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Redescription of the internal female genitalia of *Episyrphus balteatus* (De Geer) (Diptera), including a new and distinctive structure common to all Syrphidae

[Redeskription der inneren weiblichen Geschlechtsorgane von *Episyrphus balteatus* (De Geer) (Diptera), einschließlich einer neuen und charakteristischen Struktur, die allen Syrphidae eigen ist]

by

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Abstract

The internal female genitalia of *Episyrphus balteatus* (De Geer, 1776) are described morphologically and histologically. Special emphasis is placed on the description of what is called here the ventral circular structure (vcs), a newly discovered small but distinctive structure situated ventrally opposite to the opening of the spermathecal ducts. A comparative study of representatives of all major clades of Syrphidae revealed the vcs to belong to the ground plan of the family. Its potential function and uncertain phylogenetic origin are discussed.

Key words

Syrphidae, *Episyrphus*, reproduction, morphology, spermatheca, ventral receptacle, fertilization chamber, vagina

Zusammenfassung

Die inneren weiblichen Geschlechtsorgane von *Episyrphus balteatus* (De Geer, 1776) werden morphologisch und histologisch beschrieben, wobei einer ventralen, runden Struktur (vcs) besondere Beachtung geschenkt wird. Hierbei handelt es sich um eine neu entdeckte, kleine, aber sehr charakteristische Struktur, die ventral direkt gegenüber der Mündung der Spermathekengänge liegt. Eine vergleichende Untersuchung von Vertretern aller wesentlichen Untergruppen der Syrphidae ergibt, dass die ventrale runde Struktur Teil des Grundbauplans der Familie ist. Ihre mögliche Funktion und ungewisse phylogenetische Herkunft werden erörtert.

Stichwörter

Syrphidae, *Episyrphus*, Reproduktion, Morphologie, Spermatheka, ventrales Rezeptakulum, Befruchtungskammer, Vagina

Introduction

Male genitalic structures generally receive particular consideration in species descriptions. They often provide a wealth of informative characters for taxonomic and phylogenetic analyses. This could also be true for the female genitalia, but they are much less often included in the descriptions. Quite frequently only the condition of the spermathecae and/or other strongly sclerotized parts are reported.

This also applies to Syrphidae (e. g. Bańkowska 1964, Borisova 1980, Thompson & Torp 1982, Doczkal 1996, Borges & Couri 2009, Miranda 2016), a family of particular relevance, not only because it is very speciose and economically important, but also because it constitutes a relevant outgroup for phylogenetic analysis of the Schizophora (Yeates et al. 2007, Wiegmann et al. 2011). There has been some pioneer work on the syrphid female reproductive tract, but it remained superficial in many respects and is sometimes misleading.

Dufour (1851) provided illustrations of the internal female genitalia of *Volucella zonaria* (Poda, 1761) and *Eristalis tenax* (Linnaeus, 1758) showing all major components i. e. paired ovaries and lateral oviducts, a median common oviduct posteriorly opening into the vagina, three round spermathecae, and two delicate long and tubular (and in *E. tenax* multibranched) accessory glands. His figures testify dissecting skills quite remarkable for his time, but he confused the interpretation of the spermathecae (his "orbicelles") and the accessory glands (his "réservoirs séminaux").

STURTEVANT (1925) corrected this misinterpretation and added results for a number of other Syrphidae with some comments on the condition of the spermathecae and their ducts. NAYAR (1965) described the reproductive system of *Episyrphus balteatus* (DE GEER, 1776) with some histological details, especially regarding the ovaries and oogenesis. Some of his illustrations are rather poor, however, and he apparently confused some structures such as the lateral oviducts, accessory glands, and spermathecal ducts.

SHATALKIN (1981) published an extensive comparative study on the syrphid male and female genitalia and their interaction. The text provides much information, especially on the internal ornamentation of the dorsal part of the vagina surrounding the opening of the spermathecal ducts (his "vaginal plate") and the posterior portion of the vagina (his "vestibulum"), which forms a prominent bilobed pouch in some of the taxa.

HIPPA (1986, 1990) introduced a method to study details of the cuticular lining of the vagina (his "copulatory pocket") by inverting it to protrude inside out from the vulva. He did not endeavor to interpret or homologize the structures thus revealed except for a dorsal prominence bearing the openings of the spermathecae and accessory glands. Some of his meticulous illustrations (HIPPA (1986): Figs 1F, 2D, 6A, 6E; HIPPA (1990): Fig. 80C) include a small unlabeled circular structure, which might correspond to the primary subject of the present study, i. e. the ventral circular structure. HIPPA's approach was pursued by other authors such as BARKALOV (1991), who (1992) further addressed the interaction of male and female genitalia during copulation, but added little detail regarding the morphology of the syrphid internal female reproductive tract. SAREEN et al. (1989) published a SEM and histochemical survey of the spermatheca of *Eristalis tenax* (LINNAEUS).

A sound knowledge of the conditions of the internal female genitalia in Syrphidae is necessary for unraveling the evolution of these organs in the Cyclorrhapha, especially because Syrphidae are a relevant outgroup for the Schizophora. Both, calyptrate and acalyptrate Schizophora, have a fertilization chamber or ventral receptacle, i. e. a specialized fertilization site in the anteroventral part of the vagina, which has not been described in other Cyclorrhapha and whose phylogenetic origin remains unclear. Here we provide a new detailed description and microphotographic documentation for the inner female genitalia of *Episyrphus balteatus*, one of the most common and widespread species of Syrphidae in the Palaearctis. We put special emphasis on the description of the ventral circular structure, a new and distinctive structure which was previously unnoticed and constitutes, as our comparative study shows, a common feature for the entire family.

Material and methods

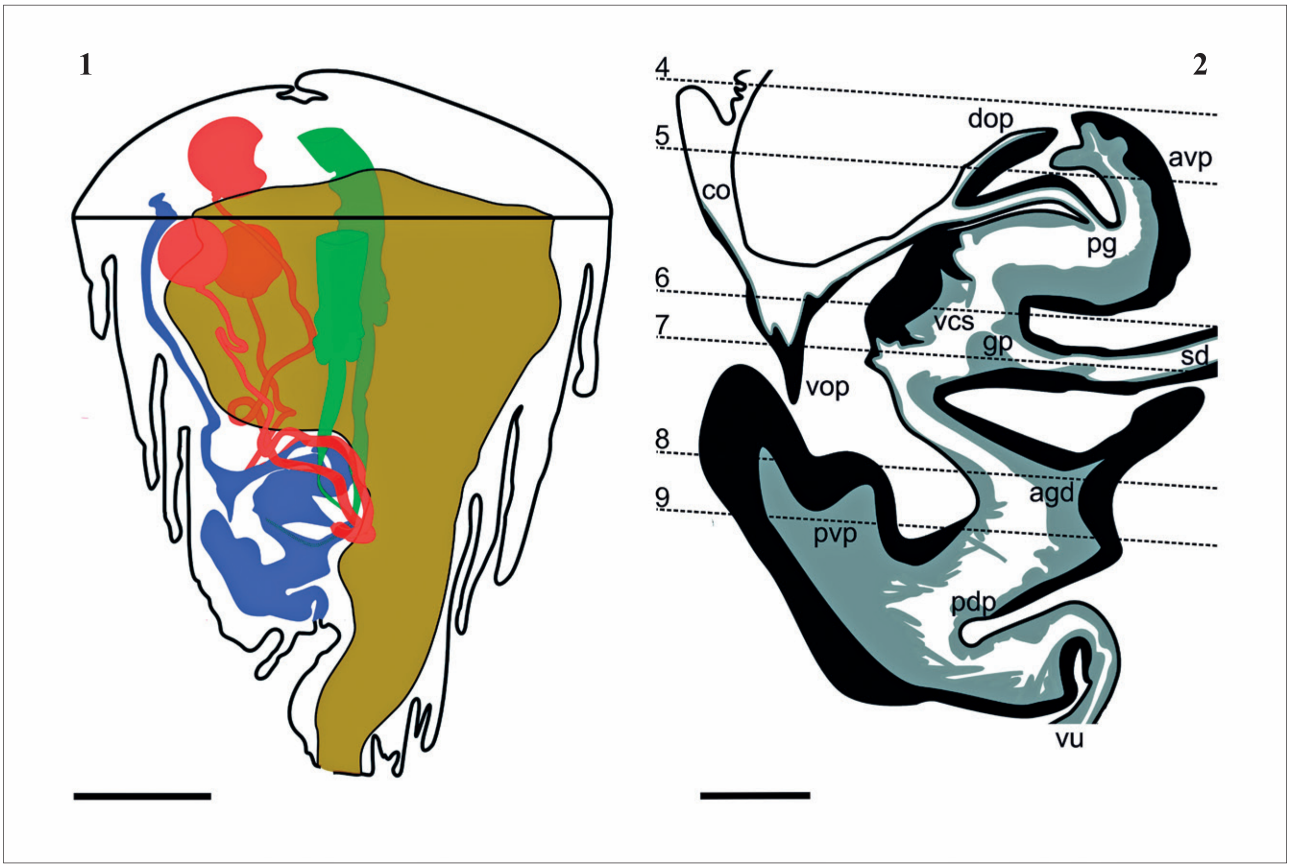
Both authors contributed equally to this study. The internal genitalia of 20 freshly killed *Episyrphus balteatus* females were dissected out and embedded as whole mounts on a microscopic slide in polyvinyllactophenol with an admixture of chlorazol black E. Postabdomina of another four freshly killed females were fixed in Bouin's fixative, rinsed in 70 % ethanol, embedded in Durcupan resin, microsectioned with a diamond knife at 1 or 2 µm (two specimens sagittal, two transversal), mounted and stained on microscopic slides with Richardson's stain (Methylene blue - Azur II), and embedded under a cover slip in Durcupan. Females of 21 other species representing all major clades of the family were dissected from freshly killed, pinned or ethanol preserved material. Pinned and ethanol preserved specimens were macerated and dissected in a 10 % solution of KOH before embedding in the above mentioned medium. Specimen details are listed in Table 1. All specimens were determined by the second author and will be deposited in the collection of the Zoologische Staatssammlung München (ZSM).

