with the second sclerites twice the length of the basal sclerite, and with the sclerites approximately equal in length. The external structure of the valvulae in various representatives of Apoidea was described in detail by Packer (2003).

However, to the best of our knowledge there are no previous studies of the functional morphology of the 3rd valvulae in the context of movements of the sting apparatus. Taking into account that no muscles attach to the 3rd valvulae in Hymenoptera (Vilhelmsen 2003; Kumpanenko & Gladun 2017), it remains unclear how they operate. The present paper aims to provide a detailed study of the external structure of the 3rd valvulae in representatives of various families of the Aculeata and discuss their biomechanics in the context of the structure of the sting apparatus. We also briefly discuss the features examined in a phylogenetic context.

2. Material and methods

2.1. Material

We examined 31 species from 26 subfamilies and 19 families of Hymenoptera (see Table 1). The family level classification follows Brothers (1999) and Peters et al. (2017). Both specimens preserved in 70% ethanol and pinned specimens (without soaking and macerating) were used for dissections. All species are common for Ukraine. The specimens were identified to species level whenever possible; for some it was only possible to identify them to genus. Identification was carried out by keys of different taxa Apocrita (Tobias 1978a, 1978b; Lelej & Schmid-Egger 2005; Dvořák & Roberts 2006; Fateryga & Shorenko 2012 and others) and confirmed by specialists (see Acknowledgements). All material examined is deposited in the collection of the Institute for Evolutionary Ecology of the National Academy of Sciences of Ukraine, Kiev.

2.2. Methods

The sting apparatus was prepared by removing it from the ethanol-preserved specimens using dissecting needles and forceps. The sting apparatus was macerated in 10% KOH and one of the 2nd valvifers together with the associated 3rd valvula was separated. These structures were placed in glycerol for light and fluorescence microscopic examination.

Light microscopy was carried out using an Olympus CX41s microscope. Fluorescence microscopy was carried out using an Olympus BX51 microscope with WU filter (excitation 330–385 nm, 420 nm longpass emission filter). Resilin-like proteins were inferred to be present in places displaying blue auto-fluorescence (Donoughe et al. 2011). The slides were photographed with a Canon EOS 600D camera. An opening was cut in the abdomen of only dried specimens with the sting shaft extended to make permanent mounts that demonstrate the position

Table 1. List of investigated species. — ***Symbols***: s – extended sting mount, v – 3rd valvula slide.

Family	Subfamily	Species	
Evaniidae		*Evania* sp.	v
Trigonalidae	Trigonalinae	*Pseudogonalos hahnii* (Spinola, 1840)	v
Bethylidae	Epyrinae	*Epyris* sp.	v
Dryinidae	Gonatopodinae	*Gonatopus cf. formicarius* Ljungh, 1810	s
Chrysididae	Chrysidinae	*Chrysis* sp.	v
Scoliidae	Scoliinae	*Scolia galbula* (Pallas, 1771)	s
		Scolia sexmaculata (O.F. Müller, 1766)	v
Pompilidae	Pepsinae	*Cryptocheilus (Adonta) versicolor* (Scopoli, 1763)	v
	Pompilinae	*Batozonellus lacerticida* (Pallas, 1771)	v
		Anoplius (Arachnophroctonus) infuscatus (Van der Linden, 1827)	s
	Ceropalinae	*Ceropales (Ceropales) maculata* (Fabricius, 1775)	v
Sapygidae	Sapyginae	*Sapyga similis* (Fabricius, 1793)	v
Tiphiidae	Tiphiinae	*Tiphia femorata* Fabricius, 1775	v s
Mutillidae	Dasylabrinae	*Dasylabris (Dasylabris) maura* (Linnaeus, 1758)	v s
	Mutillinae	*Mutilla europaea* Linnaeus, 1758	v
		Ronisia brutia (Petagna, 1787)	s
	Myrmillinae	*Myrmilla (Myrmilla) caucasica* (Kolenati, 1846)	v
Myrmosidae	Myrmosinae	*Paramyrmosa brunnipes* (Lepeletier, 1845)	v
Vespidae	Polistinae	*Polistes dominulus* (Christ, 1791)	v s
	Vespinae	*Vespula germanica* (Fabricius, 1793)	v
	Eumeninae	*Eumenes coronatus* (Panzer, 1799)	v
Formicidae	Myrmicinae	*Crematogaster schmidti* Mayr, 1853	v
		Myrmica bergi Ruzsky, 1902	v
Ampulicidae	Dolichurinae	*Dolichurus* sp.	v
Sphecidae	Ammophilinae	*Ammophila heydeni* Dahlbom 1845	v
Crabronidae	Bembicinae	*Gorytes laticinctus* (Lepeletier, 1832)	s
	Crabroninae	*Ectemnius fossorius* (Linnaeus, 1758)	v
Melittidae	Dasypodainae	*Dasypoda hirtipes* (Fabricius, 1793)	v
	Macropidinae	*Macropis europaea* Warncke, 1973	s
Apidae	Apinae	*Bombus terrestris* Linnaeus, 1758	v s
Colletidae	Hylaeinae	*Hylaeus cornutus* Curtis, 1831	v

of the 3rd valvulae in this position. To avoid the specimen destruction during preparation we immobilized the abdomen with a colophony-wax alloy. The mounts were photographed with an Olympus SZX12 microscope equipped with an Olympus DF Plapo 1x PF objective in combination with an Olympus E410 digital camera.

To explore character evolution, the traits studied were assembled in a matrix generated in Mesquite 3.6 (Maddison & Maddison 2018) and mapped on a modified version of the tree presented in Brantstetter et al. (2017: fig. S1); the topology of the Mutillidae included in the present study was resolved according to Brothers & Lelej (2017: fig. 14), that of the Pompilidae according to Waichert et al. (2015: fig. 1). The terminals not examined by us were pruned from the tree presented here (Fig. 13). Character state changes were tracked in Mesquite by implementing Trace Character History. Unambiguous character changes are mapped in Fig. 13; for a complete overview of the evolution of each character, please see individual character trees (Fig. S2). Fig. 13 was drawn in Adobe Illustrator. An electronic version of the data matrix can be downloaded from https://figshare.com/articles/Matrix_of_Aculeata_ovipositor_sheaths/9772097.

2.3. Terms and abbreviations

The terms for the sting apparatus follow Hymenoptera Anatomy Consortium (2019); see also Yoder et al. (2010). This is the standard terminology for Hymenoptera. For alternative terms applied more widely outside Hymenoptera, see Klass (2003). The following abbreviations are used: **2vf** – 2nd valvifer; **3vv** – 3rd valvula; **3vv-d** – distal part of 3rd valvula; **3vv-p** – proximal part of 3rd valvula; **3vv-o** – outgrowth of 3rd valvula; **st** – sting shaft.

3. Results

3.1. Morphology of the 3rd valvula in Aculeata

The following taxa were observed to have the 3vv sclerotisation undivided: Chrysididae, Dryinidae, Vespidae (Polistinae), some Myrmicinae (Formicidae), Apidae and Colletidae (Figs. 1B,C, 2B, 6A, 7D,E; Table 2).

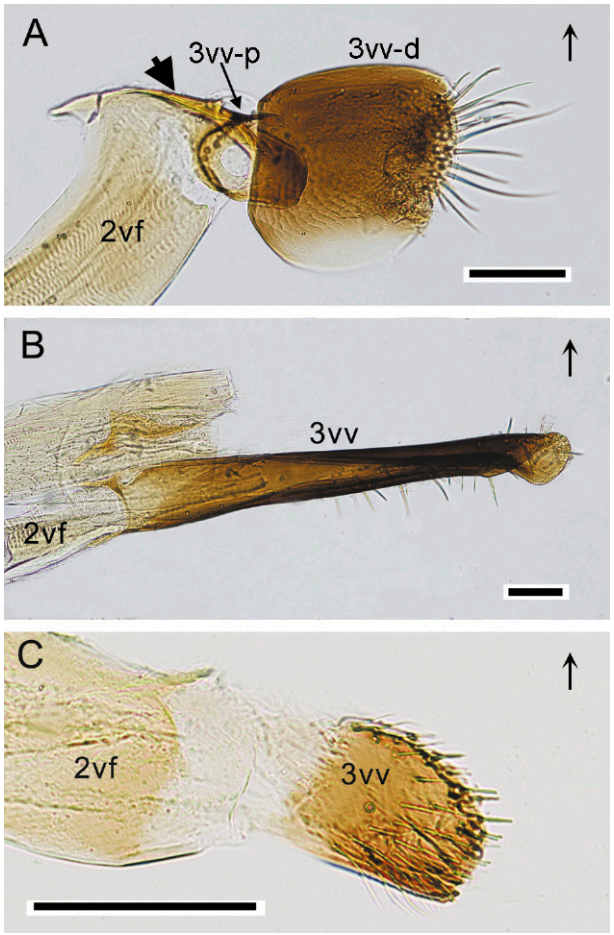

Fig. 1. The left 3rd valvula and 2nd valvifer in Chrysidoidea, lateral view, ↑ dorsal. **A**: *Epyris* sp. (Bethylidae, Epyrinae). **B**: *Chrysis* sp. (Chrysididae: Chrysidinae). **C**: *Gonatopus* cf. *formicarius* (Dryinidae: Gonatopodinae). ***Scale bar***: 0.1 mm.

