Arthropod Structure & Development 64 (2021) 101084

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

Sperm ultrastructure in arrhenotokous and thelytokous Thysanoptera

Stephanie Krueger*, Gerald Moritz

Martin-Luther University Halle– Wittenberg, Institute of Biology, Department Zoology, Heinrich-Damerow-Str.4, 06120 Halle, Germany

ARTICLE INFO

Article history:
Received 22 January 2021
Received in revised form
17 June 2021
Accepted 17 June 2021
Available online 19 July 2021

Keywords:
Endosymbionts
Haplo-diploidy
Mating system
Prospermatogeny
Synspermatogeny
Wolbachia

ABSTRACT

Thysanoptera are haplo-diploid insects that reproduce either via arrhenotoky or thelytoky. Beside genetically based thelytoky, this reproduction mode can also be endosymbiont induced. The recovery of these females from their infection again leads to the development of males. Functionality of these males ranges widely, and this might be associated with sperm structure.

We analyzed the sperm ultrastructure in three different species belonging to both suborders with different reproduction systems via electron microscopy. Beside the different reproduction modes, and adaptations to their life style, the arrhenotokous species *Suocerathrips linguis* (Thysanoptera: Tubulifera) and *Echinothrips americanus* (Thysanoptera: Terebrantia) possess typical thysanopteran-like sperm structure. But endosymbiont-cured males from the thelytokous species *Hercinothrips femoralis* (Thysanoptera: Terebrantia) possess several malformed spermatozoa and a large amount of secretions in their testes. Spermiophagy seems to be typical. It indicates a highly conserved mechanism of the male developmental pathways, despite the observed decay. However, this decay would explain why in some species no stable arrhenotokous line can be re-established.

1. Introduction

Beside the economic importance of some thrips species, the order Thysanoptera provides interesting prospects for analyzing the evolution of reproduction systems.

Thysanoptera are haplo-diploid insects. Most common is the arrhenotokous reproduction mode, in which females arise from fertilized diploid eggs, whereas males develop from unfertilized haploid ones. Some species reproduce via thelytoky, whereby females produce exclusively female progeny and only very rare males (e.g., *Heliothrips haemorrhoidales, Hercinothrips bicinctus, Hercinothrips femoralis, Helionothrips errans, Scirtothrips longipennis, Leucothrips nigripennis, Chaetanaphothrips orchidii*; Lewis 1973). Furthermore, some species show a reproductive polymorphism, where populations in different geographical regions reproduce via arrhenotoky or thelytoky (e.g., *Aptinothrips rufus, Franklinothrips vespiformis, Haplothrips tritici, Taeniothrips inconsequens, Thrips tabaci*; Arakaki et al., 2001; Nault et al., 2006; van der Kooi and Schwander, 2014a).

Thelytokous reproduction in Thysanoptera can be genetically determined (e.g., *Aptinothrips stylifer, A. karnyi*, *Thrips tabaci, Heliothrips haemorrhoidalis*; Jacobson et al., 2013; van der Kooi and Schwander, 2014b; Nguyen et al., 2015) or endosymbiont-mediated (*A. rufus, F. vespiformis, H. femoralis*; Pintureau et al., 1999; Arakaki et al., 2001; Kumm and Moritz, 2008; van der Kooi and Schwander, 2014a, b). Only Wolbachia, Cardinium and Rickettsia have been shown to induce thelytoky (Dobson et al., 2002; Kageyama et al., 2012), but there also seems to be a number of unknown endosymbionts inducing thelytoky in haplo-diploid species (Ma and Schwander, 2017).

Endosymbiont mediated thelytoky can be cured by antibiotic or heat treatment (Stouthamer et al., 1990; Kumm and Moritz, 2008), whereas males occur in haplo-diploid insects. Functionality of these males ranges widely. Whereas males in *Trichogramma* are fully functional (Stouthamer et al., 1990a,b), antibiotic-generated males of *Leptopilina clavipes* (Hymenoptera: Figitidae) and *Telenomus nawai* (Hymenoptera: Scelionidae) are able to inseminate arrhenotokous females and fertilize their eggs, but cannot do this for thelytokous females (Arakaki et al., 2000; Pannebakker et al., 2005). Also *H. femoralis* and *F. vespiformis* males are able to produce and transfer sperm, but these sperm seem not to be used for fertilization (Arakaki et al., 2001; Kumm and Moritz, 2008). A similar situation is known in some hymenopteran species (*Aphytis lingnanensis, A. diaspidis:* Zchori-Fein et al., 1995; *Eretmocercus*

* Corresponding author.
E-mail addresses: stephanie.krueger@zoologie.uni-halle.de (S. Krueger), gerald.moritz@zoologie.uni-halle.de (G. Moritz).

https://doi.org/10.1016/j.asd.2021.101084

mundus: de Barro and Hart, 2001) although in *Encarsia formosa* (Hymenoptera: Aphelinidae) males are not able to inseminate females despite sperm being produced (Zchori-Fein et al., 1992).

In most endosymbiont cured species for which functionality of sexual traits has been reported, no morphological analyses of male structures, especially the spermatozoa, are available although these might be associated with functional changes.

In this study, three representatives of Thysanoptera were selected from the two suborders (Tubulifera, Terebrantia) with different reproductive strategies and endosymbiont-infection status. The ultrastructure of spermatozoa was analyzed with regard to functionality and stability of this trait under different reproductive modes and life style.

In Thysanoptera spermatozoa are typically threadlike with a length between 60 μm (*Aeolothrips intermedius*, Paccagnini et al., 2010) and 1200 μm (*Megathrips inermis*, de Marzo, 2005), but usually measure 100–200 μm (Paccagnini et al., 2006, 2007, 2009). All spermatozoon components are helicoidally arranged along the sperm length; all components are close together. The spermatozoa consist of a bizarre flagellum of 27 microtubules, built from 9 doublets with dynein arms, 9 doublets without dynein arms and 9 singlets. These elements are arranged in a fixed asymmetric pattern of 9 groups, beginning with a single microtubular singlet and end with an arm-less microtubular doublet (Dallai et al., 1991). If less than 27 microtubules are present, this is a result of shifting of short axonemes along the length of the spermatozoa (Paccagnini et al., 2009).

Concerning the further structures, species differ in the two suborders Tubulifera and Terebrantia. In Tubulifera the cross section outline is almost circular or elliptical. The large mitochondrion flanks the nucleus and occupies one quarter in sperm cross section, and a thin acrosome is visible. In Terebrantia, spermatozoa have a bilobed transverse profile, whereas the nucleus is located laterally in one of the two lobi. The most anterior region contains the nucleus and a dense body, the posterior region bears the small mitochondrion, while an acrosome is lacking (Baccetti et al., 1969; Bode, 1988; Ananthakrishnan and Balu, 1990; Paccagnini et al., 2009, 2010).

Suocerathrips linguis Mound and Marullo, 1994 (Tubulifera: Phlaeothripidae) is used here as a representative of the Tubulifera. This species feeds on fungal hyphae and spores on *Sansevieria* species (Moritz et al., 2004; Gehlsen, 2009), and presumably originates from tropical Central Africa and dry regions in East- and South Africa (Moritz et al., 2004). *S. linguis* is arrhenotokous, subsocial and exhibiting brood care and lives in large groups on the host plant (Moritz, 2002; Moritz et al., 2004; Gehlsen, 2009). Males and females mate several times, and a single mating can last several hours (Moritz et al., 2004). Sex ratio is highly female biased: 0.25 (Gehlsen, 2009). Our lab population is partly infected with Wolbachia (Kumm and Moritz, 2008), although the impact of this infection is not yet documented.