The preparations were studied with bright field illumination or differential interference contrast (DIC) using a Zeiss Axioskop2 equipped with Plan-Apochromat 20×, Plan-Apochromat 40× and Plan-Neofluar 100× oil objectives as well as a drawing tube and a Jenoptic Progres Gryphax Subra digital camera and software. The photographs included a digital scale bar calibrated with a stage micrometer. Larger structures were measured in sketches using the drawing tube and stage micrometer.

The plates were created with CorelDRAW X5 and Corel PHOTO-PAINT X5. The line-drawings were created with Inkscape (Inkscape Community; http://www.inkscape.org). The terminology of morphological structures follows Kotrba (2000). For structures not covered there we intentionally use purely descriptive terms such as "ventral circular structure" or "anterodorsal vagina pouch" to avoid any premature implications of homology. The term "posteroventral vagina pouch" is synonymous with the "sclerotized plate" of Nayar (1965) and partially congruent with the "vestibular membrane" of Shatalkin (1981). The term "genital papilla" is synonymous with the "vaginal plate" of Shatalkin (1981). For simplicity, cuticular or epithelial layers of only about 1 or 2 µm are described as "thin". Flat single-layered epithelia are described as "simple".

Results

Originating from the paired ovaries two short lateral oviducts converge medially to meet the common oviduct, which posteriorly opens into the vagina next to the anterodorsal vagina pouch (Figs 1, 2). Two spermathecal ducts open medially into the genital papilla in the dorsal vagina wall. One bears a single spherical spermatheca, the other bifurcates to bear two slightly smaller ones. There are two tubular accessory glands whose ducts open on the genital papilla posterior to the spermathecal ducts. Directly opposite to the entry of the spermathecal ducts the ventral circular structure is embedded in the ventral vagina wall. The posterior part of the vagina ventrally bears a prominent bilobed posteroventral pouch. Dorsally this pouch is complemented by a projection of the dorsal vagina wall. After a s-shaped dorsoventral turn the vagina ends at the vulva behind segment 8.



Figs 1, **2**: *Episyrphus balteatus* (De Geer) female genitalia. – **1**: Diagram of female postabdomen with position of genital organs, ventral side left. Explanations: Blue = oviduct and vagina; red = spermathecae and spermathecal ducts; green = accessory glands and their ducts; brown = digestive tract; – **2**: Diagram of female genitalia mid-sagittal section, ventral side left. Explanations: Black = epithelium; grey = cuticular intima; white = lumen. Abbreviations: agd = accessory gland duct orifice; avp = anterodorsal vagina pouch; co = common oviduct; dop = dorsal oviduct pouch; gp = genital papilla; pdp = posterodorsal protrusion; pg = primary gonopore; pvp = posteroventral vagina pouch; sd = spermathecal duct; vcs = ventral circular structure; vop = ventral oviduct pouch; vu = vulva; dotted lines indicate levels of transversal microsections in Figs 4–9. Scale bars = 50 µm (Fig. 2); = 200 µm (Fig. 1).

The **lateral oviducts** are about 400 µm long and 100 µm wide. Their walls consist of a thin simple epithelium surrounded by some sparse musculature.

The **common oviduct** is a dorsoventrally flattened tube, about 1000 µm long and 300 µm wide. It originates in the sixth abdominal segment and runs posteriorly along the ventral side of the abdomen (Figs 1–4). Ventral to the anterior part of the vagina it takes a sharp dorsally directed turn and at the same time branches off a pouch which extends further posteriorly, ending between the two lobes of the posteroventral vagina pouch. After branching off another dorsally directed pouch, the common oviduct opens into the vagina anteriorly through a narrow transverse slit, which constitutes the primary gonopore. The walls of the common oviduct consist of a thin simple epithelium accompanied by some sparse muscle fibres. In microsections a thin cuticular intima could be discerned near its transition into the vagina, starting inside the dorsal oviduct pouch dorsally and some way anterior to the ventral oviduct pouch ventrally (Figs 2, 3).

The **vagina** occupies the ventral part of the eighth segment (Figs 1–3). When empty it is about 250 µm long and 350 µm wide. Dorsally next to the primary gonopore the vagina bears a prominent, anteriorly directed **anterodorsal vagina pouch** (Figs 2, 3, 5). This pouch is dorsoventrally compressed, about 90 µm long and about 150 µm wide at its base. The cuticular intima lining the dorsal wall of the pouch stains intensely dark blue with Richardson's stain. It forms several transverse folds and ridges. The underlying epithelium is cuboidal with large nuclei. It is surrounded by a layer of musculature.

Posterior to the primary gonopore the vaginal lumen is constricted by two consecutive large dorsal protrusions (Figs 2, 3). The anterior one is the **genital papilla**. It is formed by thick epithelium lined by a thick cuticular intima and underlaid with some musculature and some tissue containing pigment granules (Fig. 3). Laterally the genital papilla forms two longitudinal lips which are more pronounced anteriorly (Figs 6–8). Its centre forms a deep pouch which receives the spermathecal ducts at its base (Figs 2, 3, 6, 7). The posterior rim of this pouch forms a median protuberance into which the two accessory gland ducts open posteriorly (Fig. 7).

Directly opposite the genital papilla the ventral circular structure is embedded in the anteroventral vagina wall (Figs 2, 3, 6). Apart from that, the anteroventral vagina wall consists of thin simple epithelium lined by a thin cuticular intima and underlaid with some tissue containing pigment granules, but lacking any obvious musculature (Fig. 3).

Conversely, the posteroventral vagina wall is greatly enlarged to form a prominent, bilobed **posteroventral vagina pouch** (Figs 2, 3, 6–11). This pouch is about 250 µm long including the lateral lobes and about 350 µm wide. Each lobe is about 100 µm long and encloses an anteriorly directed channel. The posteroventral vagina pouch is a very voluminous structure mainly consisting of very thick (up to about 100 µm) endocuticle. It is internally ornamented with long pointed protuberances lined by a thin apical layer that stains intensely blue with Richardson's stain (Fig. 11). The underlying cuboidal epithelium consists of very large cells with very large nuclei. The posteroventral vagina pouch is dorsally complemented by the **posterior dorsal protrusion** of the dorsal vagina wall (Fig. 3), a voluminous epithelial cushion which is bilobed in cross-section.