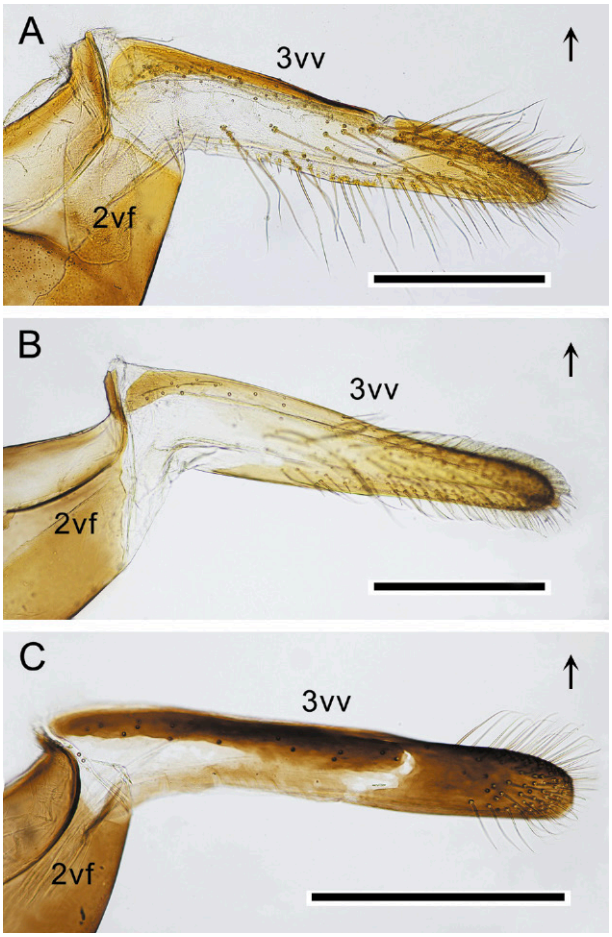

Fig. 2. The left 3rd valvula and 2nd valvifer in Vespidae, lateral view, ↑ dorsal. **A**: *Vespula germanica* (Vespinae). **B**: *Polistes dominulus* (Polistinae). **C**: *Eumenes coronatus* (Eumeninae). ***Scale bar***: 0.25 mm.

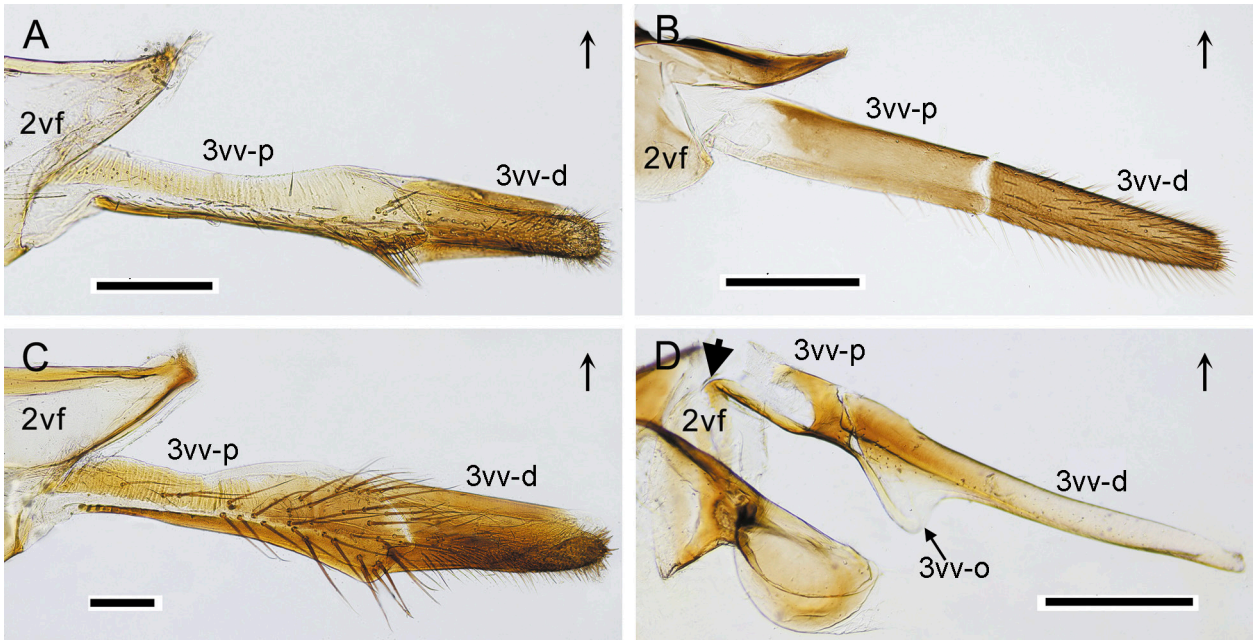

Fig. 3. The left 3rd valvula and 2nd valvifer in Pompilidae (A–C) and Sapygidae (D), lateral view, ↑ dorsal. **A**: *Cryptocheilus versicolor* (Pepsinae). **B**: *Ceropales maculata* (Ceropalinae). **C**: *Batozonellus lacerticida* (Pompilinae). **D**: *Sapyga similis* (Sapyginae). ***Scale bar***: 0.2mm.

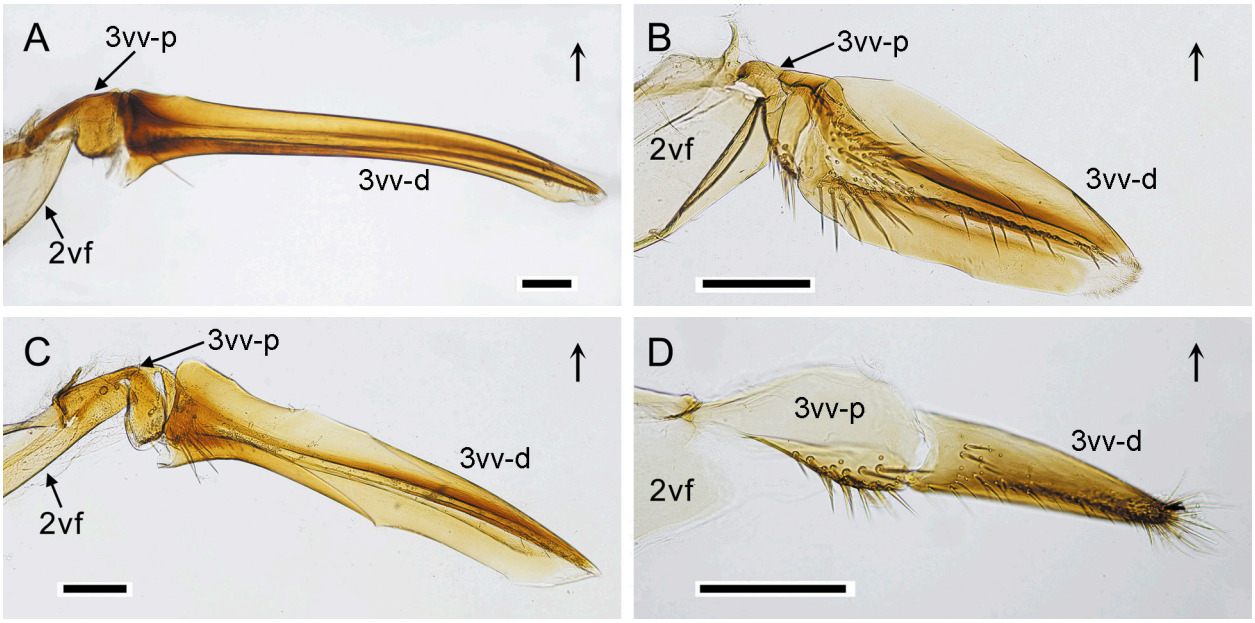

Fig. 4. The left 3rd valvula and 2nd valvifer in Mutillidae (A–C) and Myrmosidae (D), lateral view, ↑ dorsal. **A**: *Mutilla europaea* (Mutillinae). **B**: *Myrmilla caucasica* (Myrmillinae). **C**: *Dasylabris maura* (Dasylabrinae). **D**: *Paramyrmosa brunnipes* (Myrmosinae). ***Scale bar***: 0.2 mm.