Echinothrips americanus Morgan, 1913 (Terebrantia: Thripidae) and *H. femoralis* (Reuter, 1891) represent here the suborder Terebrantia. *E. americanus* is a polyphagous species with hosts in about 24 plant families (Vierbergen, 1998). Small chlorotic areas and shallow feeding punctures are typical feeding damage mainly on ornamental plants (Oetting and Beshear, 1993; Oetting et al., 1993; Trdan et al., 2003), and this species is considered a serious pest. It is native to eastern USA (Stannard, 1968) but has spread from North-Eastern USA and Canada to Europe, Asia and North–Australia (summarized in Krueger et al., 2015). This is an arrhenotokous reproducing, solitary species with a female biased sex ratio of 0.3 (Krueger et al., 2015). The lab population used here is highly infected with Wolbachia and Cardinium (Kumm and Moritz, 2008; Chuttke, 2021). Females mate only once, whereas males are polygynous (Krueger et al., 2017).

H. femoralis (Terebrantia: Thripidae) is also a polyphagous species with more than 50 known host species (summarized in Trdan et al., 2007). The species is economically important for many plants, e.g., bananas, cowpea, cucumber, sugar beet, cotton, ground nuts, figs and ornamental plants (Moritz et al., 2013). *H. femoralis* originates from Africa, but is now widespread around the world in tropical and subtropical areas (Mound, 1966; Roditakis et al., 2006), but also common in temperate areas in greenhouses (Moritz et al., 2009). *H. femoralis* reproduces via endosymbiont-induced thelytoky. As stated before, males can be produced by antibiotic treatment, and these are able to produce sperm and inseminate females, although these spermatozoa are presumably unable to effect fertilization (Kumm and Moritz, 2008).

2. Material and methods

2.1. Rearing method

Main cultures of the insects were reared at 23 ± 1 °C, 50% RH, light regime L:D 16:8 (light on 6:00 am, 5.000 lux) on species specific host plants in acrylic cages (50 × 50 × 50 cm, 2 sides covered with fine mesh).

E. americanus was fed on potted *Phaseolus vulgaris, Gossypium.* and *Hibiscus* species. Commercial organic lemon fruits, *Ocimum basilicum* and *Apium graveolens* var. *dulce* served as host plant for *H. femoralis. S. linguis* was reared *on Sansevieria* species.

2.2. Specimen collection S. linguis

S. linguis is a subsocial species (Moritz, 2002). Individual rearing or marking is not possible without massive disorder and possible negative effects on reproduction (personal communication, Moritz). Additionally, males and females are hard to distinguish. Therefore, population was checked regularly for mating pairs. When a couple was found, the individuals were picked up with a fine brush and males were fixed for electron microscopy in the same way as the other species.

2.3. Specimen collection male E. americanus individuals

Random aged females were picked from the lab culture and placed in boxes, equipped with leaves of *P. vulgaris* with petioles stuck into 1.4% agar-filled petri-dishes (Ø 6 cm, Greiner Bio-One GmbH, Austria) and a moist paper.

They were allowed to lay eggs for 3 days in the climatic chamber, afterwards females were removed, and the box checked for larvae hatching every 2–3 days. Larvae were transferred individually to Greiner plates, prepared and handled as mentioned above. They were sexed and prepared 48–72 h after adult eclosion.

2.4. Specimen collection male H. femoralis individuals

Males of *H. femoralis* were obtained after antibiotic treatment of the mother with the following protocol:

Adult random aged females from the lab culture were placed in small plastic containers (Shot glasses, 2 cl, O'canny, MäcGeiz GmbH, Landsberg, Germany), bottom side covered with gauze and top sealed with Parafilm® M (Pechiney Plastic Packaging, Chicago, IL, USA) and starved for 1d. Afterwards females were treated 2 × 3d with 100 μl antibiotic solution (rifampicin 7.5 mg/ml, in artificial diet, see Jilge et al., 2017; Chuttke, 2021), placed on the parafilm and enclosed with a second parafilm layer. The prepared containers were each placed in a second plastic container filled with 1.4% agar (*w/v*). The combined application systems were inserted into a moist cellulose paper equipped plastic box (Take-away round food cont.

450 ml, L621-3, Ø 120 × 66.5 mm, Dai Dong Tien Corporation, Vietnam) and sealed in aluminium foil. The so prepared moist chamber was kept in a climatic chamber (MLR-352h, Sanyo Electric Co., Ltd.) under the same conditions as the lab culture.

Afterwards treated females were allowed to lay eggs individually in wells of Greiner plates (CELLSTAR® cell culture multiwell plates, 12 wells, Greiner Bio-One GmbH, Austria), each equipped with 1 ml 1.4% agar (*w/v*) and a *P. vulgaris* leaf disc (Ø 1.2 cm). Females were transferred to newly prepared wells every 2–3 days until death. Plates were checked for hatching larvae every 2–3 days, which were transferred to likewise prepared Greiner wells. Animals were held under the same climatic conditions in a climatic chamber as mentioned above. Developmental stage of *H. femoralis* larvae was checked daily. They were sexed and prepared 48–72 h after adult eclosion.

2.5. Measurement of sperm length

Specimens were dissected with fine insect pins on a glass slide with Ringer solution and methylene blue to enhance contrast. Testes or vesicula seminalis in *S. linguis* were transferred to a new slide, slightly squashed with a cover glass. They were viewed either in phase contrast mode of the Leitz DMBRE (Leica, Germany) microscope fitted with a DFC 450 C camera (Leica, Germany) or were stained with DAPI (0.2 mg/ml DAPI in Aqua dest.) before squashing. As DAPI intercalates with the DNA, the measured length indicates length of the nucleus and mitochondrion, whereas under phase contrast the whole spermatozoa length is visible.

2.6. Transmission electron microscopy

Abdomina and testes of the three species were fixed in 2.5% glutaraldehyde, 3% glucose, buffered in 0.1 M Cacodylatbuffer overnight at 4 °C. After washing in buffer, samples were postfixed in 1% osmium tetroxide (2h), dehydrated and embedded in Epon 812 via acetone.

Ultrathin sections (50–70 nm) were cut using a diamond knife and an Ultracut R (Leica, Germany). Samples were stained with uranyl acetate and lead citrate (AC- 20, Leica, Germany). Observations were obtained with a JEOL TEM 1010 (at 80 kV) fitted with a Megaview III camera and software ITEM 5.0 (Soft Imaging Systems GmbH, Germany). Measurements and analyses were made with Fiji-software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997–2018).

3. Results

3.1. Suocerathrips linguis

Testes are about 800 μm in size and therefore extend almost over the whole abdominal length (Fig. 1F). They consist of one follicle, compartmentalized in various cysts with different stages of spermatogenesis (Fig. 1F). Each cyst is enclosed by a membrane and the spermatozoa within bear the same developmental stage (Fig. 1E). Stage of maturity increases from anterior to posterior. Mature sperm is transferred via the vasa deferentia to the vesicula seminalis (Fig. 1B, F), where the sperm is released from the sperm bundle and is then present individually (Fig. 1B). The cells of the vesicula seminalis are highly filled with rough endoplasmic reticulum and equipped with microvilli (Fig. 1B, D). Secreted material in different electron densities can be seen in the lumen of the seminal vesicle (Fig. 1D). Additionally, *S. linguis* possess a double paired accessory gland (acc gl I) and a single accessory gland (acc gl II, Fig. 1F). All gland ducts merge with the seminal vesicles in segment VIII and lead into the ejaculatory duct.

Sperm length in the seminal vesicle is 194.8 ± 7.2 μm (n = 97). Length under DAPI-staining reaches almost the same values: 193.7 ± 9.7 μm (n = 13). Maximum diameter of the spermatozoa is about 0.57 μm decreasing towards the posterior region to 0.27 μm. Spermatozoa are motile.

Cell organelles within the spermatozoa lie close together and are helically twisted around each other (Fig. 1C). Mitochondrion and nucleus are closely associated, whereas the acrosome is anterior to them (Fig. 1A, C, D). An electron dense layer is between the acrosome and cytoplasm membrane (Fig. 1A). Mitochondrion, nucleus and acrosome occupy a large part in the spermatozoa (Fig. 1A, C). Acrosome and mitochondrion range from the slightly anterior spermatozoa tip to the posterior spermatozoa section. The nucleus can only be seen in the more posterior region of sperm section (Fig. 3A). Diameter of the nucleus at its biggest expansion is about 0.23 μm and decrease towards the posterior region to 0.06 μm.