The three spherical **spermathecae** lie ventrolaterally in the 6th abdominal segment (Fig. 1). They are arranged asymmetrically on the two spermathecal ducts. One of the ducts bears a single spermatheca (about 130 µm diameter). The other bifurcates to bear two somewhat smaller spermathecae (about 110 µm diameter each), one lying near the single large spermatheca, the other on the opposite side of the abdomen.

The cuticular lining of the spermathecal capsules is smooth, not very thick, dark brown and heavily sclerotized (Figs 12–15). It is surrounded by stratified or pseudostratified epithelium composed of at least two distinct cell types. There are voluminous gland cells containing large nuclei and secretory reservoirs (Figs 13–15) with tiny end apparatuses that traverse the spermathecal wall. Sometimes these end apparatuses are associated with tiny hollow denticles formed by the spermathecal wall (Fig. 15). The gland cells are evenly distributed around the capsules including the area around the opening of the spermathecal ducts. They are underlaid by flat epithelial cells with flat nuclei (Fig. 13). The spermathecal epithelium may be surrounded by an additional laminar layer whose nature could not be discerned unambiguously. Finally, the entire structure is embedded in a mass of adipose tissue.

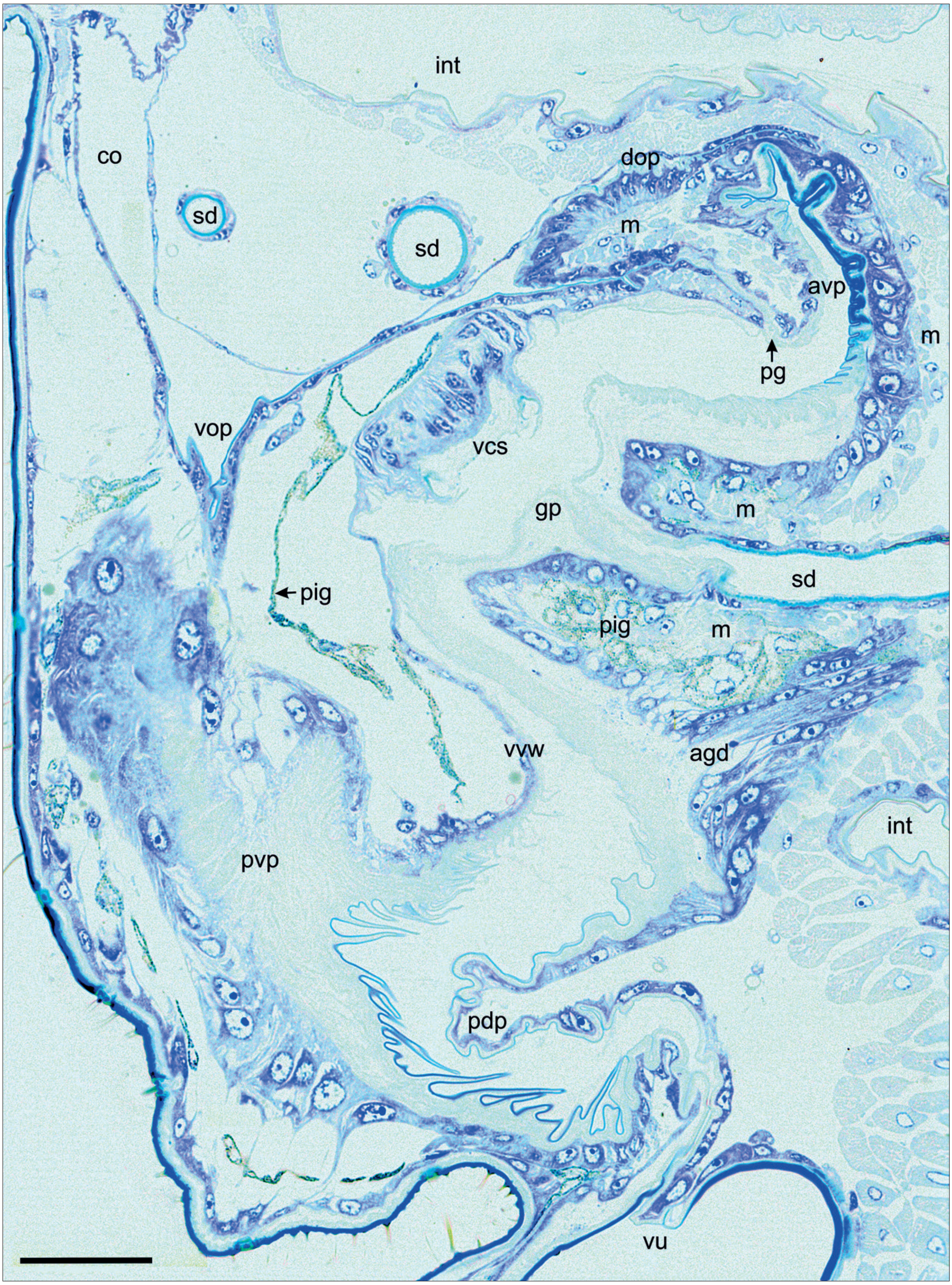
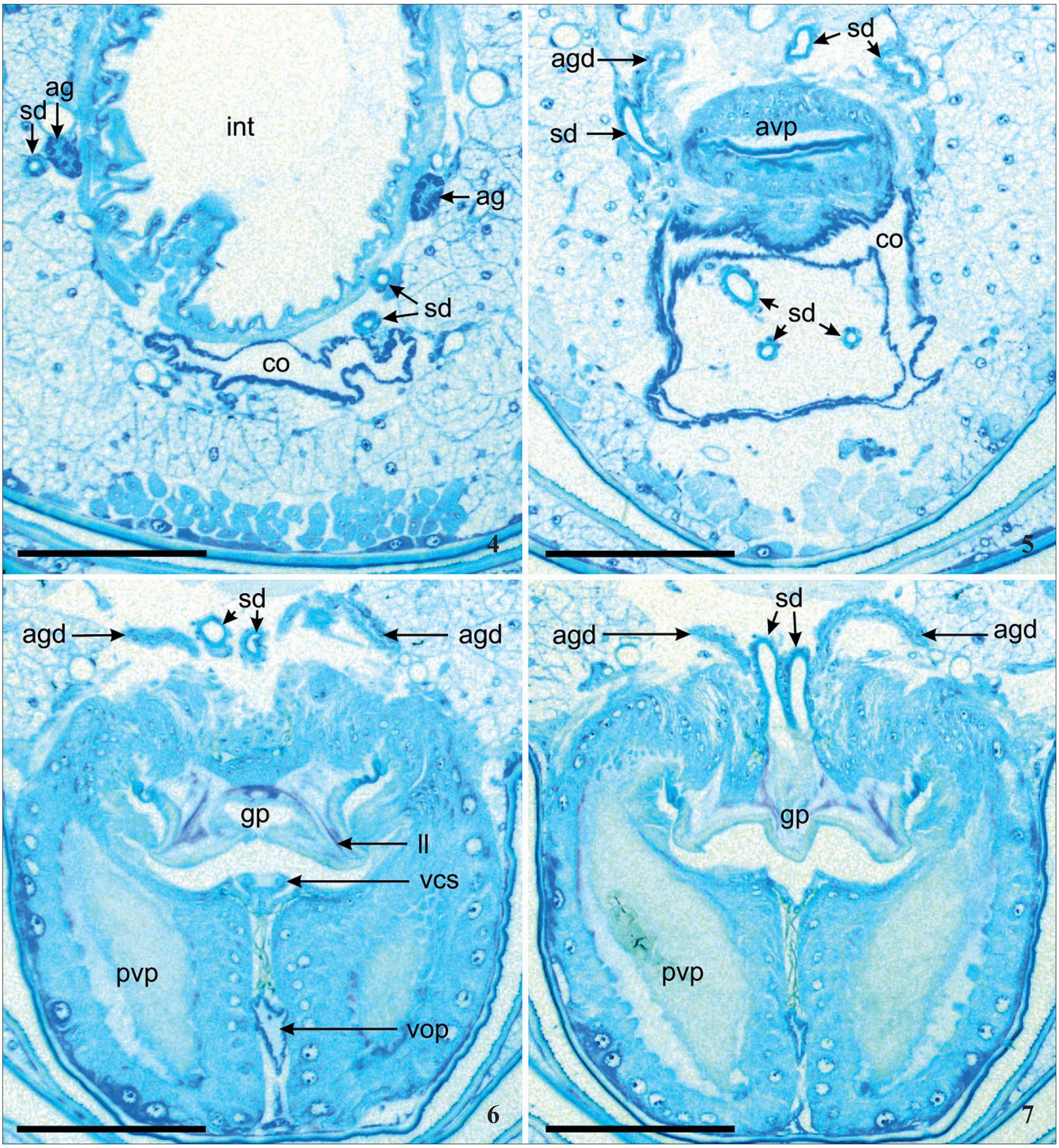
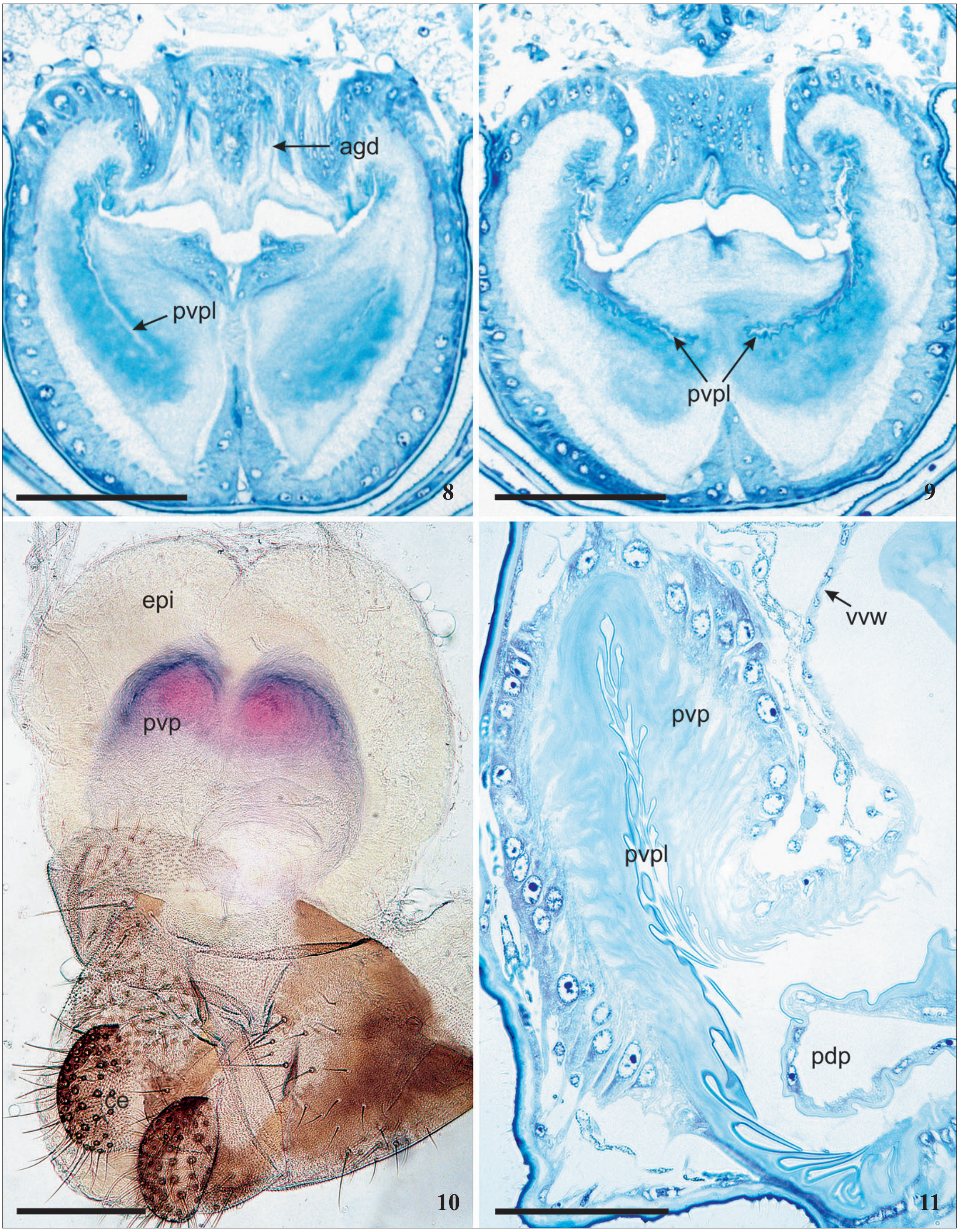


