

Sperm structure in Parasitidae mites (Parasitiformes: Mesostigmata: Gamasina)

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ABSTRACT — This contribution reviews the ultrastructure of ribbon-type sperm in 14 genera of both subfamilies (7 in Pergamasinae and 7 in Parasitinae) of the Parasitidae family (Parasitiformes: Mesostigmata: Gamasina); in total 27 species were considered, of which sperm ultrastructure was studied for the first time in 17 species and 9 genera. We found a wide range of sperm dimensions and nucleus lengths, but basic external and internal structures were substantially constant across genera. Spermatozoa are rod- or club-shaped cells with an elongated nucleus. The chromatin granules are focused in the middle zone of the nucleus. The cytoplasm around the nucleus and in the adjoining postnuclear region is filled with inclusion bodies with striated content (striated inclusion bodies, sIBs), whereas in the distant postnuclear region they are replaced by larger granular inclusion bodies (gIBs) usually containing a striated core surrounded by granular material. Mitochondria are distributed mostly subplasmalemmally in the nuclear region and between gIBs in the postnuclear region of the sperm cell. The most variable feature of the spermatozoa is the number of compound longitudinal ribbons of plasmalemmal origin alternating with subplasmalemmal cisterns: 9 (*Leptogamasus anoxygenellus*) to 21 (*Pergamasus barbarus*) in Pergamasinae and 5 (*Parasitus berlesei* and *Paracarpais loricatus*) to 30 (*Paracarpais lunulata*) in Parasitinae. In general, ribbons are electron-dense in the nuclear region but more lucent in the postnuclear region. The variation in sperm structure was not reflected in the taxonomic arrangement of genera and subfamilies within Parasitidae, but it must be emphasized that the taxonomy of Parasitidae is still awaiting a comprehensive modern revision.

KEYWORDS — mites; spermatozoa morphology; variability; ultrastructure; taxonomy

INTRODUCTION

Parasitid mites, despite their name, are predominantly free-living and predatory inhabitants of humid litter and decaying matter, feeding on immature stages and eggs of microhexapods, as well as small soil oligochaets and nematodes (Micherdziński 1969; Tichomirov 1977; Hyatt 1980; Karg 1993; Blackman 1997; Szafranek *et al.* 2013). Males and females are usually equally represented in collected material. During mating, the male uses its enlarged

second pair of legs to fix ventrally to the female, firmly holding her last legs of the 4th pair. Then, the male produces a sac-like spermatophore from the genital opening located at the anterior margin of the sternum and by manipulating the chelicerae it transfers the spermatophore into the female genital opening located in a mid-ventral position. This mode of insemination was termed tocospermy by Athias-Henriot and later specified as neotocospermy by Alberti (Athias-Henriot 1968; Alberti 2002). Parasitid spermatozoa (Figures 1, 2)

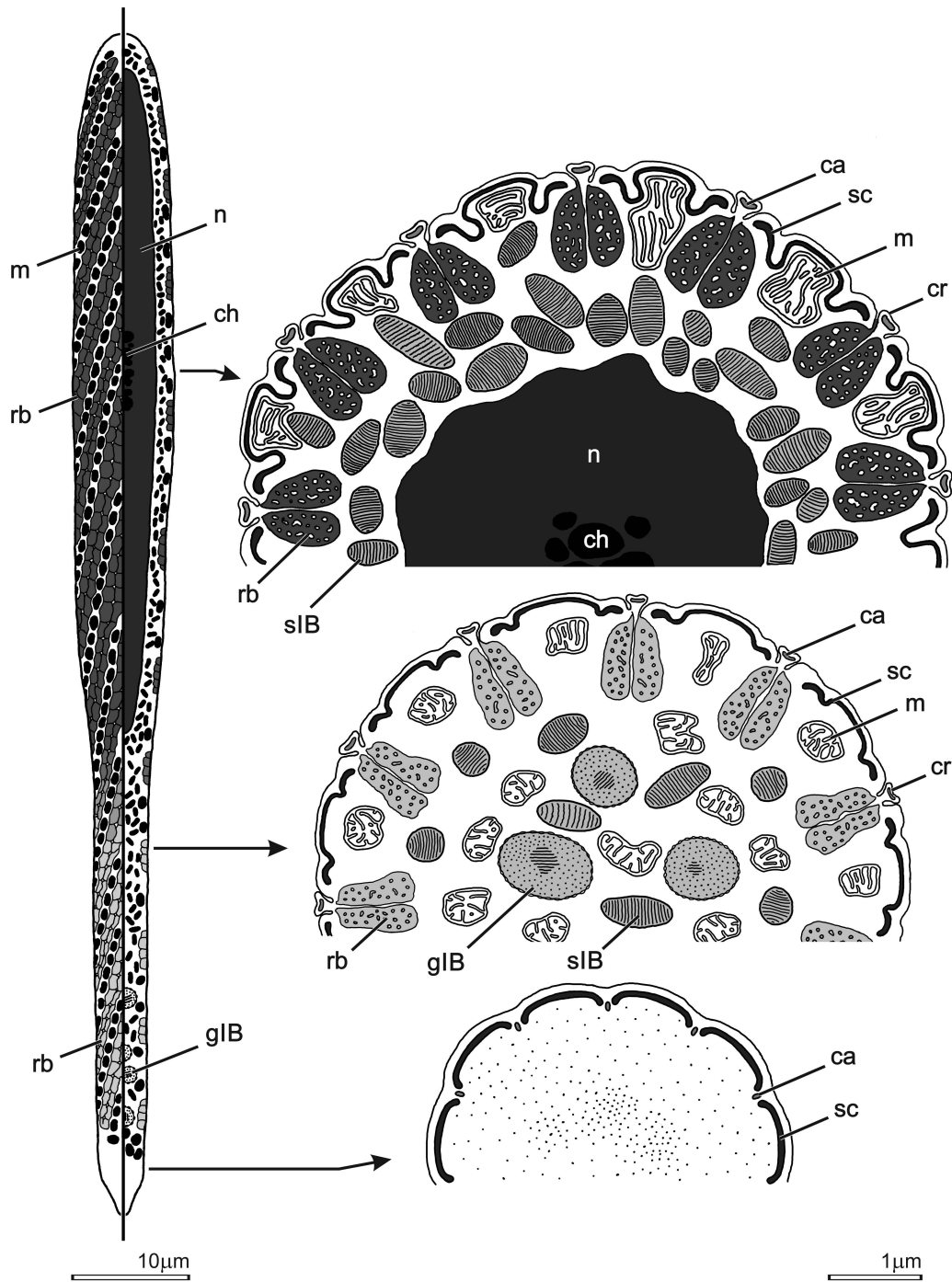


FIGURE 1: General organisation of parasitid spermatozoon (left) showing superficial and internal organization, as well as spermatozoon internal structure (right) as shown on cross sections through nuclear, postnuclear and posterior regions (based on Witaliński 1975). Abbreviations: ca – canaliculus running in longitudinal crest, ch – chromatin, cr – longitudinally running crest, glB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern, sIB – striated inclusion body

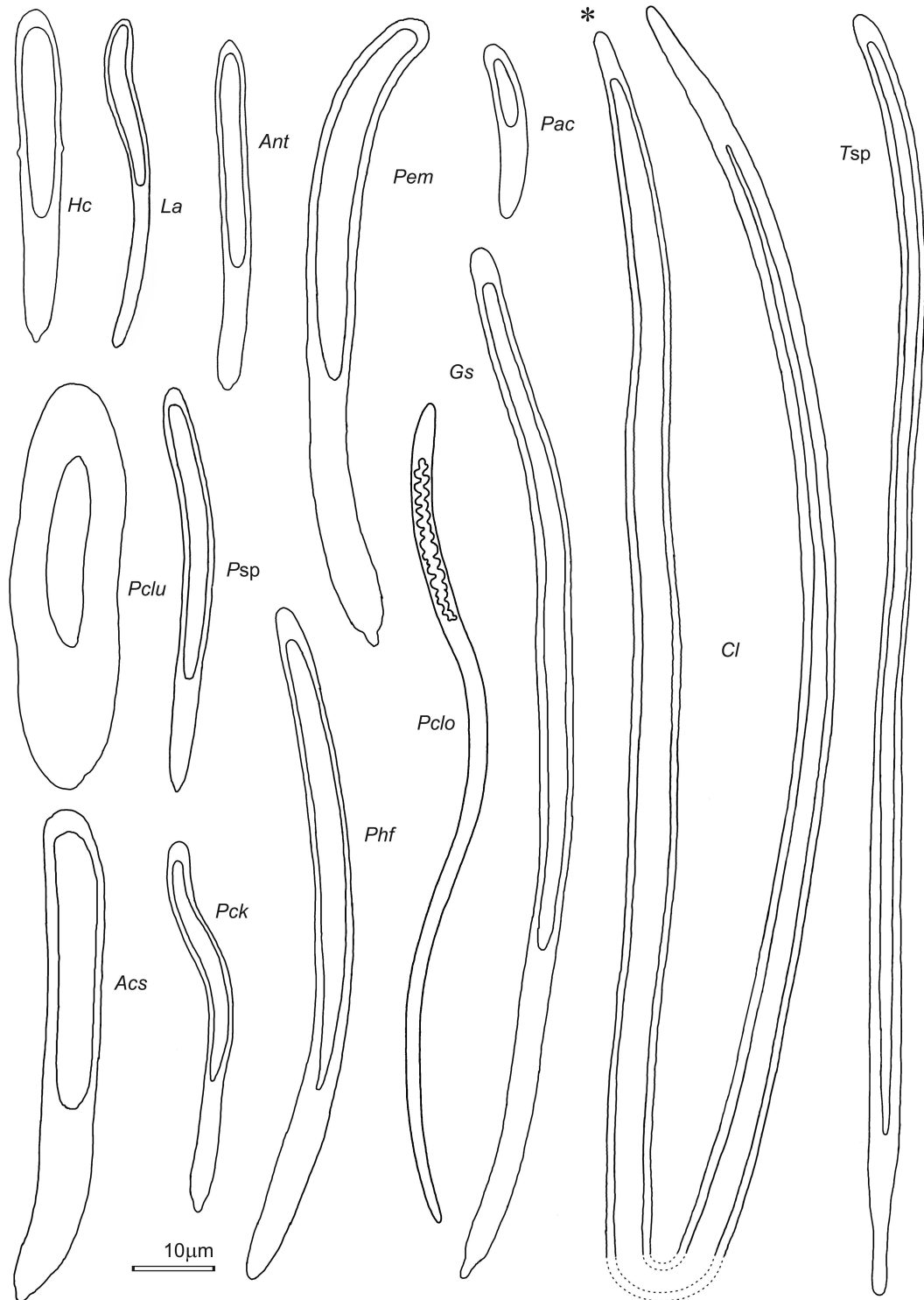


FIGURE 2: Schematic drawings of Parasitidae spermatozoa with outlined nucleus and anterior tip upwards. Abbreviations: Acs – *Aclerogamasus similis*, Ant – *Anidogamasus teutonicus*, Cl – *Cornigamasus lunaris* (asterisk marks anterior end), Gs – *Gamasodes spiniger*, Hc – *Holoparasitus calcaratus*, La – *Leptogamasus anoxygenellus*, Pac – *Parasitus coleoptratorum*, Pck – *Paracarpais kraepelini*, Pclo – *Paracarpais loricatus*, Pclu – *Paracarpais lunulata*, Pem – *Pergamasus mediocris* (sperm of *P. barbarus*, *P. brevicornis* and *P. crassipes* have similar proportions), Phf – *Phorytocarpais fimetorum*, Psp – *Paragamasus* sp., Tsp – *Trachygamasus* sp.