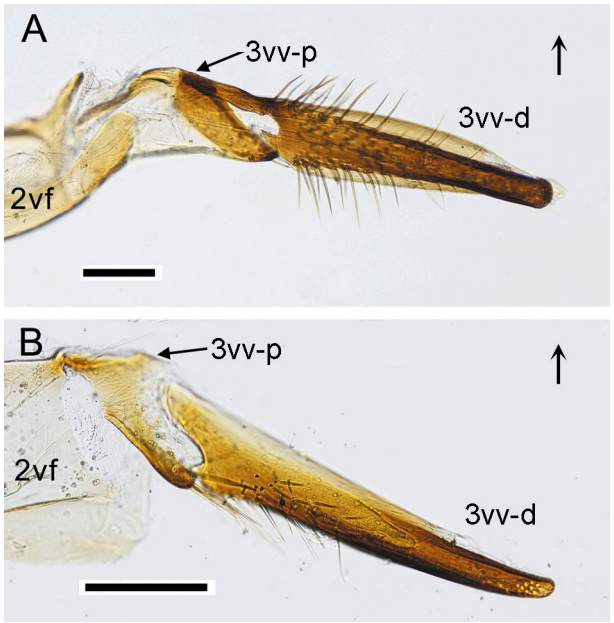

Fig. 5. The left 3rd valvula and 2nd valvifer in Scoliidae (A) and Tiphiidae (B), lateral view, ↑ dorsal. **A**: *Scolia sexmaculata* (Scoliinae). **B**: *Tiphia femorata* (Tiphiinae). ***Scale bar***: 0.2 mm.

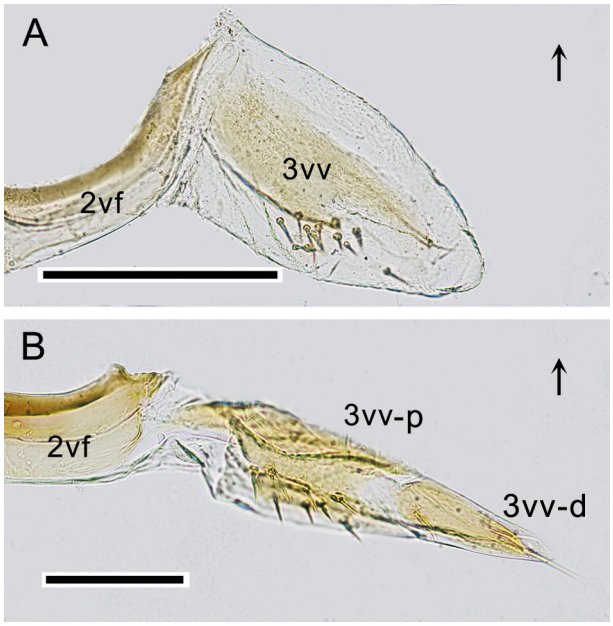

Fig. 6. The left 3rd valvula and 2nd valvifer in Formicidae, lateral view, ↑ dorsal. **A**: *Crematogaster schmidti* (Myrmicinae). **B**: *Myrmica bergi* (Myrmicinae). ***Scale bar***: 0.2 mm.

Representatives of the families Bethylidae, Scoliidae, Tiphiidae, Pompilidae (Pepsinae, Pompilinae), Sapygidae, Mutillidae, Myrmosidae, Crabronidae and Sphecidae have the 3vv sclerotisation divided into proximal (3vv-p) and distal (3vv-d) sclerites. The sclerites are movably connected (Figs. 1A, 3A,C,D, 4, 5, 7A,B; Table 2) and the degree of mobility of this articulation varies among families (see below). In Mutillidae-Dasylabrinae, Scoliidae, and Tiphiidae (Fig. 5), the interacting margins of the parts form outgrowths and troughs, which probably guide the movement in this articulation.

In Pompilidae (the cleptoparasitic subfamily Ceropalinae), Melittidae (Dasypodainae), some Ampulicidae and some Formicidae, the 3vv is divided into two parts by a region of elastic (soft-sclerotized) cuticle (Figs. 3B, 4D, 6B, 7C,F; Table 2).

In some Vespidae (Vespinae, Eumeninae), the 3vv sclerotisation consists of a more strongly sclerotized 3vv-d and a weaker 3vv-p, the latter only distinct on the dorsal side (Fig. 2A,C; Table2). A poorly sclerotized 3vv-p is also characteristic for Pompilidae (Pepsinae and Pompilinae) and Sphecidae; however, these parts are more scle-