The mitochondrion has an elliptical to triangular shape (Fig. 1A, D, 3A), and the largest expansion is in the middle sperm region with 0.39 × 0.32 μm. A reduction in mitochondrion diameter was observed (about 0.1 × 0.05 μm) at both anterior and posterior regions.

The acrosome starts in the very anterior region of the spermatozoa, and is marked by an elliptical shape and an electron dense layer between acrosome and the cell membrane (Figs. 1A and 3A). The largest expansion of the acrosome is in the anterior sperm region with 0.3 × 0.14 μm, whereas its size decreases to 0.09 × 0.05 μm in the posterior region of the middle sperm section. The electron dense layer also decreases in size to the posterior end of the acrosome.

The number of microtubules is 27, comprising 18 microtubules-doublets and 9 microtubules-singlets, although in the most anterior and posterior regions only a few microtubules can be found (Figs. 1D and 3A). The diameter of the tubules is stable over the whole spermatozoon-length (0.029 ± 0.004 μm). Some microtubular units are provided with dynein arms (Fig. 1A).

3.2. Echinothrips americanus

The paired testes of *E. americanus* are much smaller (70 μm) compared to *S. linguis*, and contain only a single cyst of germ cells. Spermatozoa are densely packed without any particular arrangement (Fig. 2C). Only mature sperm could be observed in adult males. The drop-shaped testes lead posteriorly into the vasa deferentia. Both ducts unite in the ejaculatory bulb, where the ducts of the paired accessory glands also join. The ejaculatory bulb is connected with the ejaculatory duct, which penetrates the copulatory organ above the base of the aedeagus.

Spermatozoa are 75.3 ± 6.7 μm (n = 122) in length, under DAPI-staining 49.8 ± 10.1 (n = 50), with a maximum diameter of 0.57 × 0.38 μm. Spermatozoa are divided into two areas by membrane constrictions in their anterior and middle region (Fig. 2 A, B). Paccagnini et al. (2009) call these sections lobi. The bigger lobus (maximum extension: 0.39 μm) bears the axoneme and electron dense body, the smaller one (maximum extension: 0.30 μm) is occupied by the nucleus (Fig. 2A and B). A cytoplasm strand connects axoneme and nucleus lobe.

Cell organelles are also helically twisted around each other (Fig. 2C). The anterior sperm pole is characterized by the nucleus with its maximal extension of 0.21 × 0.18 μm (Figs. 2A and 3B). It decreases in size towards the posterior region of the spermatozoa (minimum size: 0.07 × 0.06 μm) until it can no longer be detected. The electron dense body is located beside the nucleus, and seems to

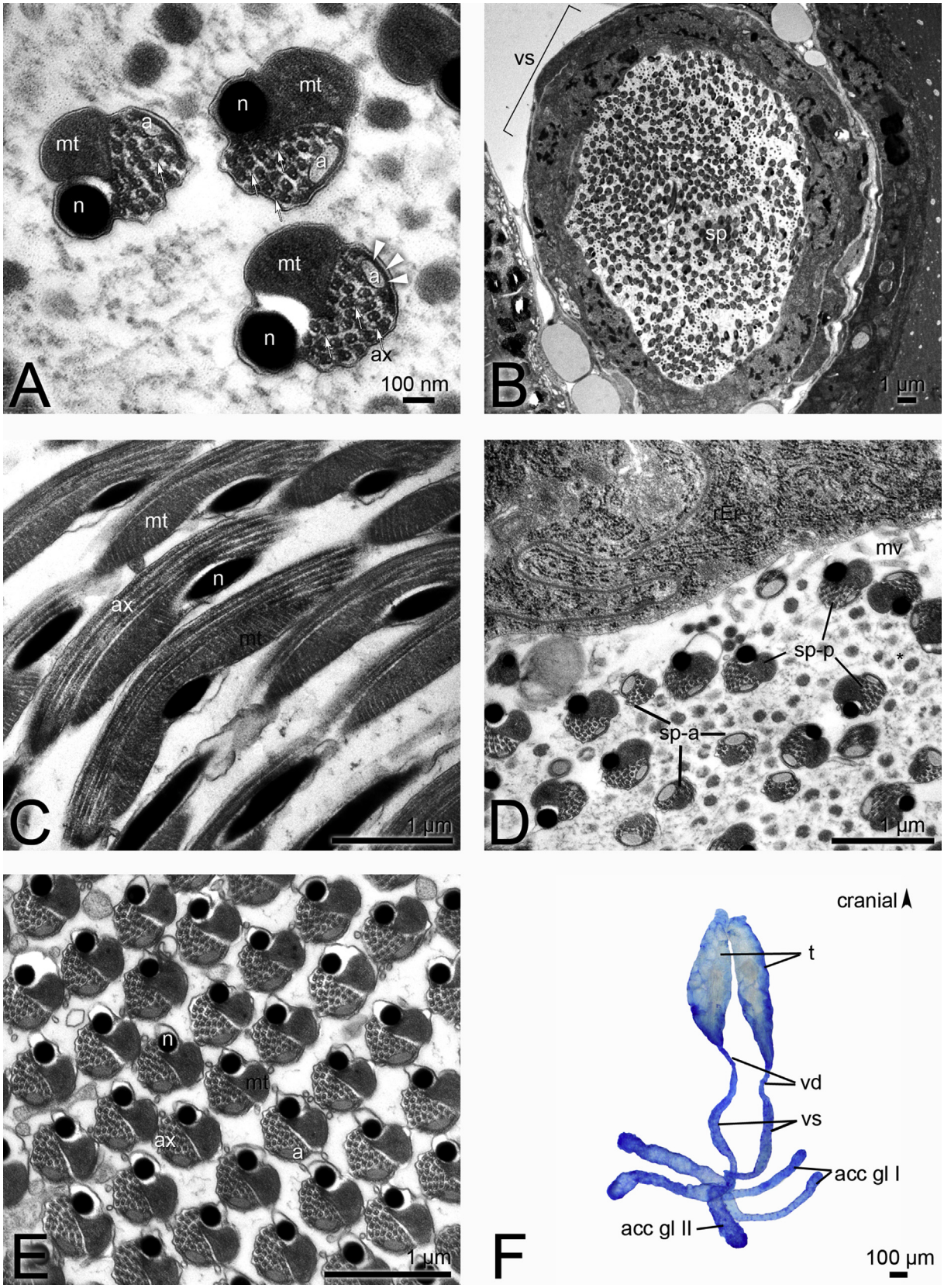

Fig. 1. *Suocerathrips linguis.* (A) Cross sections through mature spermatozoa in posterior half with acrosome (a), electron dense layer (arrowheads), axonema with dynein arms (arrows) and triangular mitochondrion (mt). (B) Cross section through a seminal vesicle (vs) with mature spermatozoa (sp) and electron dense vesicles. (C) Longitudinal section through mature spermatozoa in the seminal vesicle lumen. (D) Cross section through seminal vesical with cell wall and lumen. Rough endoplasmic reticulum (rEr) and a microvilli-border (mv) are visible in the surrounding cells. Note the different cutting levels with spermatozoa in the anterior half only with acrosome and mitochondrion (sp-a) and posterior half with nucleus (sp-p). Additionally, large amounts of secretion in the lumen can be seen (asterisk). (E) Cross section through a synspermatogenic testes cyst with uniformly arranged spermatozoa. (F) Male reproductive organs with testes(t), vasa deferentia (vd) seminal vesicle (vs) and the two accessory glands (acc gl I, acc gl II).