were observed as early as the 19th century (Winkler 1888; Michael 1892). They are easily recognizable due to their club- or rod-shape appearance and unusual, very characteristic internal structure occasionally observable under a light microscope (Sokolov 1934), but obviously better illustrated using a transmission electron microscope (TEM). They are classified as ribbon-type spermatozoa (Alberti 1980) since there are complicated systems of plasmalemmal infoldings forming so-called ribbons which are double rows of saccular structures running longitudinally along the spermatozoon from its anterior to posterior end (Sokolov 1934; Witaliński 1975, 1976, 1979). The plasmalemma above each ribbon evaginates forming a longitudinal ridge or crest containing the canaliculus. Electron-dense subplasmalemmal cisterns run longitudinally along and between ribbons. The sperm nucleus is elongated and occupies more or less half of the length of the spermatozoon. The nucleus-containing part is considered to be the anterior one, but for convenience rather than due to any physiological (e.g. movement) evidence. Around and just behind the nucleus there are many inclusion bodies with a characteristic, striated appearance (striated inclusion bodies, sIBs). The posterior part of the cell contains larger, granular inclusion bodies (gIBs) which are apparently derived from the striated ones (Witaliński 1975). Crista-type mitochondria are usually located between ribbons below subplasmalemmal cisterns in the nuclear region, but in the postnuclear region they are scattered between gIBs. Typical acrosome, flagellum, axoneme and centrioles have never been observed in parasitid sperm; an acrosomal vesicle reported in *Pergamasus* sperm (Witaliński 1975) is a result of misinterpretation.

The Parasitidae is one of the most species-rich families of mesostigmatic mites comprising at least 39 genera collected in two subfamilies, Pergamasinae Juvara-Bals, 1972 (23 genera) and Parasitinae Oudemans, 1901 (16 genera). Table 1 presents genera in both subfamilies in the taxonomic arrangement used in this study, as well as the studied species. It should be emphasized, however, that the number of genera is dependent on the taxonomist and can substantially vary since many

subgenera elevated to the genus level mainly due to the contributions of Athias-Henriot and Juvara-Bals (e.g. Athias-Henriot 1969; Juvara-Bals 1972, 1977; Juvara-Bals and Athias-Henriot 1972; Athias-Henriot 1979, 1980, 1981) are still not accepted as genera by some acarologists preferring the more simple "traditional" taxonomy as presented in e.g. Bhattacharyya 1963, Micherdziński 1969, Holzmänn 1969, Karg 1971, 1993, Evans and Till 1979, and Hyatt 1980. For example, three species recently included in *Paracarpais* (*P. loricatus*, *P. kraepelini* and *P. lunulata*) are still traditionally located in different genera (*Parasitus loricatus*, *Vulgarogamasus kraepelini* and *Porrhostaspis lunulata*). Furthermore, the phylogenetic relationships within Parasitidae, even at the generic level, were never tested with modern phylogenetic methods. Since it is commonly accepted that the structure of spermatozoa can provide a new set of characters useful for phylogenetic considerations (e.g. Afzelius 1979; Baccetti 1979; Wirth 1984; Jamieson 1987), the aim of our paper was to review the main modifications in sperm structure in 27 representatives of 14 parasitid mite genera (7 in Pergamasinae and 7 in Parasitinae), in 17 species and 9 genera for the first time at the ultrastructural level. It could also trace some evolutionary routes within family. On the other hand, future molecular taxonomic studies could be used to track general trends in sperm evolution within Parasitidae and, possibly, to allow a better understanding of the complicated sperm structures in the light of their function.

MATERIALS AND METHODS

We considered sperm structure in 27 species (Table 1) of parasitid mites from both subfamilies, Pergamasinae (16 species from 7 genera) and Parasitinae (11 species from 7 genera). Free-living species were obtained from forest litter or composted hay collected near Kraków, southern Poland, whereas the insect-associated species, *Poecilochirus carabi* G. & R. Canestrini, 1882, was collected from its host, *Nicrophorus vespilloides* Herbst, 1783, in the environs of Kraków. Males of free-living *Holoparasitus excipuliger* (Berlese, 1906) were collected from forest litter near Veszprém, Hungary. *Trachygamasus* sp. is

TABLE 1: Genera in Pergamasinae and Parasitinae subfamilies, as well as species considered. Species studied in TEM for the first time are in bold. Genera in either subfamily are listed alphabetically since there is no well-supported concept of relationships within Parasitidae at the generic level.

subfam. Pergamasinae (23 genera)		
genus	species originally studied	species known from literature
<i>Amblygamasus</i>		
<i>Ernogamasus</i>		
<i>Heteroparasitus</i>	<i>H. tirolensis</i> (Sellnick)	
<i>Holoparasitus</i>	<i>H. calcaratus</i> (Koch) <i>H. excipuliger</i> (Berlese)	<i>Holoparasitus</i> sp.: Alberti 1980
<i>Leptogamasus</i>	<i>L. anoxygenellus</i> (Micherdziński) <i>L. belligerens</i> Witaliński	
<i>Mixogamasus</i>		
<i>Ologamasiphis</i>		
<i>Oocarpais</i>		
(<i>Paragamasus</i> s.l.)		
<i>Aclerogamasus</i>	<i>A. similis</i> (Willmann)	
<i>Anchigamasus</i>		
<i>Anidogamasus</i>	<i>A. teutonicus</i> (Willmann)	
<i>Beogamasus</i>		
<i>Dyogamasus</i>		
<i>Lathragamasus</i>		
<i>Menadenogamasus</i>		
<i>Paragamasus</i> s.s.	<i>Paragamasus</i> sp.	
<i>Pergamasellus</i>		
		<i>P. alpestris</i> Berlese: Alberti 1980 <i>P. barbarus</i> Berlese (as <i>P. viator</i>): Witaliński 1975; Witaliński & Dallai 1991; this study <i>P. brevicornis</i> Berlese: Sokolov 1934; this study <i>P. crassipes</i> (Linnaeus): Alberti 1980; Alberti & Coons 1999; this study <i>P. septentrionalis</i> (Oudemans): Sokolov 1934 <i>P. truatellus</i> Athias-Henriot: Witaliński & Dallai 1991
<i>Pergamasus</i>	<i>P. mediocris</i> Berlese	
<i>Phityogamasus</i>		
<i>Taiwanoparasitus</i>		
<i>Tanygamasus</i>		
<i>Tomeogamasus</i>		
<i>Zelogamasus</i>		
subfam. Parasitinae (16 genera)		
<i>Carpaidion</i>		
<i>Colpothylax</i>		
<i>Cornigamasus</i>	<i>C. lunaris</i> (Berlese)	
<i>Cycetogamasus</i>		
<i>Eugamasus</i>		
<i>Gamasodes</i>	<i>G. spiniger</i> (Trägårdh)	
<i>Neogamasus</i>		
	<i>P. krepelini</i> (Berlese): this study ; Sokolov 1934	<i>P. loricatus</i> (Wankel) (= <i>P. niveus</i>): Witaliński 1979
<i>Paracarpais</i>	<i>P. lunulata</i> (Müller)	
<i>Parasitellus</i>		
<i>Parasitus</i>	<i>P. coleoptratorum</i> (Linnaeus)	<i>P. berlesei</i> (Willmann): Alberti 1980 <i>P. magnus</i> (Kramer): Sokolov 1934
<i>Phorytocarpais</i>	<i>Ph. fimetorum</i> (Berlese)	
<i>Poecilochirus</i>	<i>P. carabi</i> G. & R. Canestrini	
<i>Psilogamasus</i>		
<i>Rhabdocarpais</i>		
<i>Shizosthetus</i>		
<i>Trachygamasus</i>	<i>Trachygamasus</i> sp.	

a new species under description and was obtained from composted hay near Kraków.

For light (LM) and transmission electron microscope (TEM) observations the male deutonymphs (*Cornigamasus*, *Gamasodes* and *Poecilochirus*) or males (other genera) were processed as follows: mites were immersed into a droplet of Karnovsky's fixative (Karnovsky 1965) on a Parafilm-coated microscopic slide and the anteriormost region containing gnathosoma was cut off with a fine razor blade to increase the permeability of the fixative. The remaining body was transferred into fresh fixative for 24 h at 4 °C then rinsed 4 × 15 min in 0.1 M cacodylate buffer (pH 7.2) containing 8% sucrose, and postfixed with 1.4% osmium tetroxide in 8% sucrose overnight at 4 °C. After dehydration in graded ethanol and propylene oxide, the material was embedded in EponTM 812 substitute (Sigma-Aldrich). Semithin Epon sections stained with a mixture of methylene blue and azur II, examined in LM were used for male deutonymph selection in *Cornigamasus*, *Gamasodes* and *Poecilochirus*, as well as for selection of proper sites for ultrathin sectioning and TEM observations. Thin sections were contrasted with uranyl acetate and lead citrate according to standard protocols (Venable and Coggeshall 1965) and examined with JEOL JEM 100SX and JEOL JEM 2100 HT (JEOL Ltd., Tokyo, Japan) transmission electron microscopes at 80 kV. To show the distribution of DNA in sperm cells, as well as for sperm measurements, male deutonymphs were dissected in 9% sucrose on a microscopic slide with fine tungsten needles to remove and disrupt deferent ducts. Released spermatozoa were stained with DAPI (Sigma-Aldrich) then covered with a coverslip and observed/documentated with an Olympus BX51 (Olympus Corporation, Tokyo, Japan) microscope fitted with optics for DIC (differential interference contrast) and for FM (fluorescence microscopy) in UV light. Measurements were taken on DIC-obtained photos, using the measurement option implemented in Corel Draw X6.

RESULTS

Spermatozoa in the studied species are similarly club-shaped in all Pergamasinae and most Parasiti-

nae genera, but in *Cornigamasus*, *Gamasodes*, *Trachygamasus*, *Parasitus berlesei* and *Paracarpais lorica-tus* they are more or less worm-like, *i.e.* very narrow, elongated and tapered terminally (Figures 2, 11, 13, 18 and Alberti 1980). In *Paracarpais lunulata* the spermatozoon is relatively short and thick, frequently banana-shaped (Figures 2, 15A inset). The nucleus in parasitid sperm is largely spindle-shaped with rounded ends, but in *Paracarpais lorica-tus* it was helically coiled (Witaliński 1979) (Figure 2). In *Cornigamasus lunaris* and *Trachygamasus* sp., the nucleus has narrow, tapered ends and extends along nearly the whole length of the sperm. In most cases, the nuclear material is electron-dense and homogenous, but in *Paragamasus* sp. (Figure 8C) it contains large electron-lucent spots of flocculent material, corresponding to darker spots observed in FM after DAPI staining (Figure 8B). Similar vacuoles can be observed at the nuclear periphery in *Anidogamasus teutonicus* which also contains many small electron-lucent spots making nuclear material inhomogeneous (Figure 7); such heterogeneity is also present in *Heteroparasitus tirolensis* (Figures 3A-B), *Cornigamasus lunaris* (Figures 12A-C), and *Paracarpais kraepelini* (Figures 14A-B).