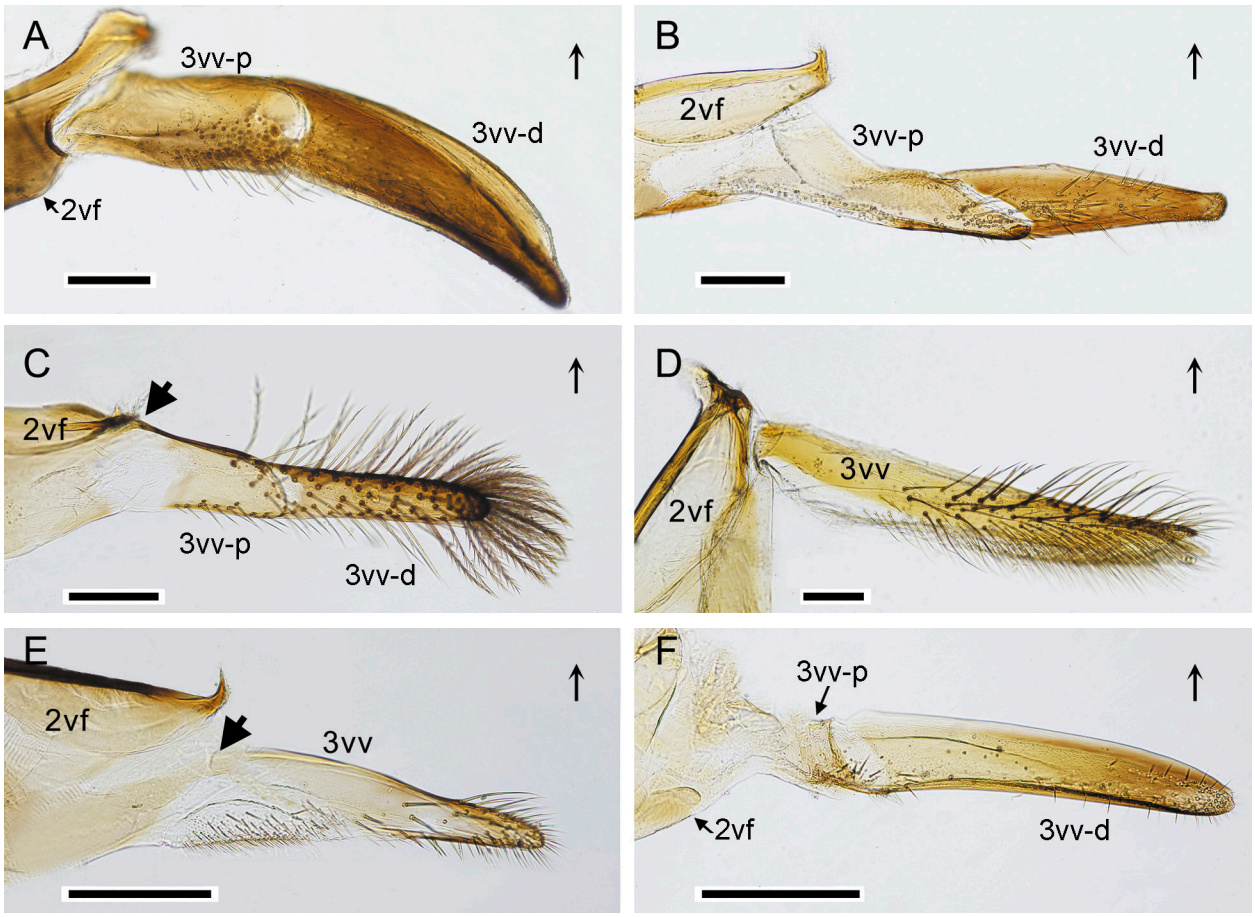

Fig. 7. The left 3rd valvula and 2nd valvifer in Apoidea, lateral view, ↑ dorsal. **A**: *Ectemnius fossorius* (Crabronidae: Crabroninae). **B**: *Ammophila heydeni* (Sphecidae: Ammophilinae). **C**: *Dasypoda hirtipes* (Melittidae: Dasypodainae). **D**: *Bombus terrestris* (Apidae: Apinae). **E**: *Hylaeus cornutus* (Colletidae: Hylaeinae). **F**: *Dolichurus* sp. (Ampulicidae: Dolichurinae). ***Scale bar***: 0.2 mm.

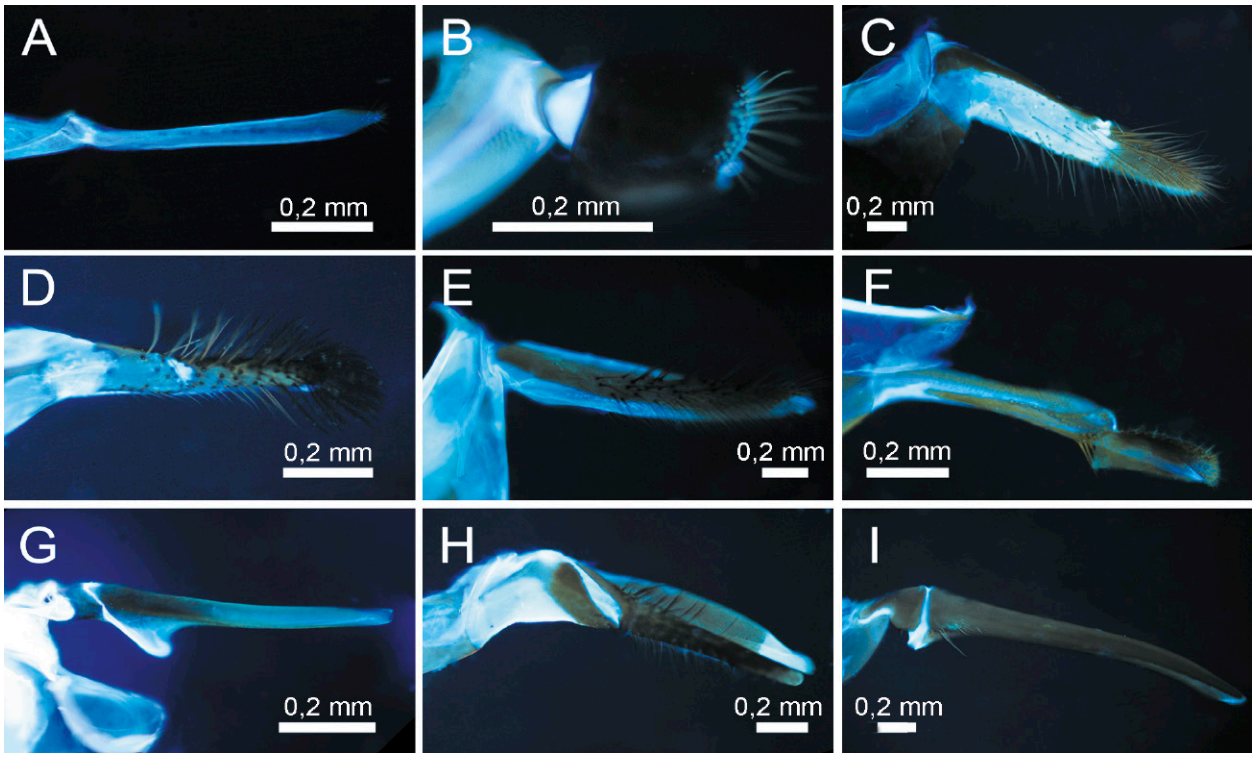

Fig. 8. Resilin-like structures in the 3rd valvula, lateral view. **A**: *Evania* sp. (Evaniidae). **B**: *Epyris* sp. (Bethylidae). **C**: *Vespula germanica* (Vespidae). **D**: *Dasypoda hirtipes* (Melittidae). **E**: *Bombus terrestris* (Apidae). **F**: *Cryptocheilus versicolor* (Pompilidae). **G**: *Sapyga similis* (Sapygidae). **H**: *Scolia sexmaculata* (Scoliinae). **I**: *Mutilla europaea* (Mutillidae). ***Scale bar***: 0.2 mm.

Table 2. Features of the 3rd valvula in Aculeata. [1] = according to Oeser (1961) and Hermann (1975).

Family	Subfamily	3vv configuration	Ratio, proximal (p) and distal (d) part	Sclerotization, proximal (p) and distal (d) part	2vf-3vv junction	Junction between 3vv parts	Proximal part of 3vv relative to sting during stinging	Sting shape	Sting function besides defense
Evaniidae		entire (0)	—	—	wide membrane (1)	—	?	decurved (0)	
Dryinidae	Gonatopodinae	entire (0)	—	—	wide membrane (1)	—	?	decurved (0)	immobilization (2)
Chrysididae	Chrysidinae	entire (0)	—	—	sclerotized bar (2)?	—	?	decurved (0)	defense only (0)
Bethylidae	Epyrinae	subdivided (1)	p < d (0)	p < d (0)	sclerotized bar (2)	articulation (0)	touching (2)	decurved (0)	immobilization (2)
Scoliidae	Scoliinae	subdivided (1)	p < d (0)	p = d (1)	narrow membrane (0)	articulation (0)	touching (2)	decurved (0)	immobilization (2)
Tiphiidae	Tiphiinae	subdivided (1)	p < d (0)	p = d (1)	narrow membrane (0)	articulation (0)	touching (2)	decurved (0)	immobilization (2)
Thynnidae [1]	Methochinae	subdivided (1)	p < d (0)	?	?	?	?	decurved (0)	immobilization (2)
	Diamminae	subdivided (1)	p < d (0)	?	?	?	?	decurved (0)	immobilization (2)
Sapygidae	Sapyginae	subdivided (1)	p < d (0)	p > d (2)	sclerotized bar (2)	membrane (1)	touching (2)	decurved (0)	?
Mutillidae	Dasylabrinae	subdivided (1)	p < d (0)	p = d (1)	narrow membrane (0)	articulation (0)	touching (2)	coiled (1)	defense only (0)
	Mutillinae	subdivided (1)	p < d (0)	p = d (1)	narrow membrane (0)	articulation (0)	touching (2)	coiled (1)	defense only (0)
	Myrmillinae	subdivided (1)	p < d (0)	p = d (1)	narrow membrane (0)	articulation (0)	?	coiled (1)	defense only (0)
Myrmosidae	Myrmosinae	subdivided (1)	p = d (1)	p = d (1)	narrow membrane (0)	articulation (0)?	?	decurved (0)	defense only (0)
Pompilidae	Pepsinae	subdivided (1)	p > d (2)	p = d (1)	narrow membrane (0)	articulation (0)	rotated (1)	decurved (0)	immobilization (2)
	Pompilinae	subdivided (1)	p > d (2)	p = d (1)	narrow membrane (0)	articulation (0)	rotated (1)	decurved (0)	immobilization (2)
	Ceropalinae	subdivided (1)	p = d (1)	p = d (1)	wide membrane (1)	membrane (1)	?	decurved (0)	defense only (0)
Vespidae	Polistinae	entire (0)	—	—	narrow membrane (0)	—	lifted (0)	decurved (0)	killing (1)
	Vespinae	subdivided (1)	p > d (2)	p < d (0)	narrow membrane (0)	membrane (1)	?	decurved (0)	killing (1)
	Eumeninae	subdivided (1)	p > d (2)	p < d (0)	narrow membrane (0)	membrane (1)	?	decurved (0)	immobilization (2)
Chyphotidae [1]		subdivided (1)	p > d (2)	?	?	?	?	decurved (0)	?
Formicidae	Myrmicinae	variable (0/1)	p > d (2)	p = d (1)	wide membrane (1)	membrane (1)	?	decurved (0)	killing (1)
Ampulicidae	Dolichurinae	subdivided (1)	p < d (0)	p = d (1)	wide membrane (1)	membrane (1)	?	decurved (0)	immobilization (2)
Sphecidae	Ammophilinae	subdivided (1)	p = d (1)	p = d (1)	narrow membrane (0)	articulation (0)	?	decurved (0)	immobilization (2)
Crabronidae	Bembicinae	subdivided (1)	p < d (0)	p = d (1)	narrow membrane (0)	articulation (0)	rotated (1)	decurved (0)	immobilization (2)
	Crabroninae	subdivided (1)	p < d (0)	p = d (1)	narrow membrane (0)	articulation (0)	?	decurved (0)	immobilization (2)
Melittidae	Dasypodainae	subdivided (1)	p < d (0)	p = d (1)	wide membrane (1)	membrane (1)	?	decurved (0)	defense only (0)
	Macropidinae	entire (0)	—	—	sclerotized bar (2)	—	lifted (0)	decurved (0)	defense only (0)
Apidae	Apinae	—	—	—	narrow membrane (0)	—	?	decurved (0)	defense only (0)
Colletidae	Hylaeinae	subdivided (1)	p < d (0)	p = d (1)	sclerotized bar (2)	membrane (1)	?	decurved (0)	defense only (0)