Fig. 3: *Episyrphus balteatus* (De Geer) female genitalia mid-sagittal microsection, ventral side left. Abbreviations: agd = accessory gland duct orifice; avp = anterodorsal vagina pouch; co = common oviduct; dop = dorsal oviduct pouch; gp = genital papilla; int = intestine; m = musculature; pdp = posterodorsal protrusion; pg = primary gonopore; pig = tissue containing pigment granula; pvp = posteroventral vagina pouch; sd = spermathecal duct; vcs = ventral circular structure; vop = ventral oviduct pouch; vu = vulva, vvw = ventral vagina wall. Scale bar = 50 µm.



Figs 4–7: *Episyrphus balteatus* (De Geer) female genitalia, transverse microsections at levels indicated in Fig. 2, ventral side down. Abbreviations: ag = accessory gland; agd = accessory gland duct; avp = anterodorsal vagina pouch; co = common oviduct; gp = genital papilla; int = intestine; ll = lateral lips of genital papilla; pvp = posteroventral vagina pouch; sd = spermathecal duct; vcs = ventral circular structure; vop = ventral oviduct pouch. Scale bars = 100 µm.

The **spermathecal ducts** insert tangentially at the spermathecal capsules without any obvious constrictions or valves. From there they run posteriorly with some loops, first ventrolaterally and then ventrally between gut and common oviduct, finally turning dorsolaterally anterior to the vagina and meeting the vagina dorsomedially to open into the genital papilla (Figs 1, 4–7). Their total length is about 1000 µm, of which the ducts bearing the smaller two spermathecae share a common section of about 300 µm.



Figs 8–11: *Episyrphus balteatus* (De Geer) female genitalia. – **8**, **9**: Transverse microsections at levels indicated in Fig. 2, ventral side down; – **10**: Posteroventral vagina pouch and terminalia, whole mount, ventral view; – **11**: Parasagittal microsection through lateral lobe of posteroventral vagina pouch, ventral side left. Abbreviations: agd = accessory gland duct; ce = cerci; epi = epithelial lining of posteroventral vagina pouch; pdp = posterodorsal protrusion; pvp = posteroventral vagina pouch; pvpl = posteroventral vagina pouch lumen; vvw = ventral vagina wall. Scale bars = 50 µm (Fig. 11); = 100 µm (Figs 8, 9); = 200 µm (Fig. 10).

The spermathecal ducts consist of two structurally distinct portions (Figs 12, 16–19). The anterior portions are only about 7 to 10 µm wide with a thin transversely wrinkled cuticular intima, whose basal layer stains intense blue with Richardson's stain, while the apical (i. e. internal) layer remains unstained and probably consists of sclerotized exocuticle (Figs 16, 18). They are lined by thin simple epithelium and lack musculature. The posterior portions have a wider lumen of about 20 µm diameter (Figs 17, 19). Their thin cuticular intima stains azure blue with Richardson's stain. It is ornamented by delicate longitudinal ridges apically. Basally it is transversely wrinkled, lined by a thin simple epithelium and accompanied by sparse longitudinal muscles. The transition between the two portions is at about half the duct length in the duct bearing a single spermatheca. In the other duct it is at the point of bifurcation.