DNA-containing chromatin granules have high electron density and occupy a small area in the middle part of the nucleus as shown in TEM (Figures 3A, 5A, D, 6A, 7, 10, 12C, 16D-E, 17A-B, 18C), FM after DAPI staining (Figures 8B, 9A-B, 11E, 15B inset) and LM after acetoorcein staining (Figure 11C). In *Paracarpais kraepelini*, however, chromatin granules are large and located one by one along the sperm nucleus (Figures 14A-B). In general, nuclear margins are well visible in LM, but in TEM the nuclear envelope in most cases cannot be easily discerned.

Inclusion bodies occur in two forms: striated (sIB) and granular (gIB) (e.g. Figures 3A-B). Striated IBs are distributed around the nucleus and just behind its posterior end. They are ellipsoidal and well delimited, and show a characteristic internal stratification (Figure 15B). In the postnuclear region of spermatozoa occur gIBs that are derivatives of sIBs. They are roundish and larger than sIBs, filled with dense granules surrounding a striated core and delimited with an evident two-layered envelope (Fig-

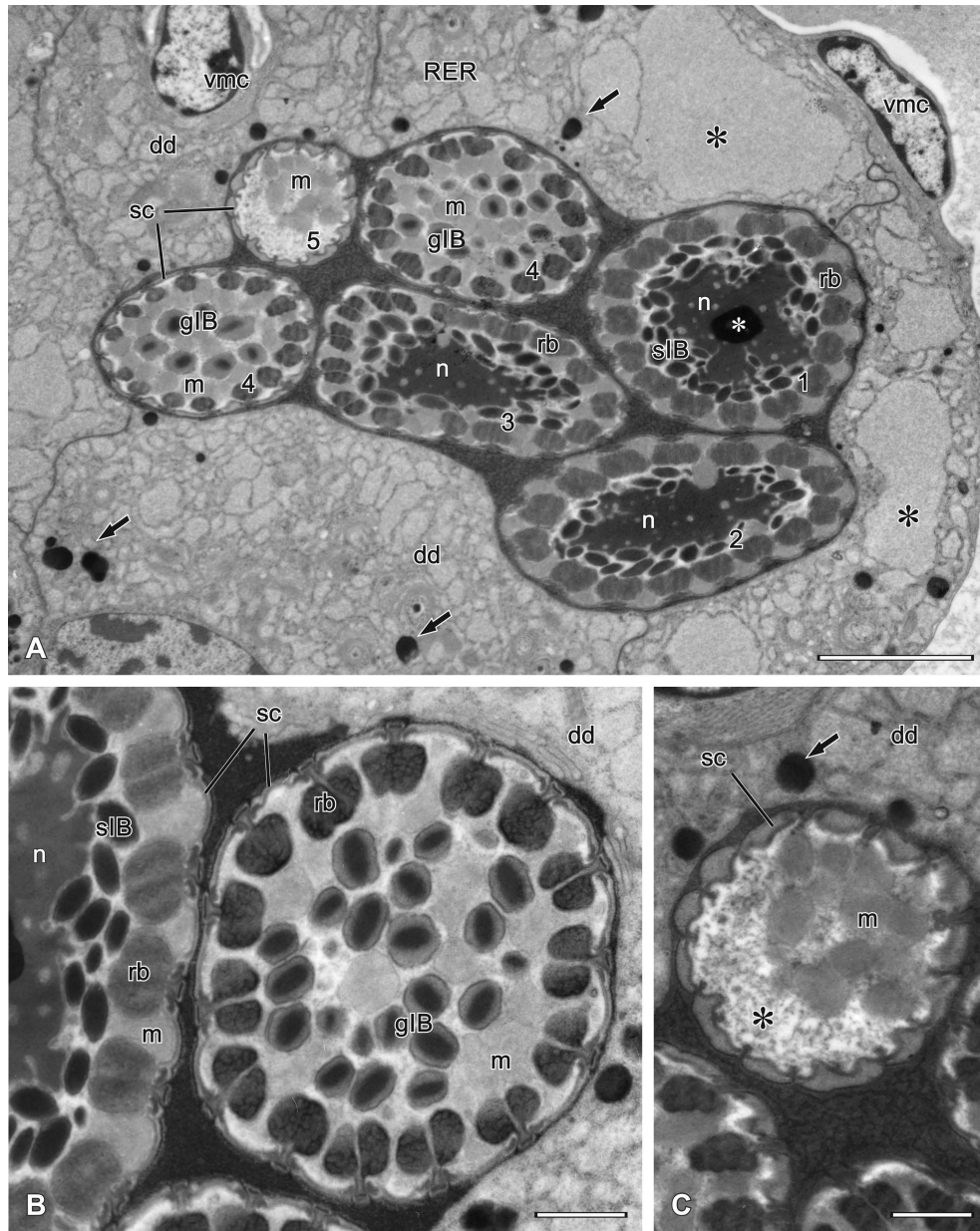


FIGURE 3: *Heteroparasitus tirolensis*, TEM. (A) Deferent duct (dd) containing several spermatozoa cross-sectioned at different levels. Section through nuclear region (1) shows nucleus (n) containing chromatin (white asterisk), sIBs surrounding nucleus and subplasmalemmal ribbons (rb). Sperm sectioned more caudally (2) shows the same organization; note different appearance of ribbons (left and right side) in sperm section close to the caudal end of nucleus (3). In sections at postnuclear region (4) gIBs are scattered among mitochondria (m). Posterior end of spermatozoon (5) is filled with flocculent material; there are also some mitochondria (m) and subplasmalemmal cisterns (sc). Deferent duct cells contain conspicuous RER filled with a moderately dense material (black asterisks) and electron-dense granules (arrows) in cytoplasm. Deferent duct is surrounded by visceral muscle cells (vmc). (B) Sperm section through nuclear region (left) showing nuclear material (n), sIBs, ribbons (rb) alternating with mitochondria (m), and subplasmalemmal cisterns (sc). Section of postnuclear region (right) adhering to deferent duct cell (dd) shows ribbons (rb) and subplasmalemmal cisterns (sc) surrounding gIBs and mitochondria (m). Note different density of ribbons in both sperm regions. (C) Posterior end of spermatozoon as (5) in (A), enlarged. Regularly distributed cisterns (sc) surround flocculent material (asterisk) with several mitochondria (m). Arrow indicates electron-dense, apparently secretory granules in deferent duct (dd) cell. Scale bars: 5 µm (A); 1 µm (B, C). Abbreviations: dd – deferent duct cell; gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, RER – rough endoplasmic reticulum in deferent duct cell, sc – subplasmalemmal cistern, sIB – striated inclusion body, vmc – visceral muscle cell

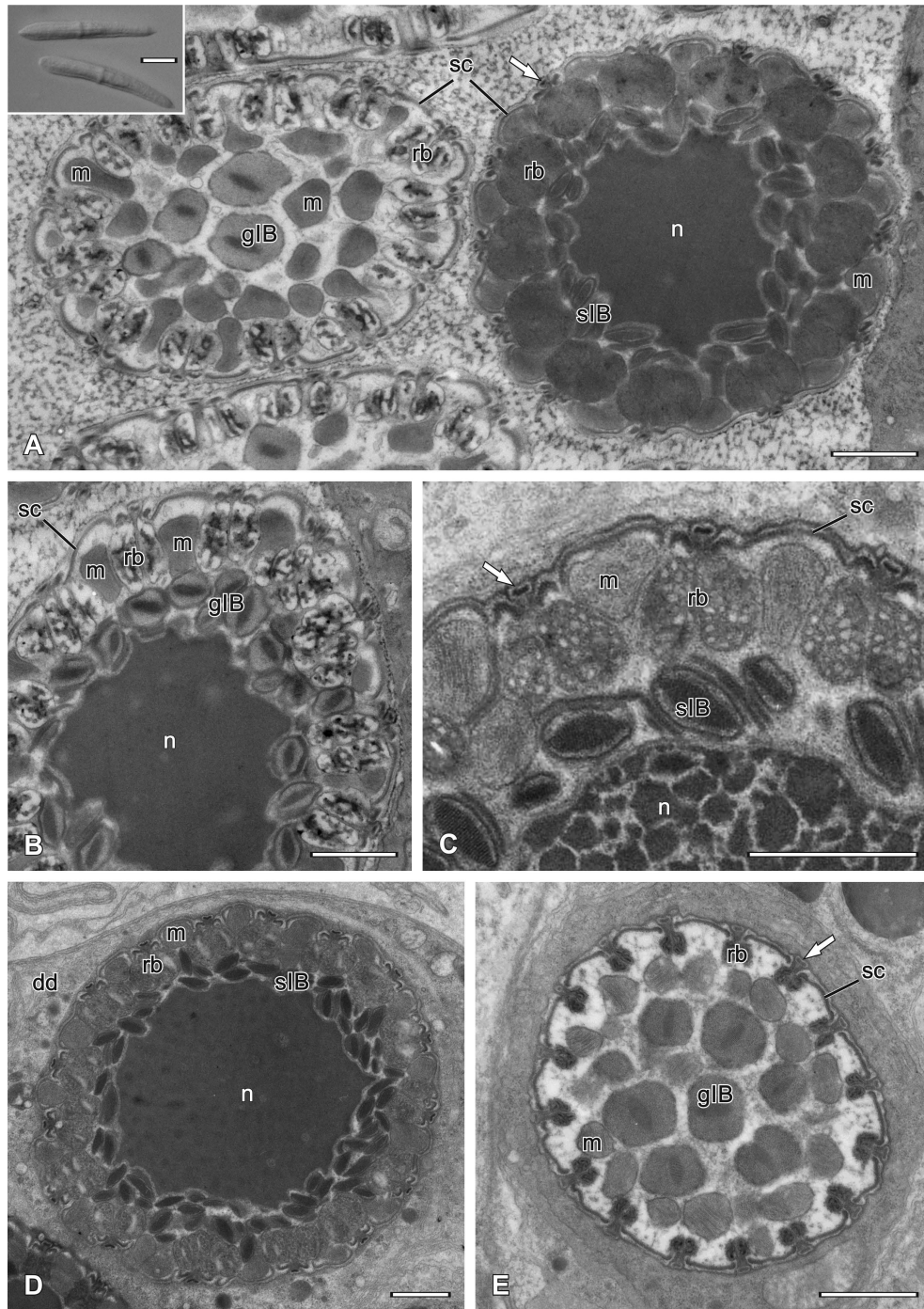


FIGURE 4: *Holoparasitus calcaratus* (A, B) and *H. excipuliger* (C-E), deferent duct spermatozoa except (C) which shows a testicular spermatid, TEM. (A) Sperm cells sectioned at nuclear region (right) and postnuclear region (left). Arrow indicates canaliculus running along longitudinal crest located above ribbon (rb). Inset: Two spermatozoa in DIC with nuclear (anterior) regions to the left. (B) Section at the level of posterior end of nucleus (n) surrounded by gIBs. Ribbons (rb) alternate with mitochondria (m), but ribbon appearance corresponds to postnuclear region rather than nuclear region. (C) Fragment of late spermatid sectioned at nucleus level. Nuclear material (n) is inhomogeneous due to incomplete condensation. Arrow indicates canaliculus running in longitudinal crest. Nuclear (D) and postnuclear (E) region of spermatozoon; note different compartment appearance in both regions. Arrow indicates canaliculus running in longitudinal crest. Scale bars: 1 μ m (A-E); 10 μ m (A, inset). Abbreviations: dd – deferent duct cell; gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern, slB – striated inclusion body