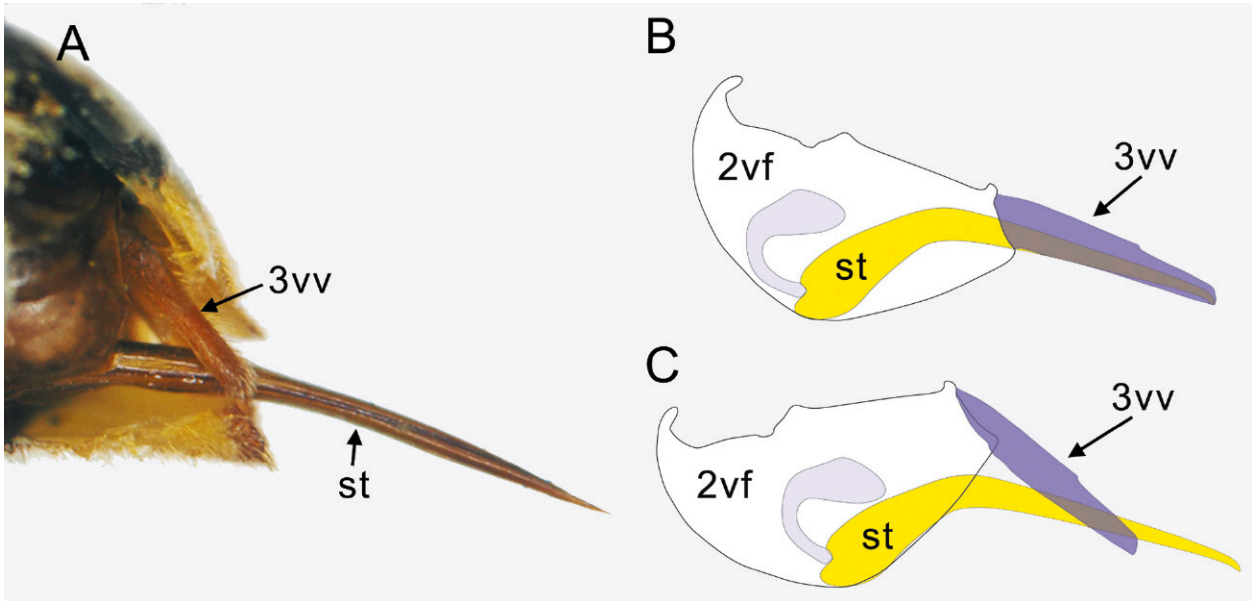

Fig. 9. Extreme positions of the 3rd valvulae (3vv) during stinging in *Polistes dominulus* (Vespidae), lateral view. **A**,**C**: Position of 3vv when sting is extended. **B**: Position of 3vv when sting is at rest.

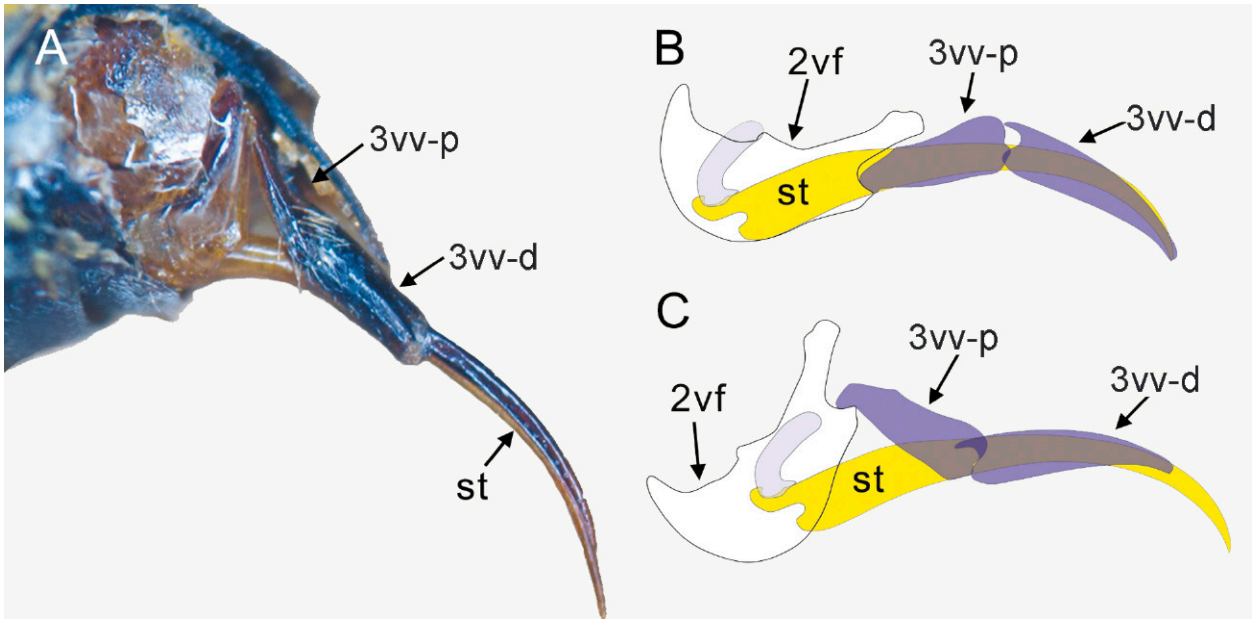

Fig. 10. Extreme positions of the 3rd valvulae (3vv) during stinging in *Gorytes laticinctus* (Crabronidae), lateral view. **A**,**C**: Position of 3vv when sting is extended. **B**: Position of 3vv when sting is at rest.

rotized ventrally (Figs. 3A,C, 7B, Table 2).In Sapygidae, the 3vv-d is less sclerotized than the 3vv-p.

The ratio between the lengths of the 3vv-p and 3vv-d varies strongly among the different families of Aculeata (Table2). In Bethylidae, Sapygidae, Mutillidae, Scoliidae, Tiphiidae and Ampulicidae, the 3vv-d is much longer than the 3vv-p (Figs. 1A, 3D, 4A–C, 5, 7F). In Pompilidae-Ceropalinae, Myrmosidae, some Crabronidae, Sphecidae and Melittidae the parts are more or less equal in length (Figs. 3B, 4D, 7A–C). In most Vespidae (Vespinae, Eumeninae) and Pompilidae (Pepsinae, Pompilinae) (Figs. 2A,C; 3A,C), the 3vv-d is significantly shorter than the 3vv-p.