The paired **accessory glands** also consist of two morphologically and anatomically distinct portions (Figs 1, 20). The anterior, glandular portions are tubular and very long (up to about 3.500 µm long and about 100 µm wide). They consist of glandular epithelium which stains very intensely with Richardson's stain and contains large nuclei and secretory reservoirs with distinct end apparatuses (Fig. 21). The narrow lumina are lined by a thin cuticular intima. The posterior portions constitute the tubular gland ducts, about 300 µm long and about 30 µm wide, with a wider part of about 50 µm near the vagina. They are composed of a thin cuticular intima lined by thin simple epithelium and surrounded by a layer of musculature (Fig. 22). In one of the microsectioned specimens, the ducts contained a two-component secretion consisting of a blue staining liquid with apparently empty, and therefore probably lipidoid droplets suspended in it (Fig. 23). When empty the duct lumina can be very narrow and hard to detect, especially where the ducts traverse the vagina wall posterolateral to the spermathecal ducts. The serial microsections show that the ducts are continued within the cuticular intima of the vagina by narrow channels which converge anteromedially and open next to each other on the genital papilla, closely behind the orifice of the spermathecal ducts (Fig. 28).

The **ventral circular structure** (vcs) lies in the ventral vagina wall just across from the genital papilla (Figs 2, 3, 6). In dorsoventral view it is characterized by its perfectly circular structure of about 45 µm in diameter (Figs 24–29), consisting of three concentric areas: a round central area of smooth, homogenous appearance (area A, diameter about 25 µm); a surrounding circular area which has very distinct inner and outer edges and is ornamented with sparse tiny spicules (area B, width about 5 µm); an outer circular area, which appears homogenous with a somewhat indistinct outer edge (area C, width about 5 µm).

Microsections (Figs 25–29) reveal that the central area (A) is a circular pad of thick endocuticle. The surrounding area (B) corresponds to a circular trench which is lined by denser cuticle and internally ornamented by tiny spicules. Distally the trench is partially covered by a delicate cuticular lip which extends from its outer margin. The outer circular area (C) is formed by a distal extension of the underlying epithelium. The structure of the entire vcs is thus somewhat reminiscent of a sucker or a press stud. It is born on a base of columnar epithelial cells which are much higher and narrower, but otherwise similar to those of the surrounding vagina wall epithelium.

In all four microsection series the vcs is positioned exactly opposite the genital papilla and its shape exactly fits the area surrounding the orifice of the spermathecal ducts (Figs 3, 6, 25, 27–29). In whole mounts, however, the position of the vcs is often shifted due to distortions of the flexible vagina wall.

The vcs was also found in other syrphid taxa, covering all major clades of the family (Fig. 30, Tab. 1). The structure could be unambiguously identified due to its clearly defined circular shape and its position in the anteroventral part of the vagina. Only in 2 out of 19 studied species we were not able to detect this structure,

Figs 12–23: *Episyrphus balteatus* (De Geer), spermathecae and accessory glands. – **12**: Two of the three spermathecae, whole mount. Explanation: Arrows indicate transition between anterior and posterior portions of spermathecal ducts; – **13–15**: Microsections of spermathecal wall showing stratified or pseudostratified epithelium, gland cells with large nuclei and end apparatus. Explanations: simple arrow = gland cell nucleus; double arrow = gland cell end apparatus. **Figs 16–19**: Microsections of spermathecal ducts, note differentiation of cuticular intima. – **16**: Anterior portion in cross-section; – **17**: Posterior portion in cross-section; – **18**: Anterior portion in longitudinal section; – **19**: Posterior portion in oblique section. **Figs 20–23**: Accessory glands. – **20**: Part of anterior glandular portion (on top) and gland duct, whole mount; – **21–23**: Microsections; – **21**: Gland in cross-section. Explanations: arrow = gland cell nucleus with nucleolus; double arrow = gland cell end apparatus; – **22**: Empty accessory gland duct in cross-section; –**23**: Accessory gland duct containing two component secretion in cross-section. Scale bars = 20 µm (Figs 13–19, 21–23); = 200 µm (Figs 12, 20).

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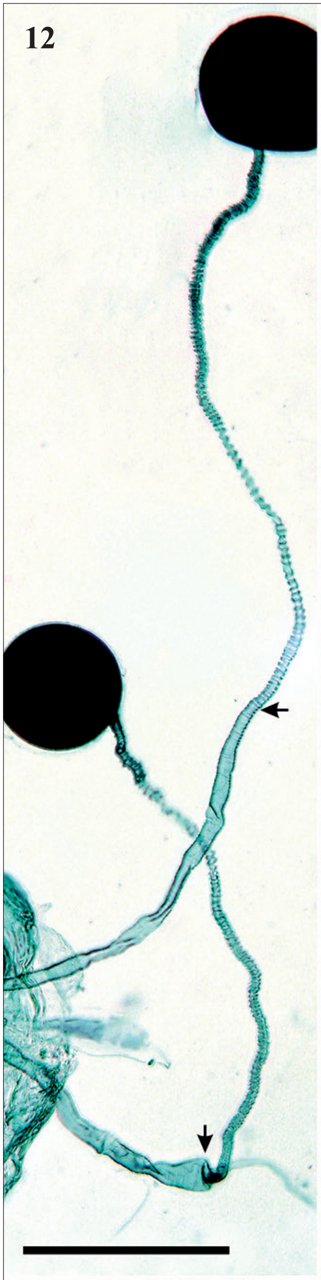

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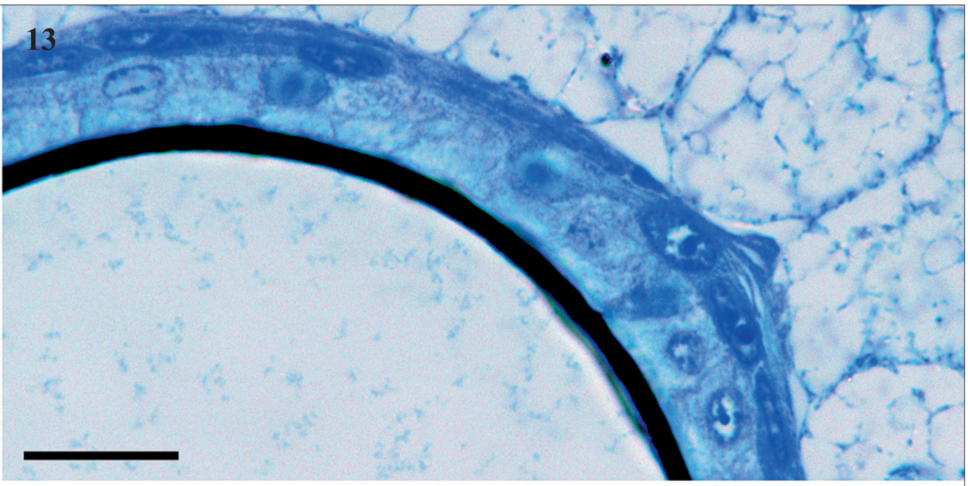

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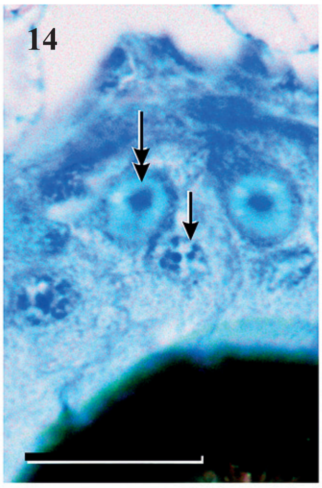