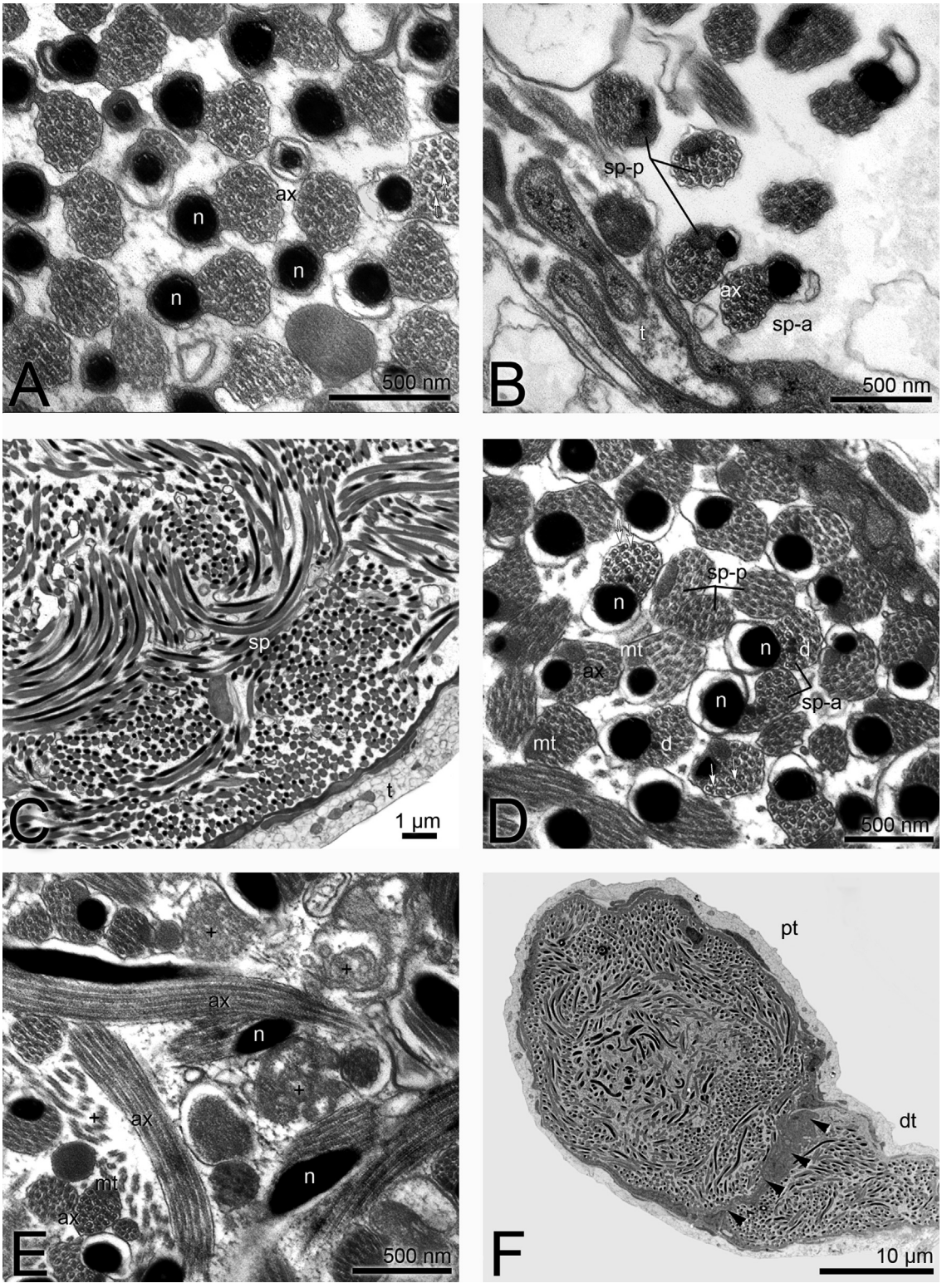

Fig. 2. A–C *Echinothrips americanus.* (A) Cross section through mature spermatozoa in the anterior half with axoneme (ax), dynein arms (arrows) and nucleus (n). Note the constriction in bigger and smaller lobus. (B) Cross section through mature spermatozoa at different levels. Sperm in the anterior region is bearing the nuclei and axoneme (sp-a), whereas in the more posterior region the mitochondrion (mt) join (sp-p). (C) Cross section through the testis (t) with randomly arranged mature spermatozoa (sp). D–F *Hercinothrips femoralis.* (D) Cross section through mature spermatozoa at different levels. Spermatozoa at anterior level (sp-a) bear nuclei (n), electron dense body (d) and axoneme (ax) with dynein arms (arrows). Spermatozoa at posterior level (sp-p) possess mitochondrion and axoneme. (E) Cross section of testis lumen with large amounts of membrane structures, malformed and damaged spermatozoa (+). (F) Cross section through a prospermatogenic testis with spermatozoa in different arrangements and granules of secretions between them. Testis is bisected in a proximal and a distal part by a membrane constriction (arrowheads).

be rod-shaped with a constant diameter of 0.38 ± 0.009 μm (Fig. 3B).

Nucleus and mitochondrion lay more behind each other and overlap only in a small area (Figs. 2B and 3B). Mitochondrion could only be seen in a small area at the rear third, and is therefore not such a dominant feature as compared to *S. linguis* (Fig. 3A and B). Beyond the nucleus region, the mitochondrion takes an ellipsoidal shape and has its maximum extension (0.2 μm × 0.17 μm) at this point (Fig. 2B).

The axoneme shows a 18 + 9 microtubulus pattern, which can be observed in a large part of the spermatozoa (Fig. 2A and B, 3B). Only in the most anterior and posterior regions less than 27 microtubules were counted (Figs. 2B and 3B). The diameter of a microtubule is 0.025 ± 0.004 μm, and some microtubule units display dynein arms (Fig. 2A).

3.3. *Hercinothrips femoralis*

The testes of *H. femoralis* are drop shaped and about 103 × 71 μm in size. Testes were bisected in a proximal and a distal part by a membrane constriction (Fig. 2F). The proximal part is much bigger than the distal one, at a ratio of about 2.2:1. The distal part leads into the vasa deferentia. Both ducts join the paired accessory gland and lead into the ejaculatory bulb and ejaculatory duct.

Only mature sperm can be seen in the testes. Length of the spermatozoa is 61.7 ± 6.8 μm, whereas in DAPI staining a length of 57.7 ± 8.7 μm can be measured.

All cell organelles were widely helically wound around each other (Fig. 2F). Cytoplasm constrictions cut the spermatozoa into two lobi, starting from the anterior sperm pole till posterior part of the middle of the sperm. The bigger lobus (Ø 0.3 μm) contains the axoneme and electron dense body, whereas the smaller one (Ø 0.2 μm) bears the nucleus (Fig. 2D).

The very anterior sperm region bears only the nucleus (Ø 0.35 μm) which decreases in size towards the posterior (0.05 μm). An electron dense body (Ø 0.07 μm) is located behind the nucleus and does not overtake this (Figs. 2D and 3C). Nucleus and mitochondrion overlap only in a small area (Figs. 2D and 3C), whereas the mitochondrion extends more posteriorly with a maximum diameter of about 0.16 μm.

The axoneme shows a 18 + 9 microtubulus pattern. Posterior microtubules are increasingly recognizable, until complete union of the three axonema (Fig. 2D).

Noticeable are differently malformed spermatozoa in the entire testes lumen (Fig. 2E). Most spermatozoa show a fuzzy membrane borderline, nuclei edges, as well as unclear axoneme structures. Some spermatozoa bear more than one nucleus and lamellar structures. Various accumulations of differently structured material can be seen in the testis lumen (Fig. 2E). In previous experiments we tested several fixation methods and chemicals with no effect on the ultrastructure of the spermatozoa, but always with a good fixation of the surrounding tissue. Possibly these are degenerated sperm cells or sperm cells with incomplete separation at the end of spermiogenesis (Fig. 2E and F). Additionally, spermiophagy can be observed frequently at the inner testis cell layer.

4. Discussion

4.1. Structure spermatozoa and special features

The structure of spermatozoa resembles that recorded in previously described species (Bode, 1988; Dallai et al., 1991; Paccagnini et al., 2006, 2007, 2009, 2010). The flagellum consists of 27 microtubules, comprising 18 doublets and 9 singlets with the typical arrangement. The helicoidal placement of the other cell organelles, the large mitochondrion and the existence of an acrosome is common in Tubulifera, whereas a small mitochondrion and lack of an acrosome is typical of Terebrantia.