The articulations of the 3vv with the 2vf differ significantly among representatives of different families of Aculeata (Table2). In most of the taxa studied, the 3vv is movably connected to the 2vf through a narrow region of transparent flexible cuticle (HERMANN 1967) (Table 2; Figs. 2, 3A,C, 4, 5, 7A,D). In Pompilidae (Pepsinae, Pompilinae), Scoliidae, Tiphiidae, Sphecidae, Crabronidae the region of flexible cuticle widens considerably towards the ventral margin of the sclerite (Figs. 3A,C, 5), or the ventral margin is less sclerotized than the dorsal one (Vespidae, Fig. 2). In Mutillidae (Fig. 4A–C), the proximal margin of the 3vv inserts into a recess distally on the 2vf, so the mobility in the articulation is probably

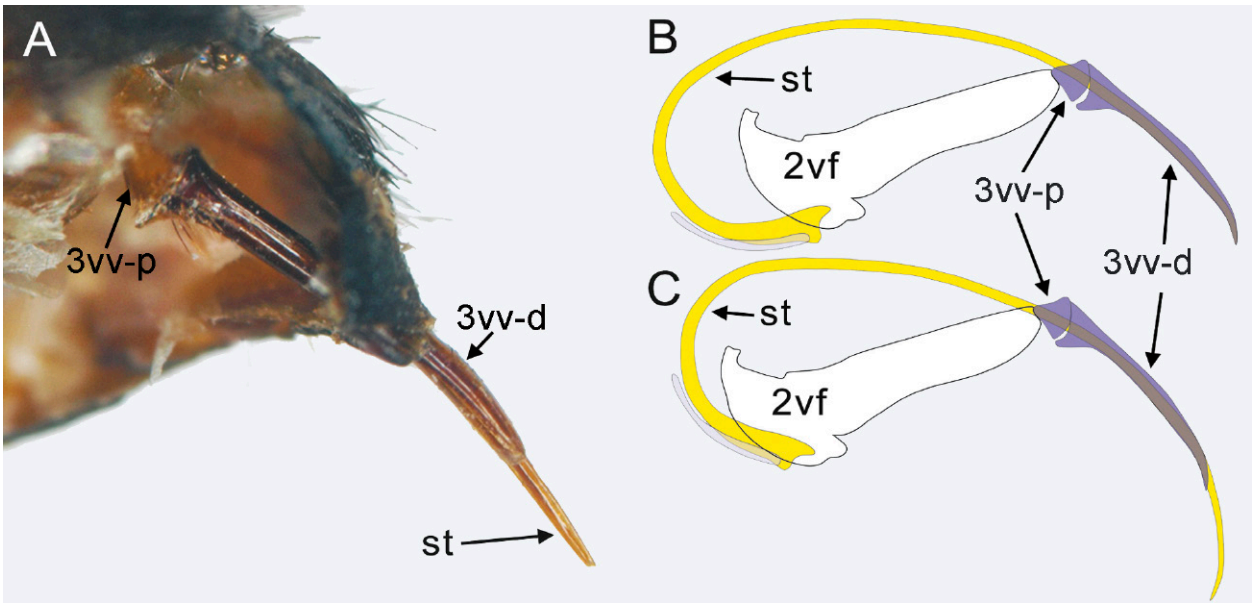

Fig. 11. Extreme positions of the 3rd valvulae (3vv) during stinging in *Ronisia brutia* (Mutillidae), lateral view. **A**,**C**: Position of 3vv when sting is extended. **B**: Position of 3vv when sting is at rest.

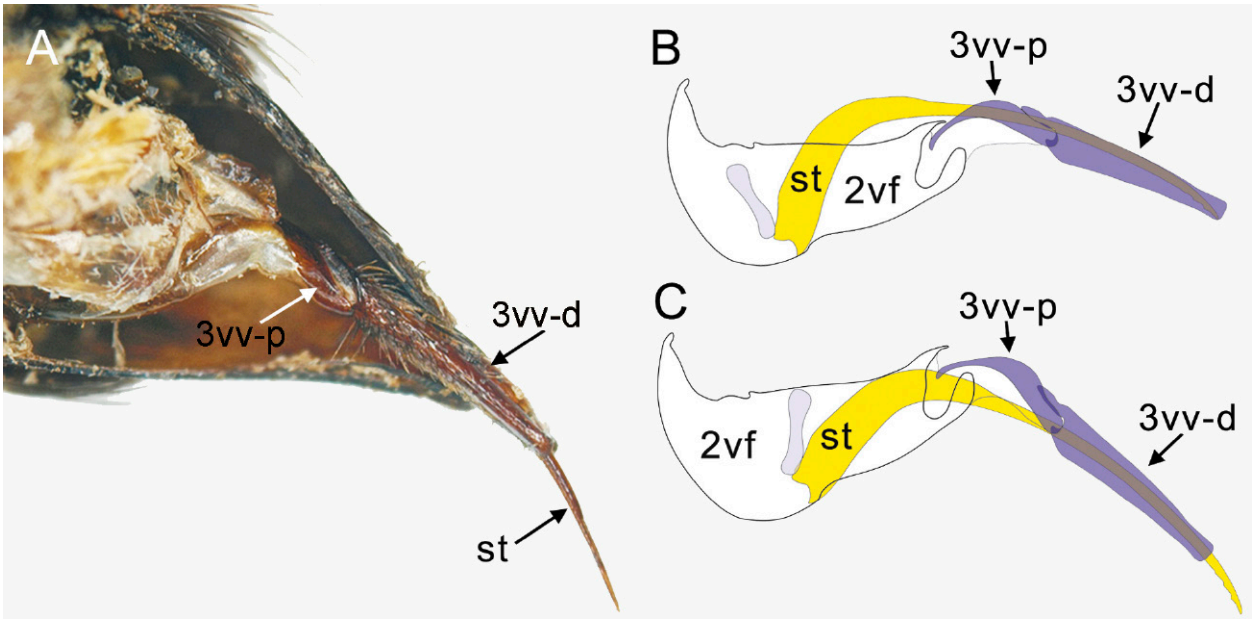

Fig. 12. Extreme positions of the 3rd valvulae (3vv) during sting extension in *Scolia galbula* (Scoliidae), lateral view. **A**,**C**: Position of 3vv when sting is extended. **B**: Position of 3vv when sting is at rest.

limited. In contrast, in the Dryinidae, Pompilidae-Ceropalinae, Formicidae, and Ampulicidae (Figs. 1C, 2B, 6, 7E,F) the 3vv and the 2vf are separated by a wide area of weakly sclerotized cuticle. In the Bethylidae, Chrysididae, Melittidae, and Colletidae the 3vv is connected with 2vf by a narrow dorsal sclerotized bar below which there is elastic cuticle (bold arrow in Figs. 1A,B, 7C,E). In Sapygidae the narrow sclerotized bar is located ventrally (bold arrow in Fig. 3D).

The examination of the 3vv with fluorescence microscopy showed blue auto-fluorescence at the articulation between the 3vv and the 2vf, between the parts of the valvula, and also the weakly sclerotized parts of the valvula, indicating the presence of resilin-like proteins (Fig. 8).

3.2. Extent of movement of the 3rd valvulae during stinging

In Aculeata the 3vv lie alongside the sting shaft at rest (retracted sting), tightly adjoining it. Two main types of the shape of the sting shaft are distinguished: **(1)** The "decurved sting", in which the sting base is the part of the sting shaft reaching furthest cephalad (e.g., Vespidae, Crabronidae) (Figs. 9, 10); **(2)** The "coiled sting", in

which the basal part of the sting shaft is rotated ventrad and posteriad through ca. 150° so that the proximal part of the sting shaft extends further cephalad than its base before the sting shaft curves back caudad (e.g., Mutillidae) (Fig. 11) (Hermann 1975). The shape of the sting shaft in Scoliidae (Fig. 12) and Tiphiidae appears intermediate: the sting shaft is strongly curved, but its proximal part does not extend cephalad beyond the level of the sting base (Hermann 1967).