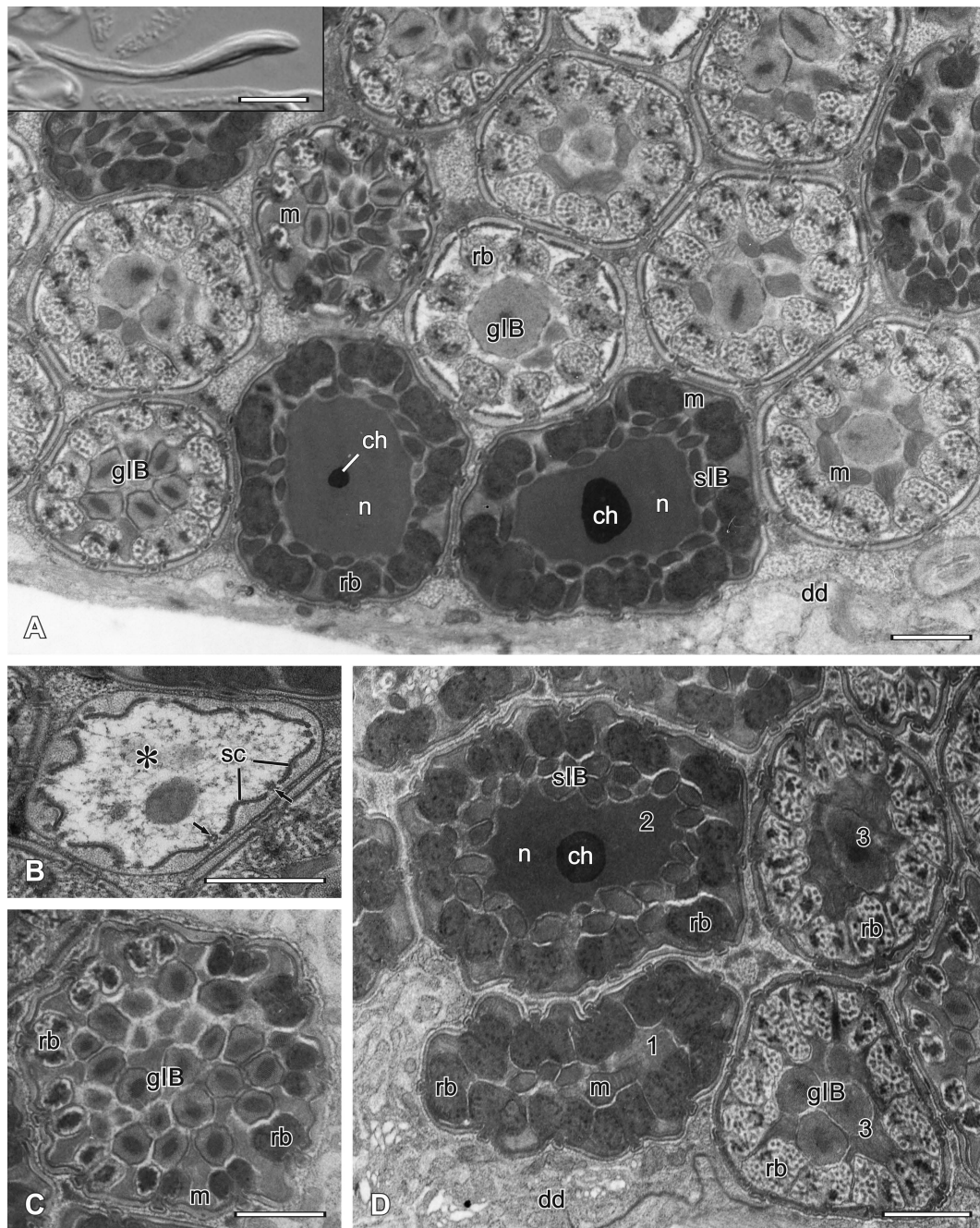


FIGURE 5: *Leptogamasus anoxygenellus* (A, B) and *L. belligerens* (C, D), deferent duct spermatozoa, TEM. (A) Many sperm sections at different levels. In two sections through the nucleus (n) condensed chromatin granules (ch) are visible. Inset: Spermatozoon with anterior end directed to the right, DIC. (B) The caudal end of a spermatozoon filled with flocculent material (asterisk). Ribbons and crests are no longer visible, but subplasmalemmal cisterns (sc) alternating with canaliculi (arrows) are still present. (C) Spermatozoon sectioned at postnuclear region. (D) Spermatozoa sectioned through the anterior (prenuclear) region (1), nuclear region (2), as well as postnuclear region (3). Scale bars: 1 μm in (A–D); 10 μm in (A, inset). Abbreviations: ch – chromatin, dd – deferent duct cell; gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern, sIB – striated inclusion body

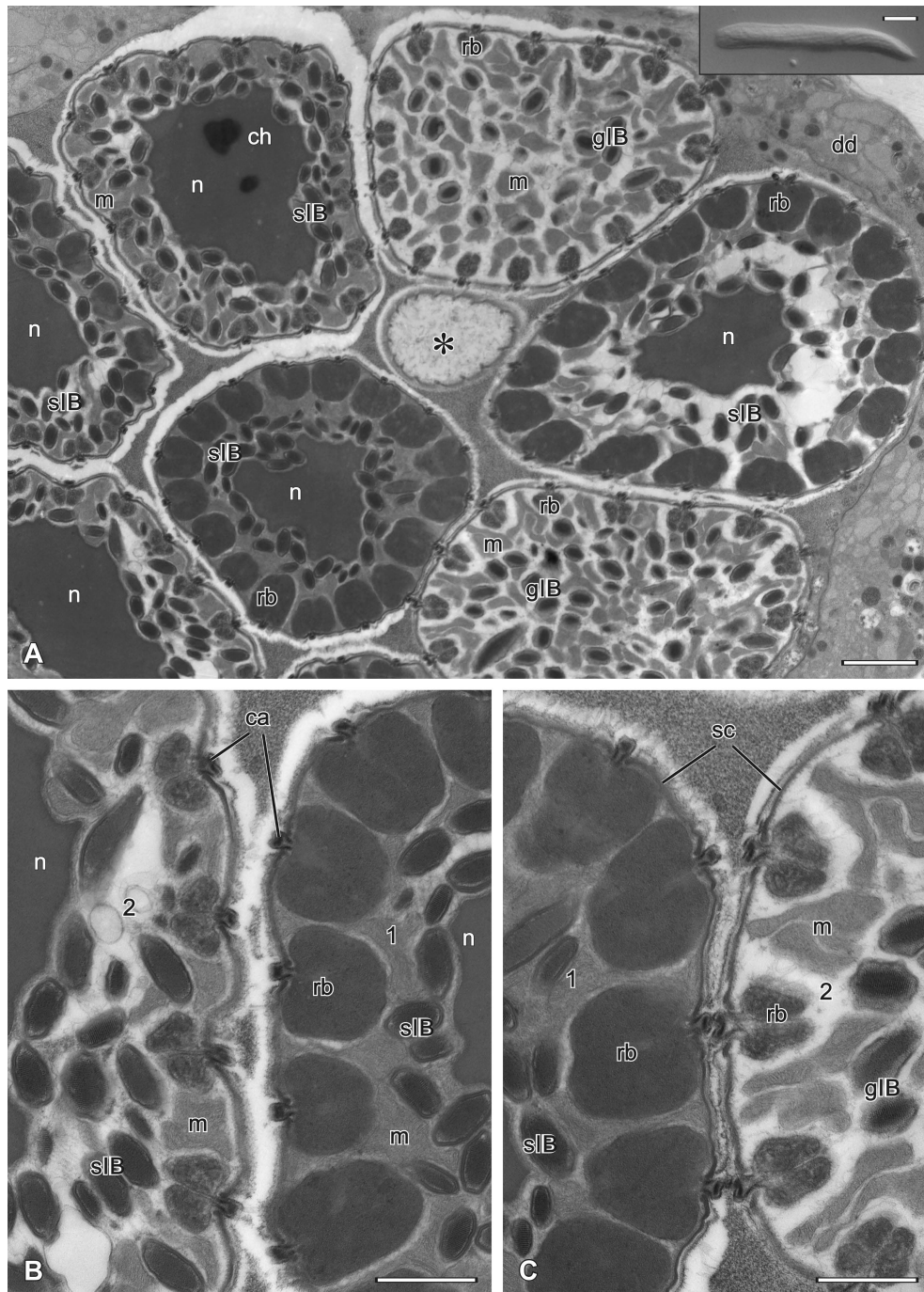


FIGURE 6: *Aclerogamasus similis*, deferent duct spermatozoa, TEM. (A) Many sperm sections at different levels: nuclear level with nuclear material (n) and condensed chromatin (ch), postnuclear level showing spermatozoon filled by gIBs and mitochondria (m), and also caudal end filled with flocculent material (asterisk) of the postnuclear region (as in Fig. 5B). Inset shows spermatozoon with anterior end directed to the left, DIC. (B) Peripheral fragments of two spermatozoa sectioned at nuclear region, at two levels – middle (1) and more posterior (2). (C) As in (B) but sections through middle nuclear region (1) adhere to postnuclear region (2). Scale bars: 2 μ m in (A); 10 μ m in (A, inset); 1 μ m in (B, C). Abbreviations: ca – canaliculus running in longitudinal crest, ch – chromatin, dd – deferent duct cell; gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern, sIB – striated inclusion body