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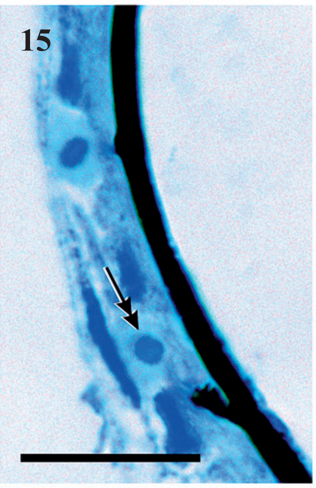

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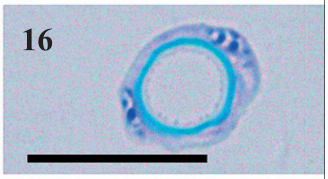

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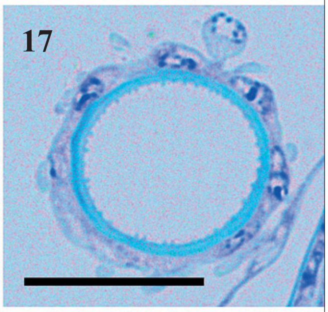

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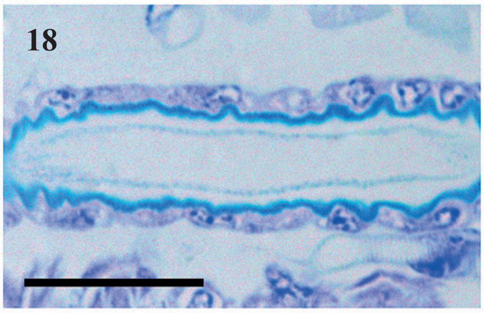

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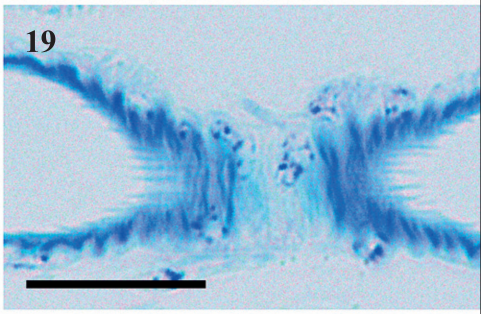

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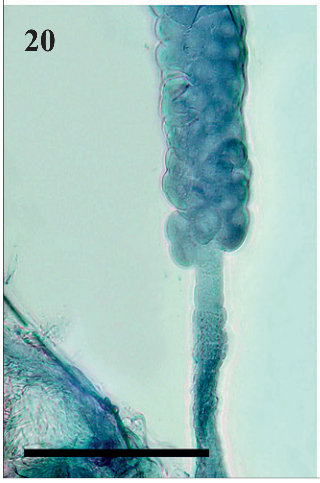

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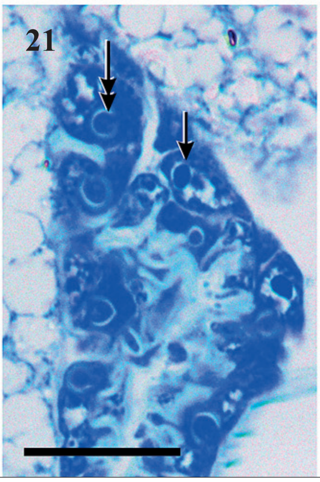

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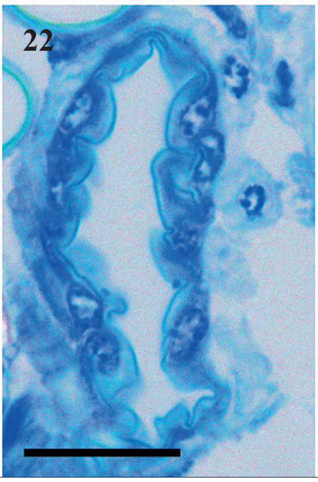

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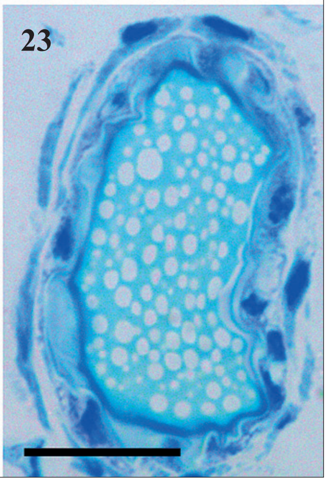

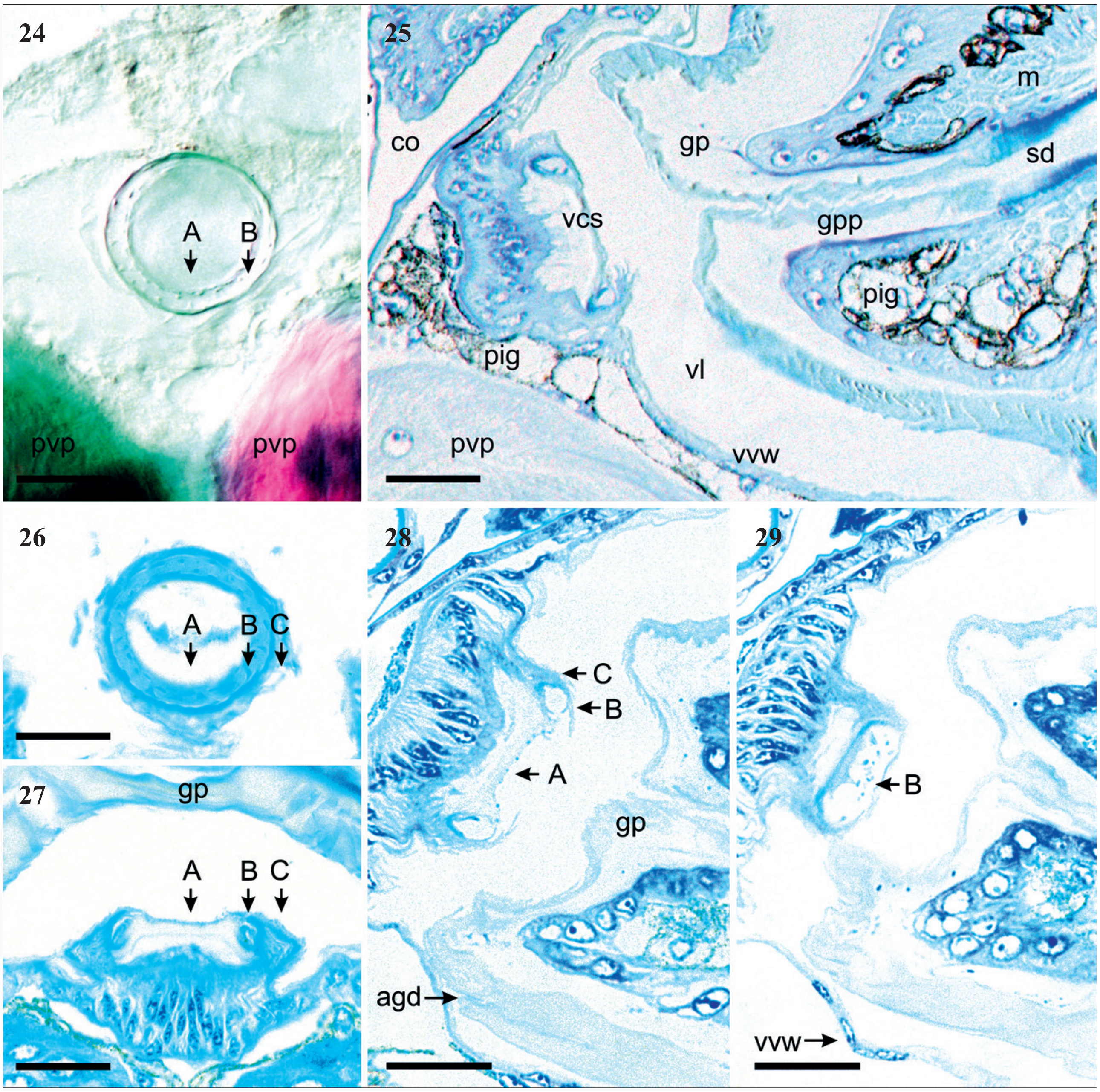

Figs 24–29: *Episyrphus balteatus* (De Geer), ventral circular structure. – **24**: Whole mount in dorsoventral view; – **25**: Mid-sagittal microsection; – **26**: Frontal microsection (reconstruction from four consecutive sections); – **27**: Transverse microsection; – **28–29**: Mid-sagittal microsections. Abbreviations: A = central area of vcs; B = circular trench with spicules; C = outer circular area; agd = accessory gland duct orifice; co = common oviduct; gp = genital papilla; gpp = genital papilla pouch receiving the two spermathecal ducts; m = muscle; pig = underlying tissue containing pigment granula; pvp = posteroventral vagina pouch; sd = spermathecal duct; vcs = ventral circular structure; vl = vagina lumen; vvw = ventral vagina wall. Scale bars = 20 µm.

possibly due to the poor quality of the available material. The appearance of the vcs varies little in the majority of the studied Syrphidae, but it tends to be somewhat smaller in the Syrphinae, while in *Microdon analis* (Macquart, 1842) it is much larger, darkened, and internally ornamented with very dense long and slender bristles (Fig. 30 top).