In the process of stinging the relative position of the 3vv and the sting shaft changes (Kumpanenko & Gladun 2017). We studied the position of the 3vv with the sting shaft extended in representatives of different families of Aculeata, and suggest four main ways of interaction between the 3vv and the sting shaft (Figs. 9–12, Table 2); these depend on the structure of the 3vv, the shape of the sting shaft as well as the functioning of the entire sting apparatus: **1.** The base of 3vv is lifted, the apex slides along the "decurved sting" (Fig. 9). **2.** The base of 3vv is lifted, but due to the articulation between 3vv-p and 3vv-d, the 3vv-d along its entire length can remain in touch with the sting shaft and only the 3vv-d slides along the "decurved sting" (Fig. 10); **3.** The 3vv slides along the "coiled sting" over of their entire length (Fig. 11); **4.** The base of the 3vv is lifted, but most of the 3vv slides along the "intermediate type of sting" (Fig. 12). Table 2 presents the features of the structure of the 3vv and their interactions with the sting shaft in representatives of various families of Aculeata.

4. Discussion

In Hymenoptera the 2vf and 3vv are formed by subdivision of the 9th gonocoxite and constitute its proximal and distal parts, respectively (Snodgrass 1956; Matushkina 2011). The degree of the mobility of the 3vv relative to the 2vf, their flexibility, relative length and mode of articulation varies significantly in Hymenoptera. Such diversity of function is a result of the ovipositor being employed for many different functions across the Hymenoptera. The main functions of the 3vv (sensory reception, protection, cleaning, and directing of the ovipositor proper/sting shaft) are common to most Hymenoptera (Quicke et al. 1999; Vilhelmsen 2003) and its morphology might facilitate quick and accurate movements of the interlocked 1st and 2nd valvulae, which compose the ovipositor proper/sting shaft (Kumpanenko & Gladun 2017).

Among "Symphyta" the 3vv are more or less separated from the 2vf by areas (membranous region) of thin elastic cuticle (Oeser 1961; Vilhelmsen 2000); only Argidae and Pergidae have the 3vv fused with the 2vf (Vilhelmsen 2000: Figs. 3E, 5E). All "Symphyta" have the 3vv undivided. The mobility of the 3vv is probably rather limited, but sufficient to provide the basic functions (sensory and protection; Smith 1972) for the penetration movements of the ovipositor.

Representatives of the parasitoid Apocrita display different ways of connecting the 3vv with the 2vf similar to "Symphyta". The 3vv are separated from the 2vf by areas (membranous regions) of elastic cuticle in Chalcidoidea (except for Eurytomidae and Mymaridae) (Copland & King 1972), Proctotrupoidea (Fergusson 1988), Ceraphronoidea (Ernst et al. 2013), and Braconidae (Dweck et al. 2008). Ichneumonidae show different states of this connection – through elastic cuticle or different degrees of fusion (Quicke 2015). In Ibaliidae and Liopteridae, which have a long, internalized, loop-like ovipositor, the 3vv and 2vf are fully fused (Fergusson 1988). The potentially close relatives of Aculeata (Trigonalidae and Evaniidae) (Zimmermann & Vilhelmsen 2016; Branstetter et al. 2017) show a very different configuration of the 3vv. The 3vv and sting shaft are reduced in Trigonalidae (Fig. S1; see also Oeser 1962), and the 3vv and 2vf are fused. Evaniidae have a short ovipositor and an undivided, weakly sclerotized, flexible 3vv (Fig. 8A). To the best of our knowledge, subdivision of the 3vv has not been recorded in any Parasitica.

In Aculeata the movements of the 3vv are achieved in a slightly different way. In most Aculeata studied by us, the 3vv are joined to the 2vf by elastic weak sclerotized cuticle, and in the majority of families they are more or less divided into two parts (Oeser 1961; Poore 1974). Among Chrysidoidea only Bethylidae have the 3vv subdivided into two parts. The 3vv can be inferred to be subdivided in the ground plan of the Vespoidea and Apoidea. Functionally, the division of the 3vv helps to improve its contact with the sting shaft during the movements of the sting apparatus. In some bee families (Apidae, Colletidae) a secondary fusion has probably occurred. It is possible that in Vespidae the partial or total loss of the mobility between the parts is also secondary.

The elastic, weakly sclerotized cuticle between the 2vf and 3vv as well as between the parts of the valvulae contains resilin-like proteins (Fig. 8). Resilin-like proteins were previously reported in other parts of the ovipositor/sting apparatus of the Hymenoptera (Smith 1972; Hermann & Willer 1986; Kumpanenko & Gladun 2017) and probably play an important role in the movements of its constituent parts. There are no muscles inserted on the 3vv. Instead, the resilin-like proteins presumably help return the 3vv to the initial position after deformation (Figs. 9–12) during stinging. Probably, a similar mechanism is available in "Symphyta" and non-aculeate Apocrita.

The Aculeata display three different modes of using the sting (Table 2): **(1)** using only for defense; **(2)** using for defense and killing prey; **(3)** using for defense and immobilization of prey.

The 3vv are in contact with the sting shaft throughout its length only when it is at rest. The functional role of the 3vv and the character of its movements are somewhat different in the various families, which probably explains the morphological variation.

In most cases the 3vv-p loses contact with the sting shaft during extension. When extended, an undivided

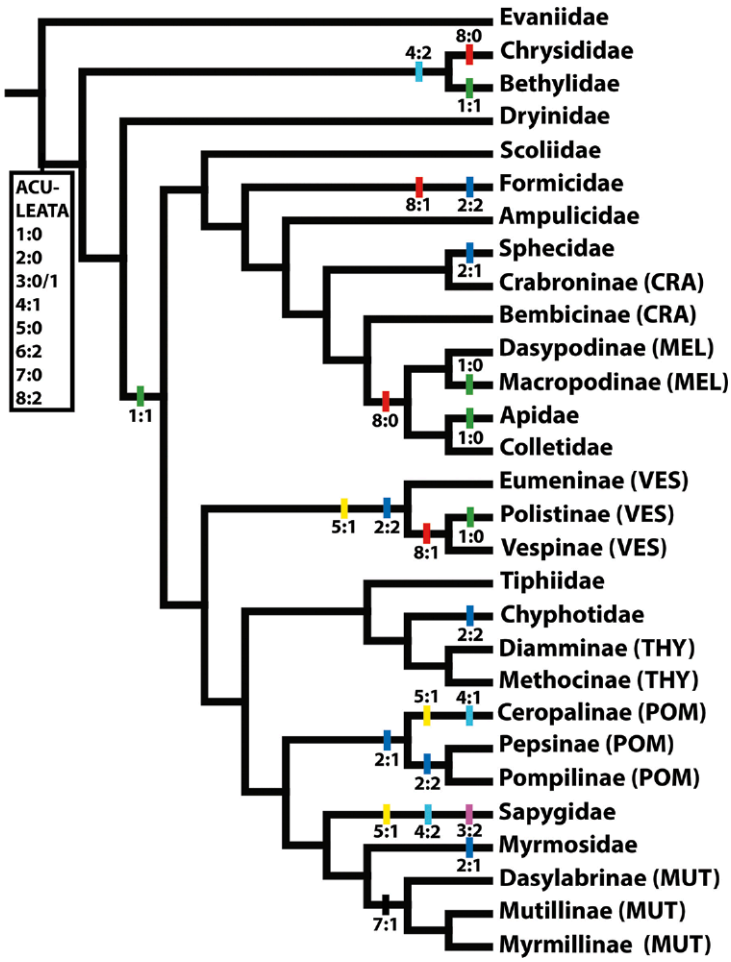

Fig. 13. Evolution of characters of the 3rd valvula in Aculeata. Mapped on the tree from Branstetter et al. (2017) modified according to Waichert et al. (2015) and Brothers & Lelej (2017). Character numbers and states according to Table 2; ground plan states indicated at base of Aculeata (inside rectangle). Only unambiguous character changes shown; for individual character evolution trees, see S2 files CharacterTrees. — ***Abbreviations***: CRA = Crabronidae; MEL = Melittidae; MUT = Mutillidae; POM = Pompilidae; THY = Thynnidae; VES = Vespidae.

3vv is in contact with the sting shaft only with the apical part (Fig. 9, Table 2).

Having a 3vv apically coupled with a decurved sting shaft is characteristic for Anthophila, Vespidae and some Myrmicinae, which use the sting to defend against predators or to kill large prey. Fine recognition of the substrate, accuracy of stinging (the guiding role of the 3vv) as well as careful cleaning of the sting shaft are probably less important for these insects.