The helicoidal arrangement of cell organelles is also known from some Carabidae- (Werner, 1965) and Lepismatidae-species (Bawa, 1964; Wingstrand, 1973; Dallai and Afzelius, 1984; Dallai et al., 2004), and was reported in Thysanoptera by Bode (1990). However, in both suborders a division into head and neck and flagellum regions is not possible (Jamieson et al., 1999; Paccagnini et al., 2009).

The electron dense layer was detected in some of the Tubulifera previously studied (Paccagnini et al., 2009), and it extends over the entire spermatozoon. The material originates from the centriole-adjunct material, which form an electron dense structure at the end of spermiogenesis (Paccagnini et al., 2010). The electron dense layer shows an association with the acrosome, as is also described in other tubuliferan species (Paccagnini et al., 2009). In mature sperm it probably has the function of preventing the disorganization of microtubule bundles during sperm movement (Paccagnini et al., 2010).

The electron dense body of the Terebrantia was visible only in the anterior sperm region. It originates from the centriole-adjunct material, as the electron dense layer in Tubulifera. But it is reduced during spermiogenesis and is therefore still in the anterior region (Paccagnini et al., 2009, 2010).

Acrosomes have only been observed in Tubulifera so far (Bode, 1988; Heming, 1995; Paccagnini et al., 2009). The size of the acrosome depends on the species. The acrosome of *S. linguis* is very large and resembles that of *Bolothrips insularis* and *Compsothrips albosignatus* (Paccagnini et al., 2009).

While the mitochondrion in *S. linguis* is rather elliptical to triangular and occupies about one third of the total area, the shape in other species is rather round and the proportion of area in cross section is only about one fourth (Paccagnini et al., 2009).

Additionally, the transverse profile of both suborders differs. Whereas tubuliferan species bear almost a circular form, Terebrantia show membrane constrictions in the anterior sperm section that divides the cross section into two lobi (Paccagnini et al., 2009, 2010.) But in contrast to the species studied so far, in *E. americanus* both lobes (nucleus lobe and axoneme lobe) are similar in size. Also, the nucleus is 0.21 μm and therefore larger than in other terebrantian species (Paccagnini et al., 2009, 2010).

The unusual number of 27 microtubules results from the fusion of three 9 + 0 axonemata during spermiogenesis (Paccagnini et al., 2007, 2009). Because of the instability of the B-subtubules the circular arrangement of 9 + 0 microtubules is broken at certain points. The resulting dyads, triads and tetrads fuse to the typical microtubule pattern (Paccagnini et al., 2007). With few exceptions, most insects bear only one axoneme (Jamieson et al., 1999). The situation with three axonemata in Thysanoptera possibly reflects the classification of the superclade Acercaria (Psocodea + Hemiptera + Thysanoptera). Parasitic Psocodea possess also two axonemata per spermatozoon, whereas in Thysanoptera an additional third one is synthesized *de novo*. Biflagellarity alone has been found also in some Hemiptera-Heteroptera and non-parasitic Psocodea (Kristensen, 1991). This would support the molecular evidence for a sister group relationship between lice and thrips (Wheeler et al., 2001).

But on the other hand, the widely accepted classification of Hemiptera and Thysanoptera (Condylognatha) as sister groups is based on the mouth-part-structures (Hennig, 1969; Kristensen, 1981) and supported by a new exhaustive genetic analysis (Misof et al., 2014). Therefore, current knowledge does not provide any information on the precise relationship and an unresolved trichotomy may still be preferable at the moment (see Kristensen, 1991).

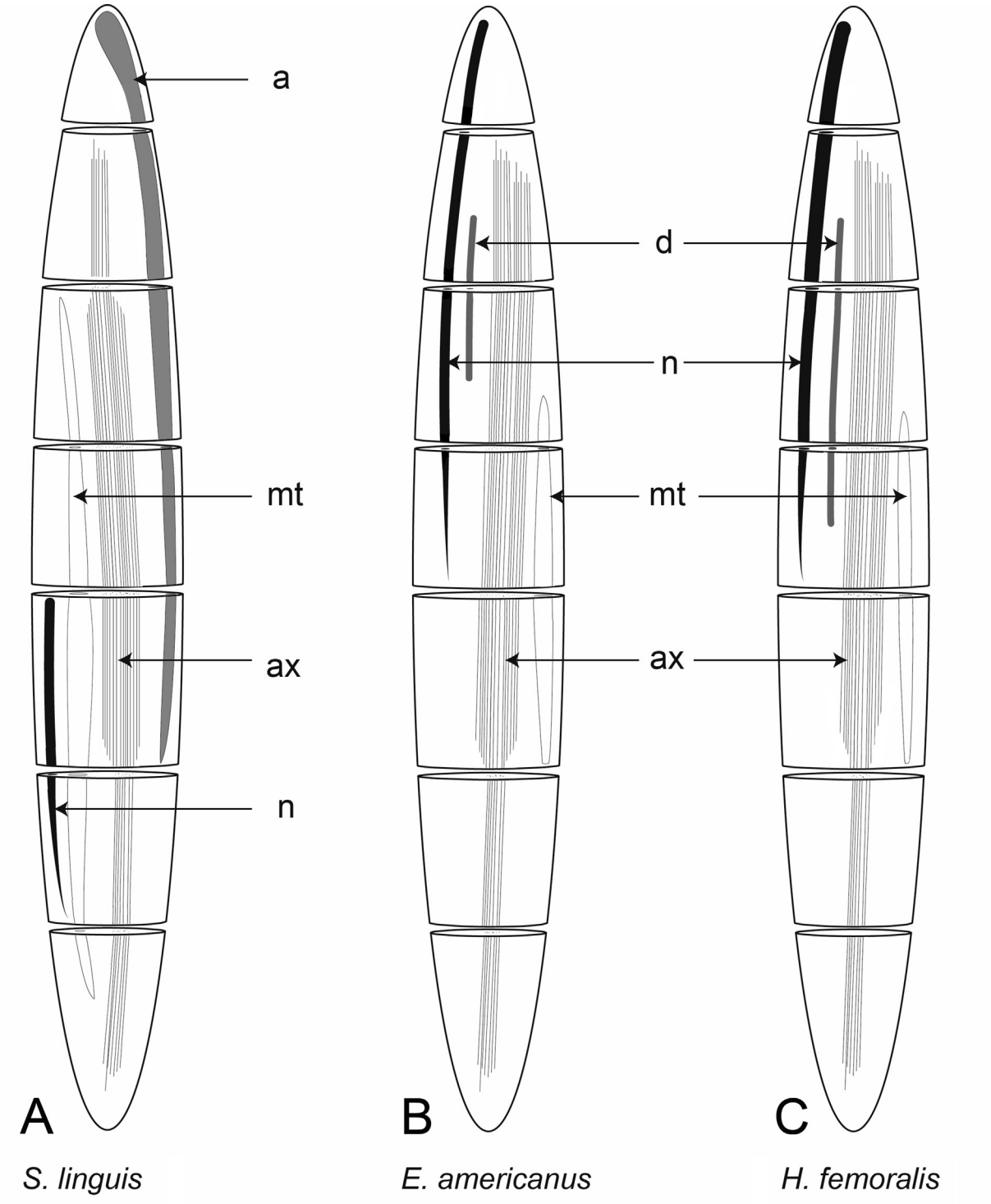

Fig. 3. Overview of ultrastructure of spermatozoa from anterior to posterior. (A) *Suocerathrips linguis*. (B) *Echinothrips americanus*. (C) *Hercinothrips femoralis*.

4.2. Endosymbiont mediated thelytoky

Whereas our populations of *S. linguis* and *E. americanus* are infected with Wolbachia and partially also with Cardinium, no detectable effect of these microorganisms can be seen (Kumm and Moritz, 2008; Chuttke, 2021), even in sperm structure. For instance, Vavre et al. (2002) mentioned that Wolbachia does not always have detectable phenotypic effects on its host. However, in *H. femoralis,* thelytoky is endosymbiont-mediated (Kumm and Moritz, 2008).