Discussion

The present study greatly expands our knowledge about the morphology and histology of the internal female genitalia of *Episyrphus balteatus* and Syrphidae in general. It also allows us to correct some previous misconceptions. In the present study, the identification of the lateral oviducts, accessory glands, spermathecae and their ducts is reliably based on individual tracing from origin to end and the respective histological findings are documented by microphotography. We therefore conclude that the obvious incongruences with the findings of Nayar (1965) and Sareen et al. (1989) are based on the confusion and/or misinterpretation of structures by these authors.

The ventral circular structure (vcs) stands out as the most important finding of our study. It was not mentioned by Nayar (1965) or any other previous publication, although some illustrations of Hippa (1986) include a small and unlabeled circular structure possibly indicating its presence. We found this new and distinctive structure in all subfamilies of Syrphidae as defined by Mengual et al. (2015), i. e. Microdontinae, “Eristalinae”, Pipizinae and Syrphinae (Fig. 30) and thus established its presence in the ground plan of the family. Whether its plesiomorphic condition more resembles the large and sclerotized form found in *Microdon analis*, a representative of the most basal clade of Syrphidae (Thompson 1972, Mengual et al. 2015), or the smaller circular form found in the other syrphid taxa remains to be resolved by outgroup comparison. So far none of the relevant aschizan families have been studied in sufficient detail. Available descriptions of the female reproductive tract of Phoridae (Goto 1983, Benner & Curtis 1988, Zacaro & Porter 2003) and Pipunculidae (Kozánek & Belcari 1997) do not mention any structure comparable to the vcs.

The lack of outgroup data impedes any speculation about the phylogenetic origins of the vcs and its potential homology with comparable structures in Schizophora. The fertilization chamber of some Calyptrates such as *Musca domestica* Linnaeus, 1758 (Leopold et al. 1978) and the ventral receptacle in many acalyptrate taxa (e. g. Sturtevant 1925, 1926; Miller 1965; Solinas & Nuzzaci 1984; Kotrba 1993, 2000) occupy an anatomically similar position opposite to the orifice of the spermathecal ducts. Remane’s (1952) positional criterion of homology is thus fulfilled. His structural criterion of homology must be negated though. None of the fertilization chambers or ventral receptacles described to date structurally resemble the vcs of *E. balteatus*, which does not constitute a chamber at all, but rather a protrusion of the vagina wall. Finally, so far, no comparable or transitional structures have been described in any of the relevant aschizan outgroups, leaving Remane’s third criterion of homology unfulfilled.

Moreover, we know nothing about the functionality of the vcs. In the schizophoran taxa, the anterior egg pole which carries the micropyle is shunted into the orifice of the fertilization chamber or ventral receptacle during oviposition and this is where fertilization occurs (e. g. Leopold et al. 1978, Miller 1965, Solinas & Nuzzaci 1984, Kotrba 1993). Considering the vast difference in length between the vagina of *E. balteatus* (about 250 µm) and its egg [about 1000 µm (Chandler 1968)], an analogous functionality of the vcs seems unlikely. Moreover, the vagina wall of *E. balteatus* lacks substantial musculature to hold and maneuver the egg. Still the vcs could be involved in the process of fertilization. Videos of *E. balteatus* oviposition show that the egg is expelled toward the rear while the abdomen is moved forward across the substrate (Gries 2000, Wyss 2013). There is a short pause when the egg is expelled about half way. In this state the anterior egg pole has a ventral position within the vagina in the vicinity of the vcs. Under these circumstances it appears possible that the vcs constitutes the fertilization site. The narrow lumen of its circular trench could accommodate a small amount of spermatozoa for this purpose, the spicules within serving as their orientation and mechanical support.

Alternatively the vcs could serve as a stopper to prevent unwarranted sperm loss from the spermathecal ducts. The microsection series show a close proximity and a perfect fit of the genital papilla and the vcs. While the circular pad in the center of the vcs (area A) rests against the orifice of the genital papilla, the cuticular lip extending from the outer margin of the circular trench (area B) and the adjacent epithelial region (area C) might help to achieve a tight seal. This seal would be in effect as long as the vagina remains empty and would be opened as soon as the ventral and dorsal vagina walls are moved apart, e. g. by an egg passing through or during copulation.

The discussed functions as a stopper in times of rest and as a fertilization site during oviposition are not mutually exclusive. The vcs could allow a limited amount of spermatozoa to enter and accommodate themselves within the ring channel while it is effectively sealing the opening of the spermathecal ducts. It would later release the spermatozoa onto the egg micropyle during oviposition.

Tab. 1: Studied material of Syrphidae with subfamily, species name and author, number of specimens, collection data, preservation method, and verification of presence of vcs. Abbreviations: CRC = Costa Rica; ECU = Ecuador; GER = Germany; USA = United States of America; n = number of specimens.

Species	n	locality	collector	date	preservation	vcs
MICRODONTINAE						
Microdon analis (Macquart, 1842)	2	GER: Bavaria, Starnberger Seegebiet	F. Stockler	25.v.1946 and 10.vi.1951	pinned	present
ERISTALINAE						
Eristalis arbustorum (Linnaeus, 1758)	2	GER: Saxony, Görlitz, Tischbrücke	R. Weniger	01.viii.2016	fresh	present
Eristalis dimidiatus Wiedemann, 1830	1	USA: MD, Catoctin Mountain Park	M. Kotrba	08.x.1994	fresh	present
Eristalis nemorum (Linnaeus, 1758)	1	GER: Saxony, Görlitz, alter Friedhof	R. Weniger	17.viii.2016	fresh	present
Neoascia meticulosa (Scopoli, 1763)	6	GER: Bavaria, Kempten, Leuthenhofener Moos	unknown	02.vii.1976	alcohol	not found
Orthonevra nitida (Wiedemann, 1830)	1	USA: MD, College Park	M. Kotrba	03.vii.1994	fresh	present
Sphegina clunipes (Fallén, 1816)	4	GER: Bavaria, Nürnberg, Reichswald	Krause	1991	alcohol	present
PIPIZINAE						
Pipiza cf. *notata* Meigen, 1822	1	GER: Bavaria, München, Nympenburg	R. Weniger	02.–11.v.2016	fresh	present
SYRPHINAE						
Epistrophe eligans (Harris, 1780)	1	GER: Bavaria, München, Nympenburg	R. Weniger	02.–11.v.2016	fresh	not found
Episyrphus balteatus (De Geer, 1776)	18	GER: Bavaria, München, Obermenzing	M. Kotrba	22.vii.2004	fresh	present
	4	GER: Bavaria, Oberstdorf, Schochen	D. Doczkal	04.vii.2014	alcohol	present
	2	GER: Bavaria, München, Nympenburg	R. Weniger	11.v.2016	fresh	present
Eupeodes americanus Wiedemann, 1830 aggr.	1	USA: VA, Colonial Beach	M. Kotrba	16.ix.1994	fresh	present
Eupeodes latifasciatus (Macquart, 1829)	2	GER: Bavaria, München, Obermenzing	R. Weniger	02.–11.v.2016	fresh	present
Ocyptamus lineatus (Macquart, 1846)	1	ECU: Tiputini Biodiversity Station	M. Kotrba	13.viii.1999	fresh	present
Melanostoma mellinum (Linnaeus, 1758)	2	GER: Bavaria, München, Obermenzing	R. Weniger	02.–11.v.2016	fresh	present
Melanostoma scalare (Fabricius, 1794)	3	GER: Bavaria, München, Obermenzing	R. Weniger	02.–11.v.2016	fresh	present
Meliscaeva auricollis (Meigen, 1822)	1	GER: Bavaria, München, Nympenburg	R. Weniger	02.–11.v.2016	fresh	present
Platycheirus albimanus (Fabricius, 1781)	1	GER: Bavaria, München, Nympenburg	R. Weniger	02.–11.v.2016	fresh	present
Platycheirus occultus Goeldlin de Tiefenau, Maibach & Speight, 1990	1	GER: Bavaria, München, Obermenzing	R. Weniger	02.–11.v.2016	fresh	present
Sphaerophoria spec.	3	GER: Bavaria, München, Obermenzing	R. Weniger	02.–11.v.2016	fresh	present
Syrphus vitripennis Meigen, 1822	1	GER: Bavaria, München, Nympenburg	R. Weniger	02.–11.v.2016	fresh	present
Toxomerus cf. *elisa* (Hull, 1951)	1	CRC: San José, San Gerardo de Dota	M. Kotrba	01.viii.1995	fresh	present
Toxomerus marginatus (Say, 1823)	3	USA: MD, College Park	M. Kotrba	11.vii.1994	fresh	present