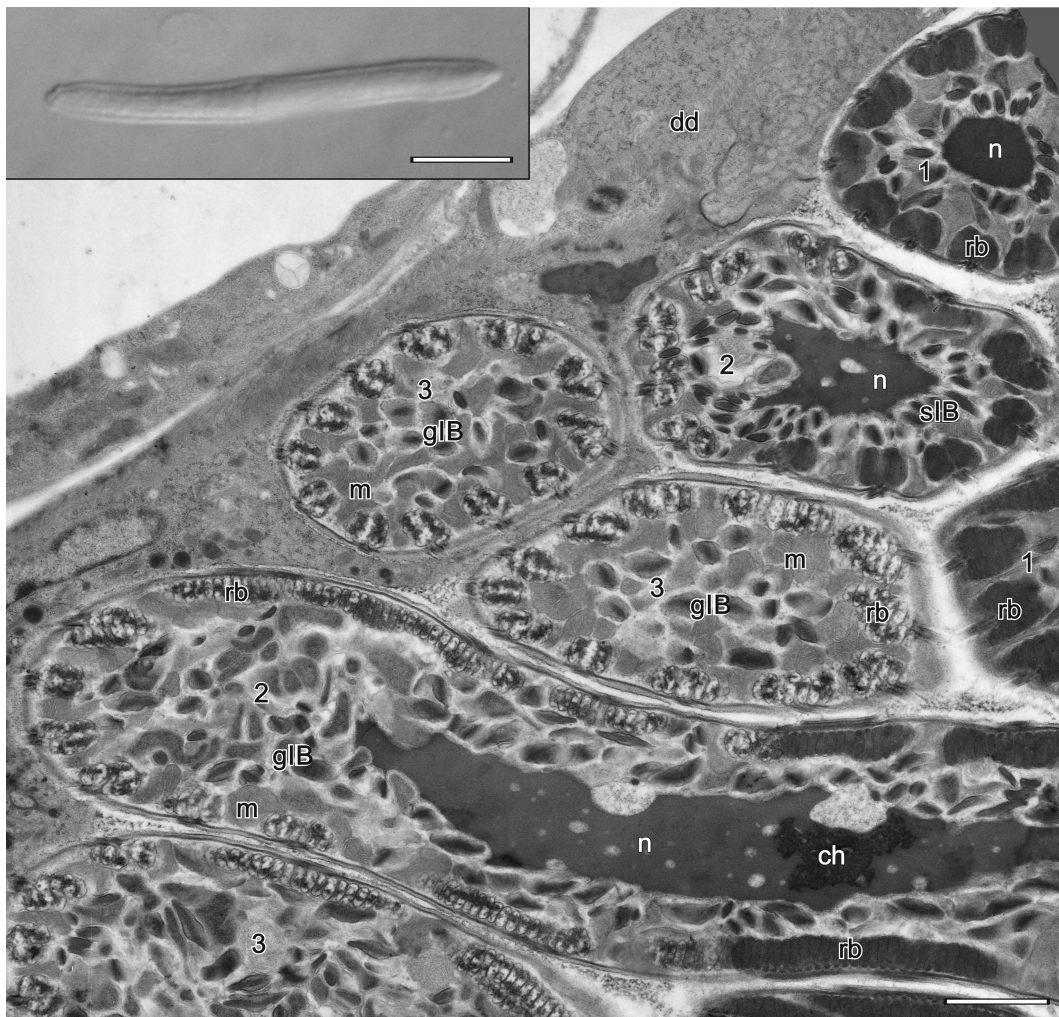


FIGURE 7: *Anidogamasus teutonicus*, spermatozoa in deferent duct (dd), TEM. Sperm cells sectioned through the middle of nuclear region (1) and more caudally (2) are spread among postnuclear sections (3). Note that sections (2) are somewhat oblique and show ribbons of different appearance, electron-dense ones – characteristic for anterior and middle parts of nuclear region, as well as electron-lucent ones, typical for the postnuclear region but also present in the posterior part of the nuclear region. Scale bar: 2 µm. Inset: spermatozoon in DIC with anterior end directed to the right. Scale bar: 10 µm. Abbreviations: ch – chromatin, dd – deferent duct cell; gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sIB – striated inclusion body

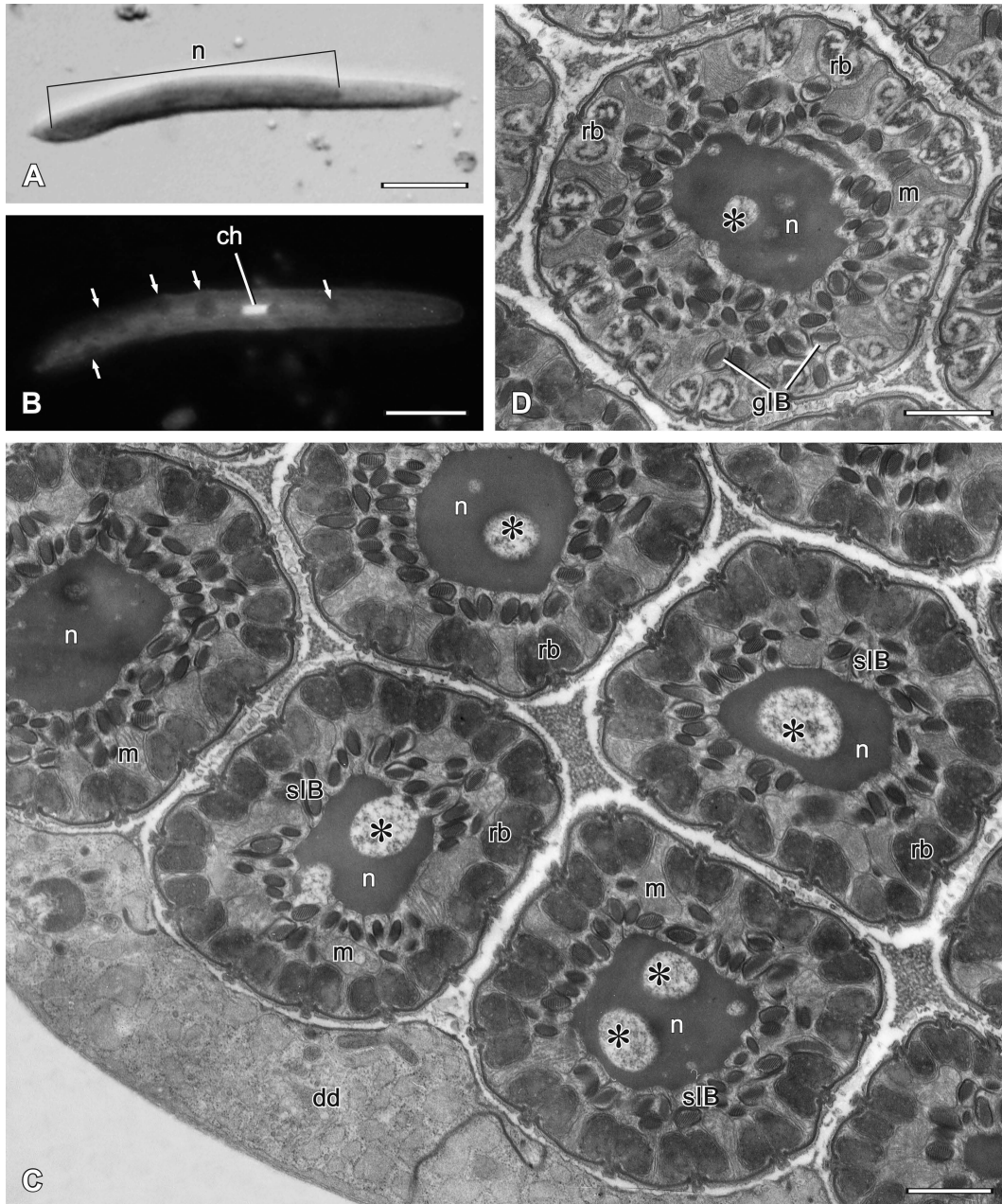


FIGURE 8: *Paragamasus* sp., isolated spermatozoon in DIC (A), in FM stained with DAPI (B), and deferent duct (dd) spermatozoa in TEM (C, D). (A) More than two-thirds of the anterior part of the spermatozoon contains the nucleus (n). (B-D) Note that nuclear material (n) contains vacuoles well discernible both in FM images (arrows) as well as in TEM (asterisks). Sections through anterior half of spermatozoa (C) show sIBs and dense ribbons (rb), whereas more posterior section (D) also contains glBs and ribbons of lower density. Scale bars: 10 μ m in (A, B); 1 μ m in (C, D). Abbreviations: ch – chromatin, dd – deferent duct cell; glB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sIB – striated inclusion body

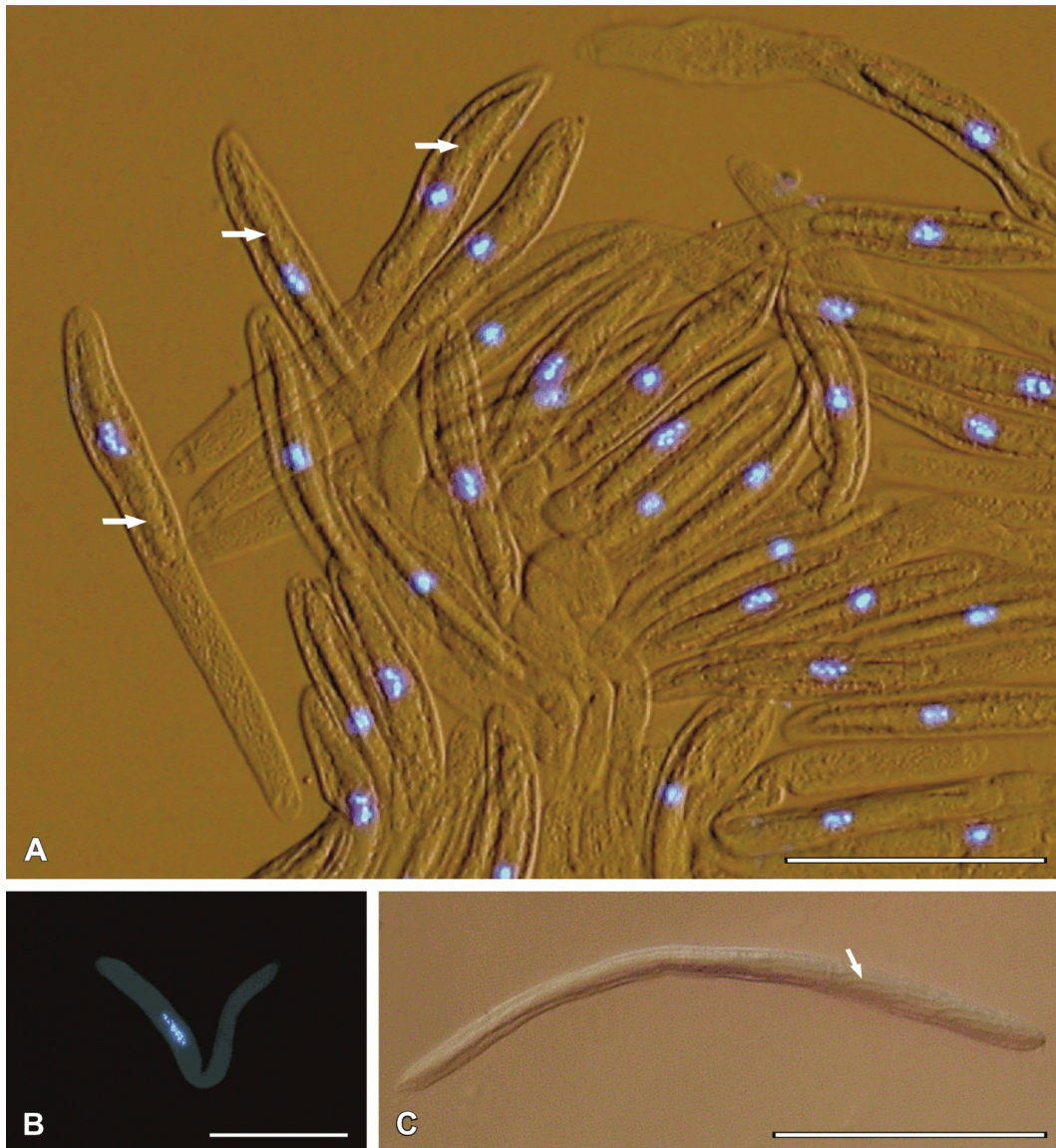


FIGURE 9: *Pergamasus barbarus* (A), *P. brevicornis* (B), and *P. crassipes* (C) isolated sperm. (A) Combined DIC and DAPI-stained fluorescence images; DNA (blue) is limited to several granules focused centrally in the elongated nuclei (arrows). (B) DNA-containing granules (blue) in DAPI-stained spermatozoon, FM. (C) Spermatozoon in DIC with a wider nucleus-containing region directed to the right and helically running ribbons visible at surface (arrow). Scale bars: 50 µm in (A-C).