In general, the ultrastructure of *H. femoralis* showed general similarities with other terebrantian species, but also a lot of malformations. Already Bode 1988 showed in *Parthenothrips dracaenae*, a species with sporadic natural occurrence of males, that the structures are largely preserved. But they contain fewer dynein arms and a smaller mitochondrial derivate (Heming, 1995) as compared to obligate arrhenotokous species.

Although some errors in spermiogenesis occur, it indicates a highly conserved mechanism that includes the unusual structures in Thysanoptera, such as instability of B-subtubules, axonemic fusion and the helical arrangement of the cell organelles. Many thelytokous lineages are able to produce males, but with different grades of functionality. So male developmental pathways can be quiescent and functional for a long time, as they are not subject to any selection under thelytoky (Schwander et al., 2013). A decay can probably only be generated by drift (van der Kooi and Schwander, 2014b). However, re-establishment of sexual lines has failed in *H. femoralis*, as also is known in all Hymenoptera, except for one genus (Trichogramma) (see Koivisto and Braig, 2003). This might be due to the partly malformed sperm structure, which may prevent fertilization of the inseminated females. Kumm and Moritz (2008) observed no change in sex ratio of offspring from mated females in *H. femoralis*.

4.3. Movement/mobility of sperm necessary

Sperm motility was observable in all three species. However, the motility is rather wave-like, and it is unclear, how far movement is possible (Bode, 1983, 1988) or if the movement is also dependent on the mitochondrion-structure. Several studies have shown a correlation between sperm motility and mitochondrial function in mammals (e.g., reviewed in Barbagallo et al., 2020), but not necessarily in insects. Some scale and stick insects are lacking mitochondria and are still motile (Baccetti et al., 1973; Paoli et al., 2015; Robison 1966). Terebrantian and tubuliferan sperm differ in the size of the mitochondria. In *S. linguis* the big mitochondria might mechanically support the movement and therefore the transport of the sperm within the complex female spermatheca, whereas the terebrantian spermatheca is much simpler and the sperm is transferred directly to the very short spermathecal entrance (Bode, 1983; Krueger et al., 2017). However, the tubuliferan species *Cryptothrips nigripes* and *Haplothrips aculeatus* show no motility, although they have similar mitochondrial structures. But both species lack dynein arms (Baccetti et al., 1969; Bode, 1983, 1988). In Terebrantia the reduction of the dynein arms is selectively at positions where no interaction is possible due to the breaking of the B- subtubule structures (Bode, 1983; Paccagnini et al., 2007). Therefore, motility is still possible, as mentioned in *Thrips validus* (Bode, 1983). Possibly, this kind of movement is rather adapted to generate a circulation of nutrient and oxygen supply and the spermatozoa might be transported passively within the female genital tract (Bode, 1983).

4.4. Background/conclusion to reproductive strategy and life history

Sperm structure is possibly related to the way of life of species in the two suborders. Across diverse taxa, the length of sperm exhibits correlated evolution with the dimension of the female sperm storage organs and/or their ducts (Pitnick et al., 2009). A paradox life style and mating strategy between the two thysanopteran suborders Terebrantia and Tubulifera can be seen. The more stable environment and long-lived breeding sites of some fungus-feeding Tubulifera, like *S. linguis*, supports the complex colonial and sub/eusocial behaviors (Evans, 1977; Terry, 1997). Cooperation in carrying the young, overlap of at least 2 generations, and division of labor are known only from tubuliferan species (Wilson, 1971; Andersson, 1984; Crespi, 1986a, b, 1988 a,b,c,d, 1993; Crespi and Yanega, 1995). Males and females seem to remate frequently (Ananthakrishnan, 1990; Crespi 1986a,b, 1988a,b). This behavior is promoted by the morphological adaptations of frequent synspermatogeny of males and large sac-like spermathecae in females. Synspermatogeny is the possibility to produce sperm over the entire life, and requires larger amounts of sperm for multiple matings. Additionally, the longer lifespan of some tubuliferan species (Crespi et al., 2004) is likely to require a constant sperm supply over the whole lifetime and indicates a stronger reproductive competition.

In comparison, many flower-living Thripidae species are more closely adapted to rapid reproduction and dispersal. Colonial lifestyles or division of labor are unknown among such thrips. Only functional and temporally limited aggregations are known among some Thripidae species, such as *Frankliniella occidentalis, Frankliniella schultzei, Thrips fuscipennis, T. major, T. atratus* or *Megalurothrips sjostedti*, which might aid in finding mates or food sources (Kirk, 1985; Terry and Gardner, 1990; Milne et al., 2002; Niassy et al., 2016). Males exhibit prospermatogeny (spermatogenesis finished with adult eclosion), which constitutes an advantage in their short lifespan and the frequently performed protandry. Females have a small and simply constructed spermatheca. Most of the examined species mate only once or with a very low frequency (except *T. tabaci*, Li et al., 2015). Thelytokous species are known only in this suborder, regardless of whether it is obligate or microorganism-initiated. Additionally, some arrhenotokous species have developed thelytokous strains (e.g., *Thrips tabaci*: Kobayashi and Hasegawa, 2012; *A. rufus*: van der Kooi and Schwander, 2014a). While males of some Tubulifera play an important part in their complex reproductive behavior, terebrantian males seem to play just a minor role and their function is more or less suppressed.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or non-for-profit sectors.

Credit author statement

Stephanie Krueger: Methodology, Investigation, Writing Original Draft, Visualization.

Gerald Moritz: Conceptualization, Resources, Writing – Review & Editing, Visualization.

Acknowledgment

We would like to thank Angelika Steller and Jessica Teuber for assistance and technical support.

References

Ananthakrishnan, T.N., 1990. The Reproductive Biology of Thrips. Indira Publishing House, Oak Park.

Ananthakrishnan, T.N., Balu, A., 1990. Aspects of spermiogenesis in *Arrhenothrips ramakrishnae* hood (Insecta: Thysanoptera). Proc. Indian Acad. Sci. 99, 91–102.