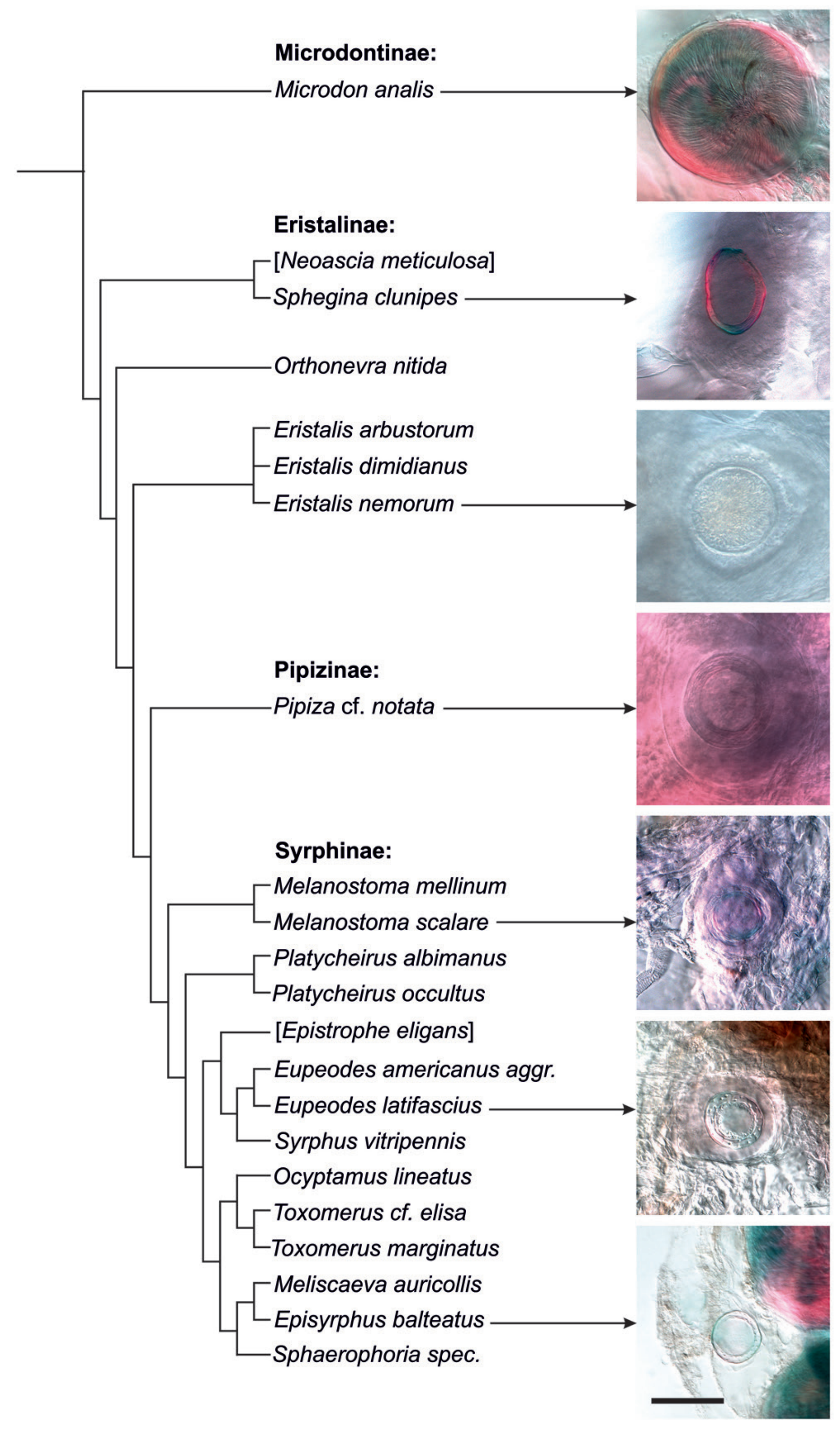

Fig. 30: Occurrence of the ventral circular structure in Syrphidae. The phylogeny follows Mengual (2015), Mengual et al. (2015) and Mengual, pers. comm. The vcs was detected in all but the two species in brackets and is illustrated for some representatives. Scale bar = 50 µm (all photographs to same scale).

Another possible functional context for the vcs, which involves a direct interaction with the male genitalia during copulation, appears much less likely. In Syrphidae the entire hypandrium is inserted into the female during copulation and the dorsal side of the male terminalia comes to rest ventrally in the vagina (Shatalkin 1981). The male's superior lobes which normally occupy a dorsal plane to the aedeagus (Nayar 1965, "harpagones") thus end up near the female's posteroventral pouch. The superior lobes are about 120 µm long and 70 µm wide in Nayar's (1965) illustrations. This roughly matches the lateral channels within the female's posteroventral pouch, into which they are probably inserted, thus anchoring the male genitalia within the female. At the same time the aedeagus, which is originally positioned ventromedially of the superior lobes, obtains a dorsally directed position within the vagina, where it connects with the genital papilla. None of the male structures described by Nayar (1965) or Shatalkin (1981) seems suited to interact with the vcs. Only in the Microdontinae is the aedeagus bifurcated into a dorsal and a ventral process (Speight 1987, Reemer & Ståhls 2013), rendering a functional interaction with the vcs possible. Concurrently we found that *Microdon analis* has a much larger vcs than all other inspected Syrphidae.

Further studies are needed to elucidate the functional context of the vcs in Syrphidae, its evolution within the family, its presence in the relevant outgroups, and its potential homology with comparable schizophoran structures such as the fertilization chamber and the ventral receptacle. Because the vcs is small and most often colorless, it is sometimes hard or impossible to detect in whole mounts and may easily be overlooked, as was the case in previous studies of Syrphidae. Therefore, special emphasis should be put on the relevant region ventrally opposite the opening of the spermathecal ducts. In higher Diptera this is the pivotal region where fertilization generally occurs, i. e. where the intra- and intersexual competition of genes for entrance into the next generation culminates. Therefore, studies on the functional morphology and physiology of the respective structures may not only provide valuable characters for the reconstruction of deeper level phylogeny, but they may also contribute to unravel the story of sexual evolution in general – a story about conflict and collaboration, about an intersexual arms race, internal courtship, sperm competition and cryptic female choice. Our study provides a sound foundation for the inclusion of these important aspects in future surveys in Syrphidae and related taxa.

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