ures 4A-B, E, 5A). The appearance of sIBs and gIBs is similar in all studied species except *Paracarpais kraepelini*, *Parasitus coleoptratorum* and *Trachygamasus* sp. In the nuclear region of *Paracarpais kraepelini* sperm (Figures 14A-B), a very narrow space free of nucleus and ribbons is filled exclusively with mitochondria, whereas sIBs are not present. In the post-nuclear region (Figures 14C-D) gIBs are present, but

paracrystalline cores are poorly discernible. In *Parasitus coleoptratorum* both nuclear and postnuclear regions contain inclusion bodies of similar appearance filled with electron-lucent flocculent material; their shape is irregular and margins are frequently unclear (Figures 16D-F). In *Poecilochirus carabi* IBs both around the nucleus and in the postnuclear region (Figure 17B) are electron-lucent and devoid

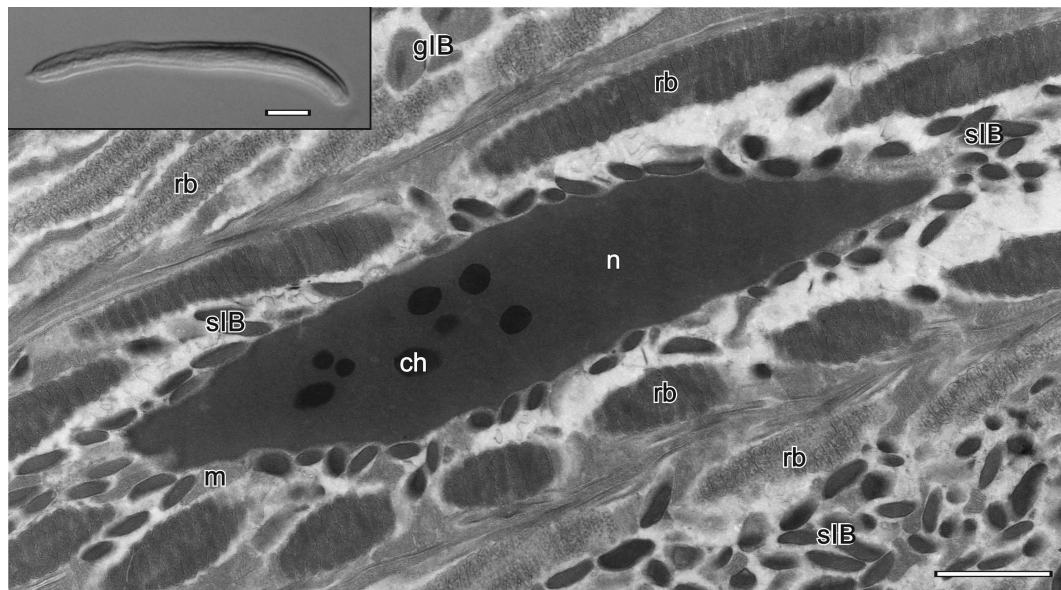


FIGURE 10: *Pergamasus mediocris* spermatozoon sectioned obliquely through nuclear region showing nuclear material (n), chromatin granules (ch), sIBs, mitochondria (m) and ribbons (rb). Fragment of section through postnuclear region just behind the nucleus with sIBs is visible in lower right corner, whereas in upper left corner there is a fragment of postnuclear region sectioned more posteriorly, containing gIBs instead of sIBs. Scale bar: 2 μ m. Inset: spermatozoon in DIC with anterior end directed to the right. Scale bar: 10 μ m. Abbreviations: ch – chromatin, gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sIB – striated inclusion body

of any stratification, but their margins are thickened and electron-dense. Most of the *Trachygamasus* sp. spermatozoon is filled with nucleus, therefore the postnuclear region is small (Figure 18A). Numerous sIBs surrounding the nucleus can be recognized mainly by paracrystalline stratification (Figure 18C), but their margins in mature sperm are usually poorly visible as compared to the spermatid (Figure 18B).

A striking feature of parasitid spermatozoa is a complex of superficial membranous structures: longitudinally oriented ribbons composed of double vesicular invaginations of plasmalemma, subplasmalemmal electron-dense cisterns located between ribbons, as well as plasmalemmal evaginations running above each ribbon and hosting a more or less flattened canaliculus (Figure 1B) (Witaliński 1975). The number of ribbons varies between genera and species, but can also vary even within the same specimen (Table 2). The number of ribbons ranges from 5 to 30. The lowest number 5 is in *Paracarpais loricatus* (= *Parasitus niveus*) (Witaliński 1979) and 5-7 in *Parasitus berlessei* (Alberti 1980), 7-

9 in *Paracarpais kraepelini* (Figures 14A, C) and 8 in *Trachygamasus* sp. (Figure 18C). In the two studied species of *Leptogamasus* the ribbon number is 9-12 (Figure 5), whereas 12 or 13 were found in *Anidogamasus teutonicus* (Figure 7), *Cornigamasus lunaris* (Figures 12A-B), *Gamasodes spiniger* (Figures 13B-C) and *Phorytocarpais fimetorum* (Figure 17A). The same number of ribbons occurs in *Parasitus coleoptratorum* (Figures 16E-F), but the exceptionally low number of 9 ribbons can also be found in this species (Figure 16D). In *Paragamasus* sp. the number of ribbons is 12-14 (Figures 8C-D). In the most studied genera/species the ribbon number is 13-17: *Aclerogamasus* (Figure 6A), *Heteroparasitus* (Figures 3A-B), *Holoparasitus* (Figures 4A, D-E) (Alberti 1980), *Pergamasus* (most species) (e.g. Figure 10), and *Parasitus magnus* (Sokolov 1934). The highest ribbon numbers were found in *Poecilochirus carabi* (22-25) (Figure 17B) and in *Paracarpais lunulata* (24-30) (Figure 15A). Also, a relatively high range and number of ribbons were observed in *Pergamasus barbarus* (16-21, with modal number 19). The number of ribbons and sperm diameter are roughly corre-

TABLE 2: Basic data on sperm morphology in studied parasitid mites. Genera and species are arranged in alphabetic order as in Table 1. N/A – data not available; in bold – original data obtained in this study ("orig.").

genus	species	sperm length x diameter of nuclear region (µm)	length of nucleus (µm)	number of ribbons	source/references
subfam. Pergamasinae					
<i>Heteroparasitus</i>	<i>tirolensis</i>	N/A x 7.8-9.0	N/A	15 or 16 (same frequency)	orig.
<i>Holoparasitus</i>	<i>calcaratus</i>	46-49 x 4.5-6.5	24-27	13	orig.
<i>Holoparasitus</i>	<i>excipuliger</i>	N/A x 7.2-8.0	N/A	16 (sometimes 14 or 15)	orig.
<i>Holoparasitus</i>	sp.	N/A	N/A	13 or 14	Alberti 1980
<i>Leptogamasus</i>	<i>anoxxygenellus</i>	40-43 x 3.8-4.2	15-18	9, sometimes 10	orig.
<i>Leptogamasus</i>	<i>belligerens</i>	55-62 x 4.5-6.5	24-34	11 or 12	orig.
<i>Aclerogamasus</i>	<i>similis</i>	64-70 x 8.2-9.5	40-43	16 or 17	orig.
(=Paragamasus s.l.)					
<i>Anidogamasus</i>	<i>teutonicus</i>	42-47 x 4.2-5.2	32-37	12 or 13	orig.
(=Paragamasus s.l.)					
<i>Paragamasus</i>	sp.	51-57 x 4.2-5.2	36-40	déc-14	orig.
(=Paragamasus s.l.)					
<i>Pergamasus</i>	<i>alpestris</i>	63 x 8.5	N/A	15 or 16	Alberti 1980
<i>Pergamasus</i>	<i>barbarus</i>			15-20	Witaliński 1975;
	(as <i>P. viator</i>)	110-113 x 8.1-9.2	48-53	(most frequently 18 and 19)	Witaliński & Dallai 1991
				16 – 21 (modal 19)	orig.
<i>Pergamasus</i>	<i>brevicornis</i>	125-130 x N/A	N/A	13 – 15 (modal 14)	Sokolov 1934 as <i>Gamasus brevicornis</i>
		111-119 x 6.7-8.2	61-72	13 – 15 (modal 14)	orig.
<i>Pergamasus</i>	<i>crassipes</i>	N/A	N/A	15 or 16 (seldom 17)	Alberti 1980; Alberti & Coons 1999
		121-130 x 6.9-7.4	65-71	15 or 16	orig.
<i>Pergamasus</i>	<i>mediocris</i>	81-98 x 6.0-7.0	42-49	14 (seldom 13 or 15)	orig.
<i>Pergamasus</i>	<i>septentrionalis</i>	100-105 x N/A	N/A	14	Sokolov 1934 as <i>Gamasus (Amblyogamasus) septentrionalis</i> var. <i>norvegicus</i>
<i>Pergamasus</i>	<i>truatellus</i>	80 x 6.8***	N/A	15 (modal) or 16	Witaliński & Dallai 1991
subfam. Parasitinae					
<i>Cornigamasus</i>	<i>lunaris</i>	322-335 x 5.0-5.7	285-298	12 or 13 (same frequency)	orig.
<i>Gamasodes</i>	<i>spiniger</i>	135-142 x 4.9-5.6	97-99	12 or 13 (similar frequency)	orig.
<i>Paracarpais</i>	<i>kraepelini</i>	70-87 x 3.0-3.7	69-83	7 or 8 (sometimes 9)	orig.
(=Vulgarogamasus)		65-70 x 4.0	N/A	06-sept	Sokolov 1934 as <i>Gamasus (Eugamasus) kraepelini</i>
<i>Paracarpais</i>	<i>loricatus</i> (=niveus)	120-130 x 3.0-3.5	25*	5	Witaliński 1979
(=Parasitus)					
<i>Paracarpais</i>	<i>lunulata</i>	49 x 8.9***	N/A	21(?)**	Witaliński & Dallai 1991
(=Porrhostaspis)		48-56 x 14.1-15.4	18-23	27 or 28 (sometimes 24, 25, 26, 29, 30)	orig.
<i>Parasitus</i>	<i>berlesei</i>	35 x 2.5	N/A	5 – 7 (modal 6)	Alberti 1980
<i>Parasitus</i>	<i>coleopratorum</i>	21-27 x 4.0-4.9	6.0-8.7	12, sometimes 13	orig.
<i>Parasitus</i>	<i>magnus</i>	60-75	N/A	13 or 14	Sokolov 1934 as <i>Gamasus (Eugamasus) magnus</i>
<i>Phorytocarpais</i>	<i>fimetorum</i>	80-90 x 4.6-5.5	58-63	13 (seldom 12)	orig.
<i>Poecilochirus</i>	<i>carabi</i>	N/A x 14.0-15.6	N/A	22 – 24 (sometimes 25)	orig.
<i>Trachygamasus</i>	sp.	204-220 x 4.5-5.2	175-200	8	orig.