Andersson, M., 1984. The evolution of eusociality. Annu. Rev. Ecol. Evol. Syst. 15, 165–189.
Arakaki, N., Noda, H., Yamagishi, K., 2000. Wolbachia-induced parthenogenesis in the egg parasitoid *Telenomus nawai*. Entomol. Exp. Appl. 96, 177–184.
Arakaki, N., Miyoshi, T., Noda, H., 2001. Wolbachia-mediated parthenogenesis in the predatory thrips *Franklinothrips vespiformis* (Thysanoptera: insecta). Proc. R. Soc. B. 268, 1011–1016.
Baccetti, B., Burrini, A.G., Dallai, R., Pallini, V., Periti, P., Piantelli, F., Rosati, F., Selmi, G., 1973. The spermatozoon of Arthropoda. XIX. Structure and function in the spermatozoon of *Bacillus rossius*. J. Ultrastruct. Res. 44 (Suppl. 1), 1–73.
Baccetti, B., Dallai, R., Rosati, F., 1969. The spermatozoon of Arthropoda. IV. Corrodentia, Mallophaga and Thysanoptera. J. Microsc. 8, 249–262.
Barbagallo, F., La Vignera, S., Cannarella, R., Aversa, A., Calogero, A.E., Condorelli, R.A., 2020. Evaluation of sperm mitochondrial function: a key organelle for sperm motility. J. Clin. Med. 9, 363.
Bawa, S.R., 1964. Electron microscope study of spermiogenesis in a fire-brat insect, *Thermobia domestica* PACK. I. Mature spermatozoon. J. Cell Biol. 23, 431–446.
Bode, W., 1983. Spermienstruktur und Spermatohistogenese bei *Thrips validus* Uzel (Insecta, Thysanoptera). Zool. Jahrb. Anat. Tiere 109, 301–318.
Bode, W., 1988. The spermatozoa of Thysanoptera and their relevance for systematics. Acta Phytopathol. Entomol. Hung. 23, 267–273.
Bode, W., 1990. Spermienstruktur bei Thysanopteren und ihre Bedeutung für die Systematik. Mitt. Dtsch. Ges. Allg. Angew. Entomol. 7, 535–540.
Chuttke, J., 2021. Economic Thrips and Their Microbiome: Importance and Distribution of Endosymbionts and Other Bacteria. D. Phil. Thesis. Martin-Luther-University Halle-Wittenberg.
Crespi, B.J., 1986a. Territoriality and fighting in a colonial thrips, *Hoplothrips pedicularius*, and sexual dimorphism in Thysanoptera. Ecol. Entomol. 11, 119–130.
Crespi, B.J., 1986b. Size assessment and alternative fighting tactics in *Elaphrothrips tuberculatus* (Insecta: Thysanoptera). Anim. Behav. 34, 1324–1335.
Crespi, B.J., 1988a. Alternative mating tactics in thrips: effects of sex ratio variation and body size. Am. Midl. Nat. 122, 142–150.
Crespi, B.J., 1988b. Risks and benefits from lethal male fighting in colonial *Hoplothrips karnyi* (Insecta: Thysanoptera). Behav. Ecol. Sociobiol. 22, 293–301.
Crespi, B.J., 1988c. Sex-ratio selection in bivoltine thrips. I. Conditional sex ratio manipulation and fitness variation. Evolution 42, 1199–1211.
Crespi, B.J., 1988d. Adaption, compromise and constraint: the development, morphometrics, and behavioural basis of a fighter-flier polymorphism in male *Hoplothrips karnyi* (Insecta: Thysanoptera). Behav. Ecol. Sociobiol. 23, 93–104.
Crespi, B.J., 1993. Sex allocation ratio selection in Thysanoptera. In: Wrensch, D.L., Ebbert, M.A. (Eds.), Evolution and Diversity of Sex Ratio in Insects and Mites. Chapman and Hall, New York, pp. 214–234.
Crespi, B.J., Yanega, D., 1995. The definition of eusociality. Behav. Ecol. 6, 109–115.
Crespi, B.J., Morris, D.C., Mound, L.A., 2004. Evolution of Ecological and Behavioural Diversity. Australian acacia Thrips as Model Organisms. CSIRO, Canberra.
Dallai, R., Afzelius, B.A., 1984. Paired spermatozoa in *Thermobia* (Insecta, Thysanura). J. Ultrastruct. Res. 86, 67–74.
Dallai, R., Afzelius, B.A., Lanzavecchia, S., Bellon, P.L., 1991. Bizarre flagellum of thrips spermatozoa (Thysanoptera, Insecta). J. Morphol. 209, 343–347.
Dallai, R., Fanciulli, P.P., Frati, F., Paccagnini, E., Lupetti, P., 2004. Sperm winding in Collembola. Pedobiologia 48, 493–501.
de Barro, P.J., Hart, P.J., 2001. Antibiotic curing of parthenogenesis in *Eretmocerus mundus* (Australian parthenogenic form). Entomol. Exp. Appl. 99, 225–230.
de Marzo, L., 2005. Lunghezza degli spermatozoi in *Megathrips inermis* priesner (Thysanoptera Phlaeothripidae). Boll. Zool. Agrar. Bachic. Ser II. 37, 179–183.
Dobson, S.L., Marsland, E.J., Veneti, Z., Boutzis, K., ONeill, S.L., 2002. Characterization of Wolbachia host cell range via the in vitro establishment of infections. Appl. Environ. Microbiol. 68, 656–660.
Evans, H.E., 1977. Extrinsic and intrinsic factors in the evolution of insect sociality. Bioscience 27, 613–616.
Gehlsen, U., 2009. Ernährungssystem, Verhalten und Wehrsekret des subsozialen Phlaeothripinen *Suocerathrips linguis* MOUND and MARULLO, 1994 (Insecta, Thysanoptera, Tubulifera). D.Phil. Thesis. Martin-Luther-University Halle-Wittenberg.
Heming, B.S., 1995. History of the germ line in male and female thrips. In: Parker, B.L., Skinner, M., Lewis, T. (Eds.), Thrips Biology and Management. International Conference on Thysanoptera: towards Understanding Thrips Management. Springer, Vermont, pp. 505–535.
Hennig, W., 1969. Die Stammesgeschichte der Insekten. Kramer, Frankfurt am Main.
Jacobson, A.L., Johnston, J.S., Rotenberg, D., Whitfield, A.E., Booth, W., Vargo, E.L., Kennedy, G.G., 2013. Genome size and ploidy of Thysanoptera. Insect Mol. Biol. 22, 12–17.
Jamieson, B.G.M., Dallai, R., Afzelius, B.A., 1999. Insects. Their Spermatozoa and Phylogeny. Science Publishers, Enfield.
Jilge, M., Krüger, S., Chuttke, J., Moritz, G., 2017. Artificial diets with ingredients and agents for pest thrips (Thysanoptera): a new method to test effects and impacts. In: 5th Symposium on Palaearctic Thysanoptera, Cracow, Poland, vol. 23.
Kageyama, D., Narita, S., Watanabe, M., 2012. Insect sex determination manipulated by their endosymbionts: incidences, mechanisms and implications. Insects 3, 161–199.
Kirk, W., 1985. Aggregation and mating of thrips in flowers of *Calystegia sepium*. Ecol. Entomol. 10, 433–440.
Kobayashi, K., Hasegawa, E., 2012. Discrimination of reproductive forms of *Thrips tabaci* (Thysanoptera: Thripidae) by PCR with sequence specific primers. J. Econ. Entomol. 105, 555–559.
Koivisto, R.K.K., Braig, H.R., 2003. Microorganisms and parthenogenesis. Biol. J. Linn. Soc. 79, 43–58.
Kristensen, N.P., 1981. Phylogeny of insect orders. Annu. Rev. Entomol. 26, 135–157.
Kristensen, N.P., 1991. Phylogeny of extant hexapods. In: Naumann, I.D., Carne, P.B., Lawrence, J.F., Nielsen, E.S., Spradberry, R.W.T., Whitten, M.J., Littlejohn, M.J. (Eds.), The Insects of Australia. A Textbook for Students and Research Workers, second ed. Melbourne University Press, Carlton, pp. 125–140.
Krueger, S., Mound, L.A., Moritz, G.B., 2015. Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* Morgan 1913 (Thysanoptera: Thripidae). J. Appl. Entomol. 140, 462–473.
Krueger, S., Jilge, M., Mound, L., Moritz, G., 2017. Reproductive behavior of *Echinothrips americanus* (Thysanoptera: Thripidae). J. Insect Sci. 17, 66.
Kumm, S., Moritz, G., 2008. First detection of Wolbachia in arrhenotokous populations of thrips species (Thysanoptera: Thripidae and Phlaeothripidae) and its role in reproduction. Environ. Entomol. 37, 1422–1428.
Lewis, T., 1973. Thrips. Their Biology, Ecology, and Economic Importance. Academic Press, London and New York.
Li, X.-W., Fail, J., Shelton, A.M., 2015. Female multiple matings and male harassment and their effects on fitness of arrhenotokous *Thrips tabaci* (Thysanoptera: thripinae). Behav. Ecol. Sociobiol. 69, 1585–1595.
Ma, W.J., Schwander, T., 2017. Patterns and mechanisms in instances of endosymbiont-induced parthenogenesis. J. Evol. Biol. 30, 868–888.
Milne, M., Walter, G.H., Milne, J.R., 2002. Mating aggregations and mating success in the flower thrips, *Frankliniella schultzei* (Thysanoptera: Thripidae), and a possible role for pheromones. J. Insect Behav. 15, 351–368.
Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., et al., 2014. Phylogenomics resolves the timing and pattern of insect evolution. Science 346, 763–767.
Moritz, G., 2002. The biology of thrips is not the biology of their adults: a developmental view. In: Mound, L.A., Marullo, R. (Eds.), Thrips and Tospovirus: Proceedings of the 7th International Symposium on Thysanoptera. CSIRO, Canberra, pp. 259–267.
Moritz, G., Schäfer, E., Kumm, S., Steller, A., Tschuch, G., 2004. Der Alien- Thrips *Suocerathrips linguis*- Biologie und Verhalten. Mitt. Dtsch. Ges. Allg. Angew. Entomol. 14, 177–181.
Moritz, G., ODonnell, C., Parrella, M.P., 2009. Pest Thrips of North America - Associated with Domestic and Imported Crops. CD-ROM, CBIT Publishing, Queensland, Australia.
Moritz, G., Brandt, S., Triapitsyn, S., Subramanian, S., 2013. Identification and Information Tools for Pest Thrips in East Africa, Version Number: 1.1 04/2013; QBIT, QAAFI Biological Information Technology. The University of Queensland, Australia. ISBN: 978-1-74272-067-8.
Mound, L.A., 1966. The genus *Hercinothrips* (Thysanoptera) with one new species. Ann. Mag. Nat. Hist. 13, 243–247.
Nault, B.A., Shelton, A.M., Gangloff-Kaufmann, J.L., Clark, M.E., Werren, J.L., Cabrera-la Rosa, J.C., Kennedy, G.G., 2006. Reproductive modes in onion thrips (Thysanoptera: Thripidae) populations from New York onion fields. Environ. Entomol. 35, 1264–1271.
Nguyen, D.T., Spooner-Hart, R.N., Riegler, M., 2015. Polyploidy versus endosymbionts in obligately thelytokous thrips. BMC Evol. Biol. 15, 23.
Niassy, S., Ekesi, S., Maniania, N.K., Moritz, G.B., de Kogel, W.J., Subramanian, S., 2016. Active aggregation among sexes in bean flower thrips (*Megalurothrips sjostedti*) on cowpea (*Vigna unguiculata*). Entomol. Exp. Appl. 158, 17–24.
Oetting, R.D., Beshear, R.J., 1993. Biology of the greenhouse pest *Echinothrips americanus* morgan (Thysanoptera: Thripidae). Int. J. Pure Appl. Zool. 4, 307–315.
Oetting, R.D., Beshear, R.J., Liu, T.-X., Braman, S.K., Baker, J.R., 1993. Biology and identification of thrips on greenhouse ornamentals. Geo. Agr. St. Bull. 414, 20.
Paccagnini, E., de Marzo, L., Giusti, F., Dallai, R., 2006. The aberrant spermatogenesis of the *Haplothrips simplex* (Buffa) (Thysanoptera): ultrastructural study. Tissue Cell 38, 177–186.
Paccagnini, E., Mencarelli, C., Mercati, D., Afzelius, B.A., Dallai, R., 2007. Ultrastructural analysis of the aberrant axoneme morphogenesis in thrips (Thysanoptera, Insecta). Cell Motil Cytoskeleton 64, 645–661.
Paccagnini, E., Lupetti, P., Afzelius, B.A., Dallai, R., 2009. New findings on sperm ultrastructure in thrips (Thysanoptera, Insecta). Arthropod Struct. Dev. 38, 70–83.
Paccagnini, E., Mercati, D., Giusti, F., Conti, B., Dallai, R., 2010. The spermatogenesis and the sperm structure of Terebrantia (Thysanoptera, Insecta). Tissue Cell 42, 247–258.
Pannebakker, B.A., Schidlo, N.S., Boskamp, G.J.F., Dekker, L., van Dooren, T.J.M., Beukeboom, L.W., Zwaan, B.J., Brakefield, P.M., van Alphen, J.J.M., 2005. Sexual functionality of *Leptopilina clavipes* (Hymenoptera: Figitidae) after reversing Wolbachia-induced parthenogenesis. J. Evol. Biol. 18, 1019–1028.
Paoli, F., Roversi, P.F., Mercati, D., Marziali, L., Cocco, A., Dallai, R., 2015. The ultrastructure of spermiogenesis in four species of Coccoidea (Insecta, Homoptera). Zool. Anz. 258, 69–81.
Pintureau, B., Lassabliére, F., Khatchadourian, C., Daumal, J., 1999. Parasitoïdes oophages et symbiotes de deux thrips européens. Ann. Soc. Entomol. Fr. 35, 416–420.
Pitnick, S., Hosken, D.J., Birkhead, T.R., 2009. Sperm morphological diversity. In: Birkhead, T.R., Hosken, D.J., Pitnick, S. (Eds.), Sperm Biology: an Evolutionary Perspective. Academic Press, Burlington, pp. 69–149.
Robison, W.G., 1966. Microtubules in relation to the motility of a sperm syncytium in an armored scale insect. J. Cell Biol. 29, 251–265.