Another state is demonstrated by the 3vv with a very short 3vv-p. Mutillidae use the sting for defense against bees (*Bombus*, Halictidae, etc.) and Crabronidae in whose nests they lay their eggs (Lelej 1985). In the process of movement the 3vv is in contact with the sting shaft throughout its entire length (Fig. 11B,C). The freedom of movement in the articulation between the 3vv-p/3vv-d and between the 2vf and 3vv is very limited. The movement of the 3vv-p is limited by the structure of the 2vf (Fig. 4A,C). Probably, in this case in addition to the functions mentioned above the heavily sclerotized 3vv presumably also support the sting shaft when used against targets with hardened cuticle.

We assume the movements of the sting apparatus in Mutillidae to differ significantly from that of other Aculeata. The movement of the sting shaft along the 3vv (without rotating between the 2vf and sting base: Acre et al. 1980; Kumpanenko & Gladun 2017) is probably possible due to its specific configuration ("coiled sting" according to Hermann 1975).

Scoliidae, Tiphiidae and Thynnidae have a significantly curved (but not coiled) sting shaft (Steinberg 1962; Billen et al. 2017; Fig. 12), which is used to immobilize prey and to defend against predators. The sting shaft is guided and strengthened by the rigid 3vv. However, the connection of the proximal part of the 3vv with 2vf is more flexible than that in Mutillidae. This structure combines rigidity with some mobility. Although the sting apparatus of Scoliidae, Tiphiidae and Mutillidae are functionally similar, we assume that the mechanism of the movements in Scoliidae and Tiphiidae is the same as in other Aculeata with a decurved (not coiled) sting shaft. Probably, the movement of the sting shaft along the 3vv is possible due to the significant curvature of the sting shaft, which, however, is not coiled and completely directed backward (Fig. 12).

The 3vv in Sapygidae (Fig. 3D) are distinguishable from the ones of other Aculeata studied by the presence of a flat, probably elastic (Fig. 8G) outgrowth (3vv-o) on the ventral edge of the 3vv-d. The purpose of this outgrowth is unknown. We assume that it can facilitate passage of the egg during oviposition. These wasps have a strongly curved sting shaft. The overall structure of the sting apparatus differs significantly from other Aculeata and the features of its movements have not been studied.

The mechanism of sting movement has not been studied in Chrysididae, Bethylidae and Dryinidae. We assume that the range of their sting shaft mobility is very limited. Chrysididae use the sting for defense only rarely, whereas Bethylidae and Dryinidae use it to paralyze their prey. Bethylidae sting their prey (larvae of some Coleoptera and Lepidoptera) many times in certain muscle groups (Rathmayer 1978) and probably need a larger range of sting shaft mobility. An increase of sting shaft mobility is maintained in Bethylidae by subdividing the 3vv (Fig. 1A). In addition, according to Oeser (1961) and Brothers (1975), in Bethylidae the 2vf is divided into two movable parts. In our opinion, this structure increases the mobility of the sting shaft and, respectively, of the 3vv, which does not lose contact with the sting shaft during movement. A similar structure of the 2vf is also characteristic of the Chrysididae, Dryinidae (Brothers 1975) and Myrmosidae (Kumpanenko et al., unpublished data).

In Pompilidae, Crabronidae and Sphecidae, the connection via the steering articulation between the 3vv-p and 3vv-dis much more mobile than that of the other Aculeata. Only the 3vv-d retain contact through out their length with the sting shaft when it moves (Fig. 10, Kumpanenko & Gladun 2017). These wasps use the sting for defense and immobilization of prey (Table 2). In some cases prey (e.g., spiders) can be dangerous to handle, so for quick immobilization stinging must be very accurate and fast. In addition, representatives of these families use the sting repeatedly. The well sclerotized 3vv-d determines the point of stinging via the receptors, and provides additional rigidity of the sting shaft as well as cleaning it. Probably, this configuration of the 3vv is the most functionally advanced.

In Fig. 13, we map the characters we have explored on a modified version of the tree presented in Brantstetter et al. (2017; see also Material and Methods). As can be seen, there are a number of character changes that are potentially phylogenetically relevant. Having the 3vv subdivided is a putative synapomorphy of Apoidea and Vespoidea (character 1, state 1; Fig.7A; paralleled in Bethylidae, reversed in Polistinae and some bees, e.g., Apidae).

The length ratio between the 3vv-p and 3vv-d (character 2) is variable, having the 3vv-p shortest (state 0; Fig. 4A) is the ground plan state. Short 3vv-p (state 2; Fig. 3A) are observed in Chyphotidae, Formicidae, and Vespidae, approx. equal length of 3vv-p and 3vv-p (state 1; Fig. 7B) in Myrmosidae and Sphecidae. In Pompilidae, Ceropalinae has state 1 whereas Pepsinae and Pompilinae have state 2, suggesting that the 3vv-d has been progressively shortened within this family.

The relative degree of sclerotisation of the 3vv-p and 3vv-d (character 3) is difficult to interpret, even the ground plan state for Aculeata being uncertain (state 0; Fig. 2; or 1; Fig. 4A). The only unambiguous change for this character is in Sapygidae, which has the 3vv-p more sclerotized than the 3vv-d (state 2; Fig. 3D). Having the 3vv-p less sclerotized than the 3vv-d (state 0) is definitely the ground plan state for Vespidae, but it cannot be decided whether it is apomorphic or plesiomorphic.

The configuration of the junction between 2vf and 3vv (character 4) is variable, but it seems to be a ground plan condition of Aculeata to have them separated by a wide stretch of elastic cuticle (state 1; Fig. 1C), although the majority of the taxa examined have only a narrow stretch of cuticle (state 0; Fig. 7A) in this position; the occurrence of state 1 in Ceropalinae (Pompilidae) is a reversal. The 2vf and 3vv are connected by a sclerotized bar (state 2; Fig. 7E) in Bethylidae + Chrysididae and Sapygidae as well as in Colletidae and Macropodinae (Melittidae), but this character is variable in bees (Packer 2003).

Having the two parts of the 3vv connected by an articulation (character 5, state 0; Fig. 4A) is the ground plan state for Apoidea + Vespoidea. The articulation is replaced by a narrow stretch of elastic cuticle (state 1; Fig. 3B) in Ceropalinae (Pompilidae), Sapygidae, Vespidae, as well as in Formicidae and some Apoidea.

The position of the 3vv-p during stinging (character 6) has only been observed in very few taxa and hence it is not possible to pinpoint any unambiguous changes in the Aculeata. Having the 3vv in contact with the sting shaft/ovipositor proper proximally as well as distally during stinging (state 2; Fig. 12) seems to be the ground plan feature.

Having the sting shaft coiled (character 7, state 1; Fig. 11) is an autapomorphy of Mutillidae, all other taxa examined having the sting shaft decurved.

The function of the sting (character 8) in the ground plan of Aculeata is both to serve in defense and to immobilize prey (state 2). The sting is for defense only (state 0) in bees, Chrysididae and Ceropalinae (Pompilidae), as well as for Myrmosidae and all Mutillidae included. Finally, in Formicidae and the social Vespidae, the sting is used for defense and for killing prey (state 1).

In the Aculeata functional adaptations of the 3vv are associated with the use of the sting. Although the 3vv completely lack intrinsic musculature, the animals are able to reversibly move them during the process of stinging. The 3vv are also used for a number of other tasks like protecting, cleaning and directing of the sting shaft, receptor function and also may contain some glands. The complexity of the morphology of the 3vv reflects the range of different tasks that are performed in the process of stinging by various groups. Some morphological adaptations developed independently in different families.

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File 1: kumpanenko&al-aculeatastingsheaths-asp2019-electronic supplement-1.pdf — **Fig. S1.**The 2vf+3vv in *Pseudogonalos hahnii* (Trigonalidae), lateral view, ↑ dorsal. — DOI: 10.26049/ASP77-2-2019-08/1

File 2: kumpanenko&al-aculeatastingsheaths-asp2019-electronic supplement-2.pdf — **Fig. S2.** Individual character evolution trees. Character states mapped in different colors, as indicated. Grey indicates that information is missing for the relevant branches. — DOI: 10.26049/ASP77-2-2019-08/02