* nucleus length is ca. 85 µm but in bent native form its length is ca. 25 µm.

** in Witaliński and Dallai (1991) number of ribbons is 21, which is not in accordance with the presented results and may be due to 1) different methods of sperm preparation and ribbon counting – counting of crests on sperm surface close to posterior end of spermatid in SEM (Witaliński & Dallai 1991), and on sperm cross sections in TEM (this study). Close to the posterior end of sperm cells (and spermatids) the number of ribbons can be lower than in the remaining part of the cell. 2) In *Paracarpais lunulata* at least two slightly different populations are observed; the Italian specimens studied by Witaliński and Dallai (1991) differ from Polish specimens (this study). It is possible that both morphologically different populations are actually different species with different sperm structure.

*** approximate dimensions were obtained from measurements of SEM-processed spermatozoon (in Witaliński and Dallai 1991, scale bar in Figs. 18 and 19 should be read 20 µm instead of 2 µm).



FIGURE 11: *Cornigamasus lunaris*. (A) Fragment of deferent duct with extending spermatozoa, DIC. (B) Isolated spermatozoon with anterior pole directed upward, DIC. (C) Spermatozoon stained with acetoorcein to show nuclear material (n) and chromatin (ch) in light microscope. (D,E) The same spermatozoon after DAPI staining in DIC (D) and in FM (E) to localize DNA in chromatin (ch) within nuclear material (n). Scale bars: 100 µm in (A); 50 µm in (B-E). Abbreviations: ch – chromatin, n – nucleus.

lated (Figure 19).

Ribbon internal structure usually varies along the length of the sperm and, in general, they are more electron-dense in the nuclear region than in the postnuclear region, as in *Holoparasitus*, *Lep-togamasus*, *Aclerogamasus*, *Anidogamasus*, *Pergama-sus* and *Poecilochirus* (Figures 4-7, 10, 17B). In other

genera, the differences in ribbon appearance be-tween nuclear and postnuclear regions are less pro-nounced or evidently absent, as in *Cornigamasus*, *Gamasodes*, *Paracarpais*, *Parasitus coleoptratorum* and *Trachygamasus* (Figures 12-16, 18). Moreover, the double structure of each ribbon on cross-section can be clearly visible (in e.g. *Heteroparasitus* – Figure

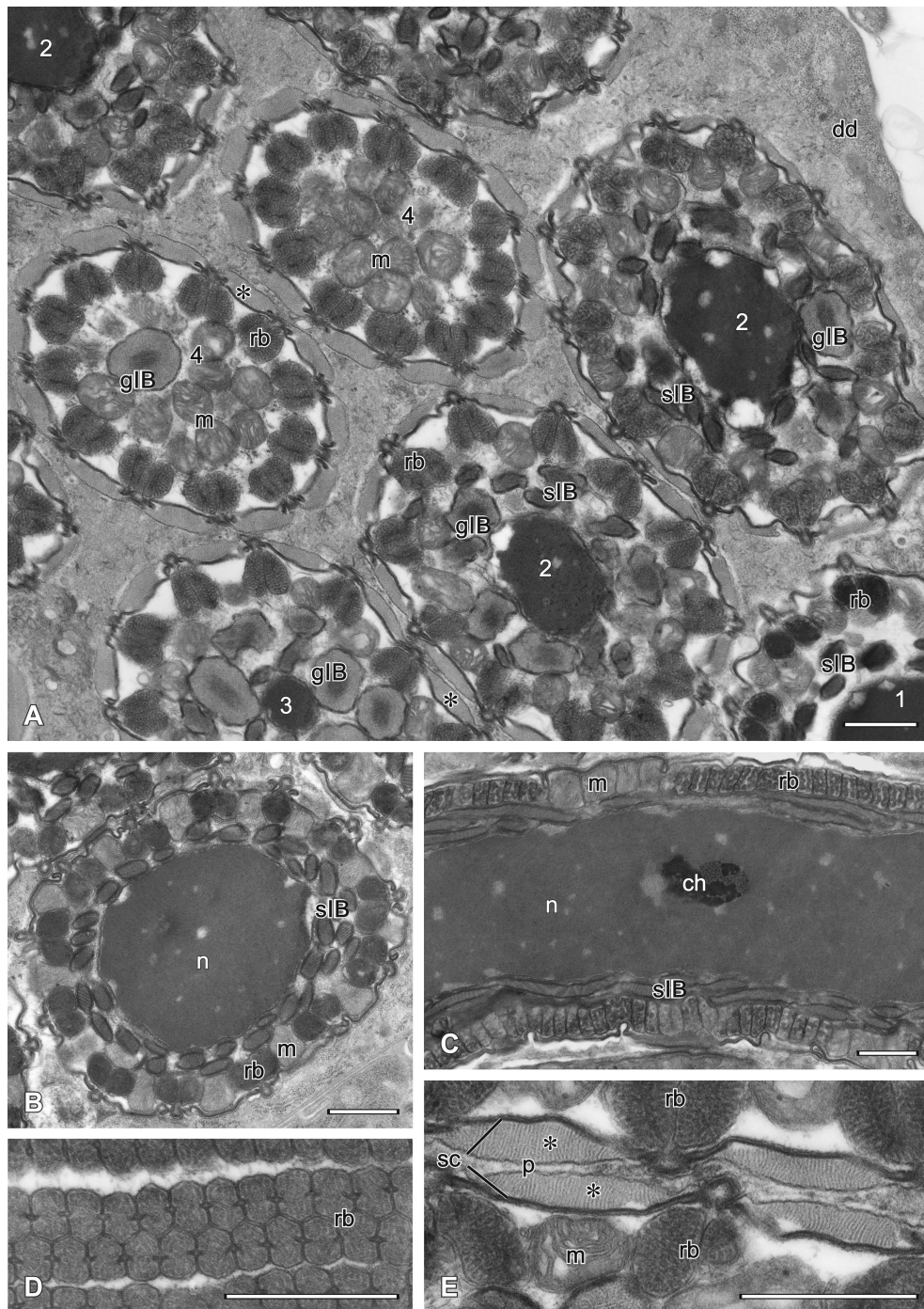


FIGURE 12: *Cornigamasus lunaris*, TEM. (A) Deferent duct (dd) spermatozoa sectioned at different nucleus levels in anterior-posterior sequence (1-3) and through postnuclear region (4). Note some material (asterisks) located between plasmalemma and subplasmalemmal cisterns (compare with E). (B) Cross section through spermatozoon at nuclear region. (C) Nearly longitudinal section of sperm nuclear region (as in B) showing nuclear material (n), chromatin (ch), ribbons (rb) and mitochondria (m). (D) Tangential section of ribbon (rb) showing a double row of vesicular bodies. (E) Superficial fragments of two adhering cross-sectioned spermatozoa. Note regularly arranged material (asterisks) in space between sperm plasmalemma (p) and subplasmalemmal cisterns (sc). Scale bars: 1 μm in (A-E). Abbreviations: ch – chromatin, dd – deferent duct cell, glB – granular inclusion body, m – mitochondrion, n – nucleus, p – sperm plasmalemma, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern, sIB – striated inclusion body