Roditakis, E., Mound, L.A., Roditakis, N.E., 2006. Note: first record in Crete of *Hercinothrips femoralis* in greenhouse banana plantations. Phytoparasitica 34, 488–490.

Schwander, T., Crespi, B., Gries, R., Gries, G., 2013. Neutral and selection-driven decay of sexual traits in asexual stick insects. Proc. R. Soc. Biol. Sci. 280, 20130823. https://doi.org/10.1098/rspb.2013.0823.

Stannard, L.J., 1968. The thrips, or Thysanoptera, of Illinois. Bull. Ill. Nat. Hist. Surv. 29, 215–552.

Stouthamer, R., Luck, R.F., Hamilton, W.D., 1990a. Antibiotics cause parthenogenetic *Trichogramma* (Hymenoptera/Trichogrammatidae) to revert to sex. Proc. Natl. Acad. Sci. Unit. States Am. 87, 2424–2427.

Stouthamer, R., Pinto, J.D., Platner, G.R., Luck, R.F., 1990b. Taxonomic Status of thelytokous forms of *Trichogramma* (Hymenoptera: Trichogrammatidae). Ann. Entomol. Soc. Am. 83, 475–481.

Terry, I., 1997. Host selection, communication and reproductive behaviour. In: Lewis, T. (Ed.), Thrips as Crop Pests. CAB International, New York, pp. 65–118.

Terry, I., Gardner, D., 1990. Male mating swarms in *Frankliniella occidentalis* (pergande) (Thysanoptera: Thripidae). J. Insect Behav. 3, 133–141.

Trdan, S., KuŽnik, L., Vidrih, M., 2007. First results concerning the efficacy of entomopathogenic nematodes against *Hercinothrips femoralis* (Reuter). Acta Agric. Slov. 89, 5–13.

Trdan, S., Milevoj, L., Raspudic, E., Zezlina, I., 2003. The first record of *Echinothrips americanus* morgan in Slovenia. Acta Phytopathol. Entomol. Hung. 38, 157–166.

van der Kooi, C.J., Schwander, T., 2014a. Evolution of asexuality via different mechanisms in grass thrips (Thysanoptera: Aptinothrips). Evolution 68, 1883–1893.

van der Kooi, C.J., Schwander, T., 2014b. On the fate of sexual traits under asexuality. Biol. Rev. 89, 805–819.

Vavre, F., Fleury, F., Varaldi, J., Fouillet, P., Boulétreau, M., 2002. Infection polymorphism and cytoplasmic incompatibility in Hymenoptera-Wolbachia associations. Heredity 88, 361–365.

Vierbergen, G.B., 1998. *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). Proc. Exp. Appl. Entomol. 9, 155–160.

Werner, G., 1965. Untersuchungen über die Spermiogenese beim Sandläufer, *Cicindela campestris* L. Z. Zellforsch. Mikrosk. Anat. 6, 255–275.

Wheeler, W.C., Whiting, M., Wheeler, Q.D., Carpenter, J.M., 2001. The phylogeny of the extant hexapod orders. Cladistics 17, 113–169.

Wingstrand, K.G., 1973. The spermatozoa of the thysanuran insects *Petrobius brevistylis* carp. And *Lepisma saccharina* L. Acta Zool. 54, 31–52.

Wilson, C.R., 1971. The Insect Societies. Harvard University Press, Cambridge.

Zchori-Fein, E., Faktor, O., Zeidan, M., Gottlieb, Y., Czosnek, H., Rosen, D., 1995. Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae). Insect Mol. Biol. 4, 173–178.

Zchori-Fein, E., Roush, R.T., Hunter, M.S., 1992. Male production induced by antibiotic treatment in *Encarsia formosa* (Hymenoptera: Aphelinidae), an asexual species. Experientia 48, 102–105.