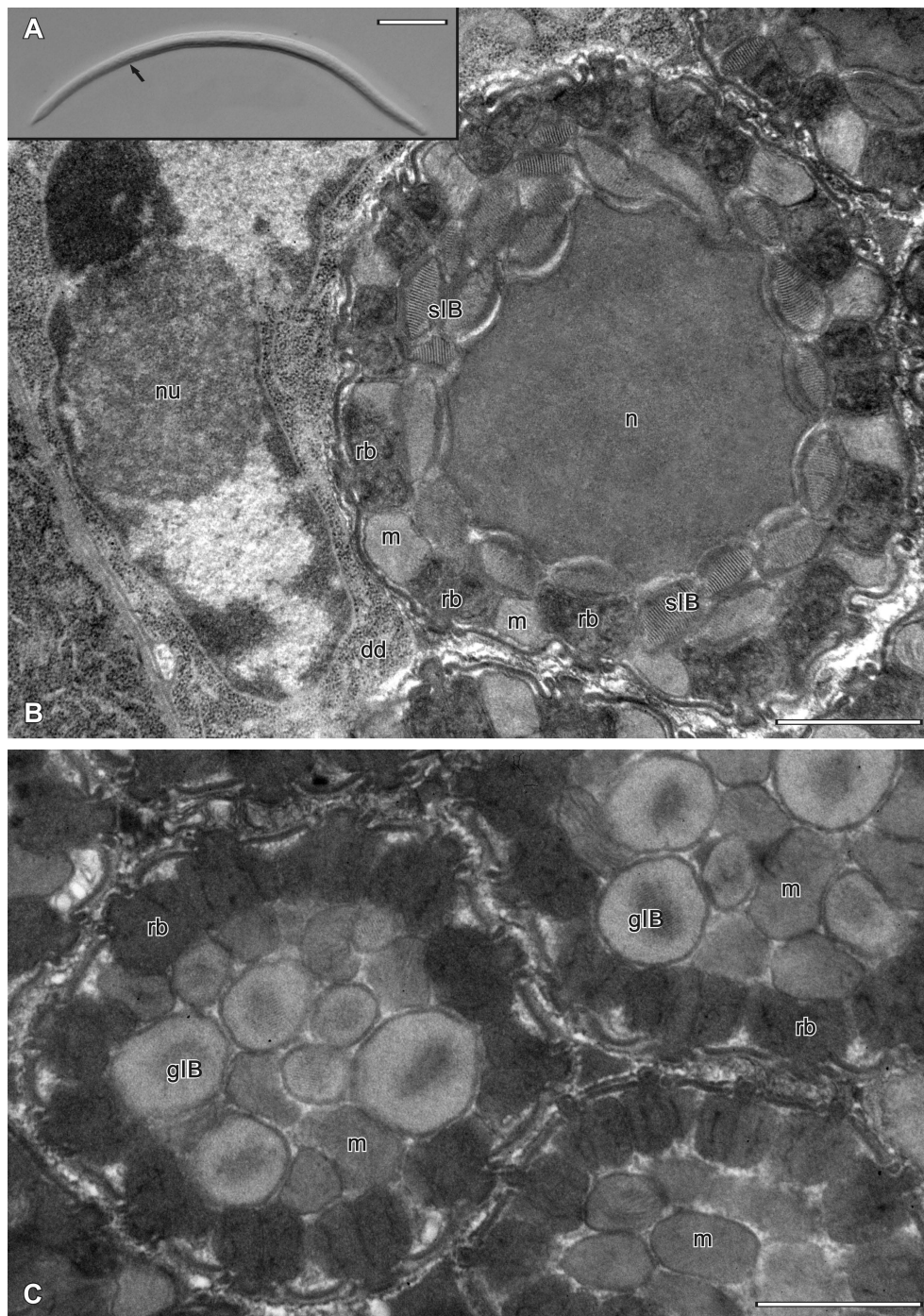


FIGURE 13: *Gamasodes spiniger*, DIC (A), TEM (B, C). (A) Isolated spermatozoon with anterior end directed to the right. Arrow indicates posterior end of nucleus. (B) Deferent duct spermatozoon cross-sectioned through the nucleus filled with homogenous nuclear material (n) and surrounded by sIBs. More peripherally, 13 ribbons (rb) alternating with mitochondria (m) are present. Nucleus of deferent duct cell (dd) contains a prominent nucleolus (nu). (C) Deferent duct spermatozoa sectioned at postnuclear region, containing gIBs and mitochondria (m) surrounded by ribbons (rb); note only 12 ribbons in left sperm section; sperm cell sectioned more posteriorly (lower right corner) is filled with mitochondria (m), whereas gIBs are no longer present. Scale bars: 20 μm in (A); 1 μm in (B, C). Abbreviations: dd – deferent duct cell, gIB – granular inclusion body, m – mitochondrion, n – nucleus, nu – nucleolus in nucleus of deferent duct cell, rb – subplasmalemmal longitudinal ribbon, sIB – striated inclusion body

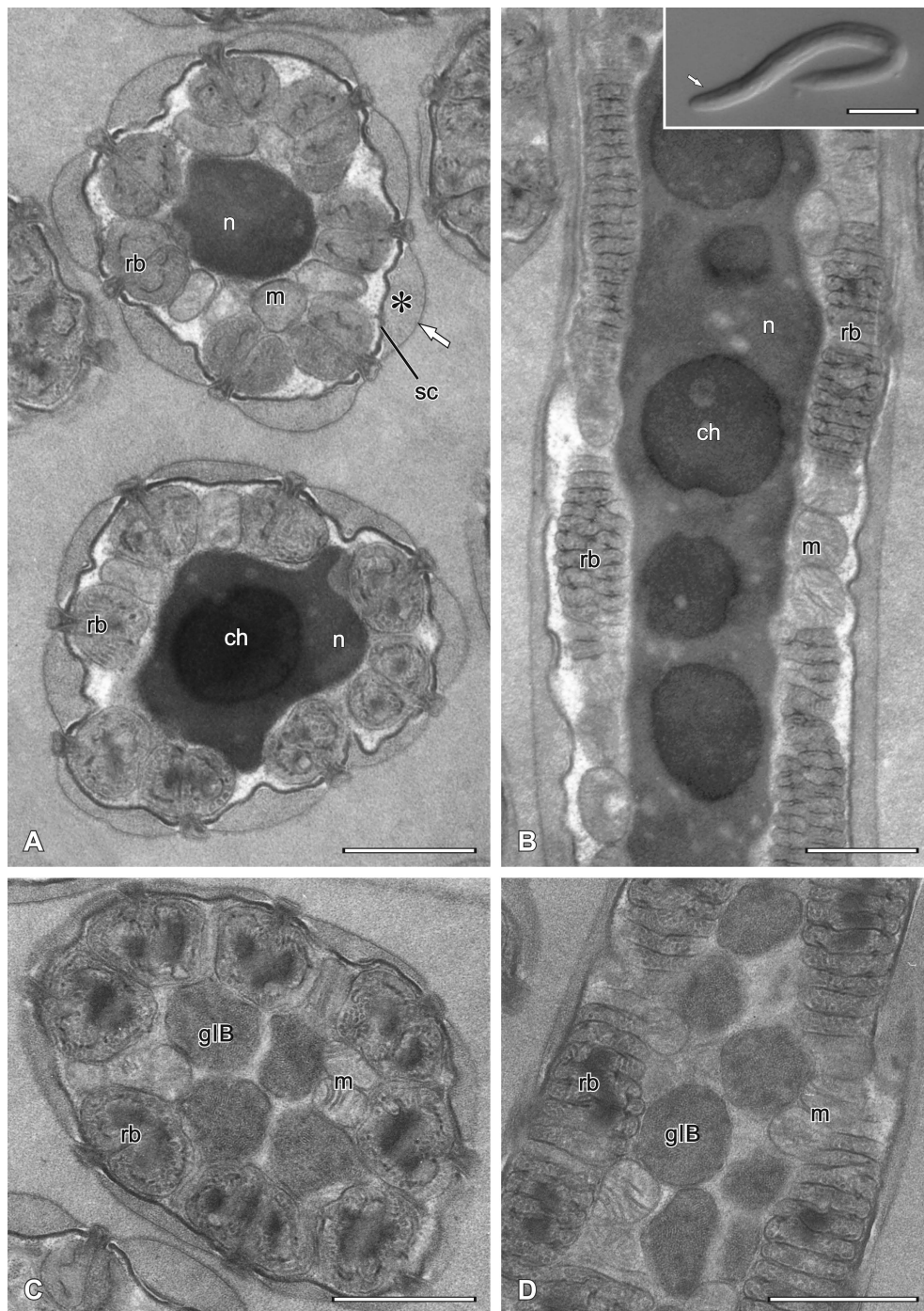


FIGURE 14: *Paracarpais kraepelini*, spermatozoa, TEM. (A) Two spermatozoa sectioned through nuclear region showing different numbers of ribbons (rb); nucleus (n) with a large chromatin granule (ch) occupies most of the internal space, thus mitochondria (m) are mostly between ribbons since sIBs are absent. Note granular material (asterisk) located between plasmalemma (arrow) and subplasmalemmal cisterns (sc). (B) Nearly axial section through nuclear region demonstrating several large chromatin granules (ch) in nuclear material (n). Inset: bent spermatozoon with anterior end marked (arrow). (C, D) Postnuclear region in cross (C) and axial (D) section. Granular inclusion bodies (gIB) contain poorly organized granular material. Scale bars: 1 μm in (A-D); 10 μm in (B, inset). Abbreviations: ch – chromatin, gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern

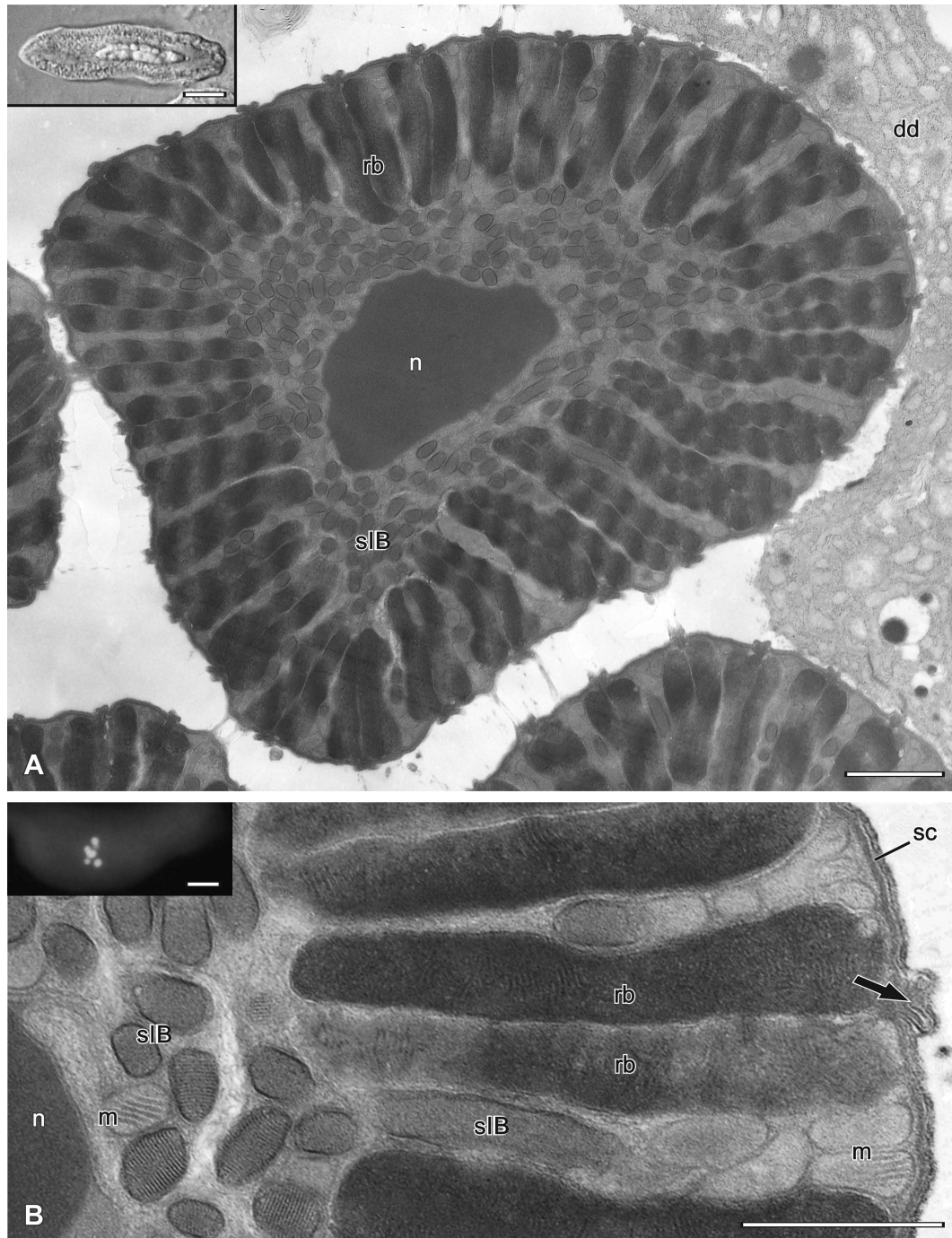


FIGURE 15: *Paracarpais lunulata*, cross-sectioned deferent duct spermatozoa, TEM. (A) Sperm cell with nucleus (n), sIBs, and ribbons (rb) deeply penetrating into the sperm cell. Inset: Spermatozoon with anterior pole directed to the right, DIC. (B) Higher magnification of spermatozoon as in (A). Note nucleus (n), sIBs, mitochondria (m), and double vesicular bodies of ribbons (rb) which penetrate the cell in this species 3.0–4.0 μm deep. Subplasmalemmal cisterns (sc), as well as longitudinal crest running above ribbon and containing canaliculus (arrow) are also present. Inset: DNA-containing granules in nucleus of spermatozoon, DAPI staining, FM. Scale bars: 2 μm in (A); 10 μm in (A, inset); 1 μm in (B); 5 μm in (B, inset). Abbreviations: dd – deferent duct cell, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern, sIB – striated inclusion body

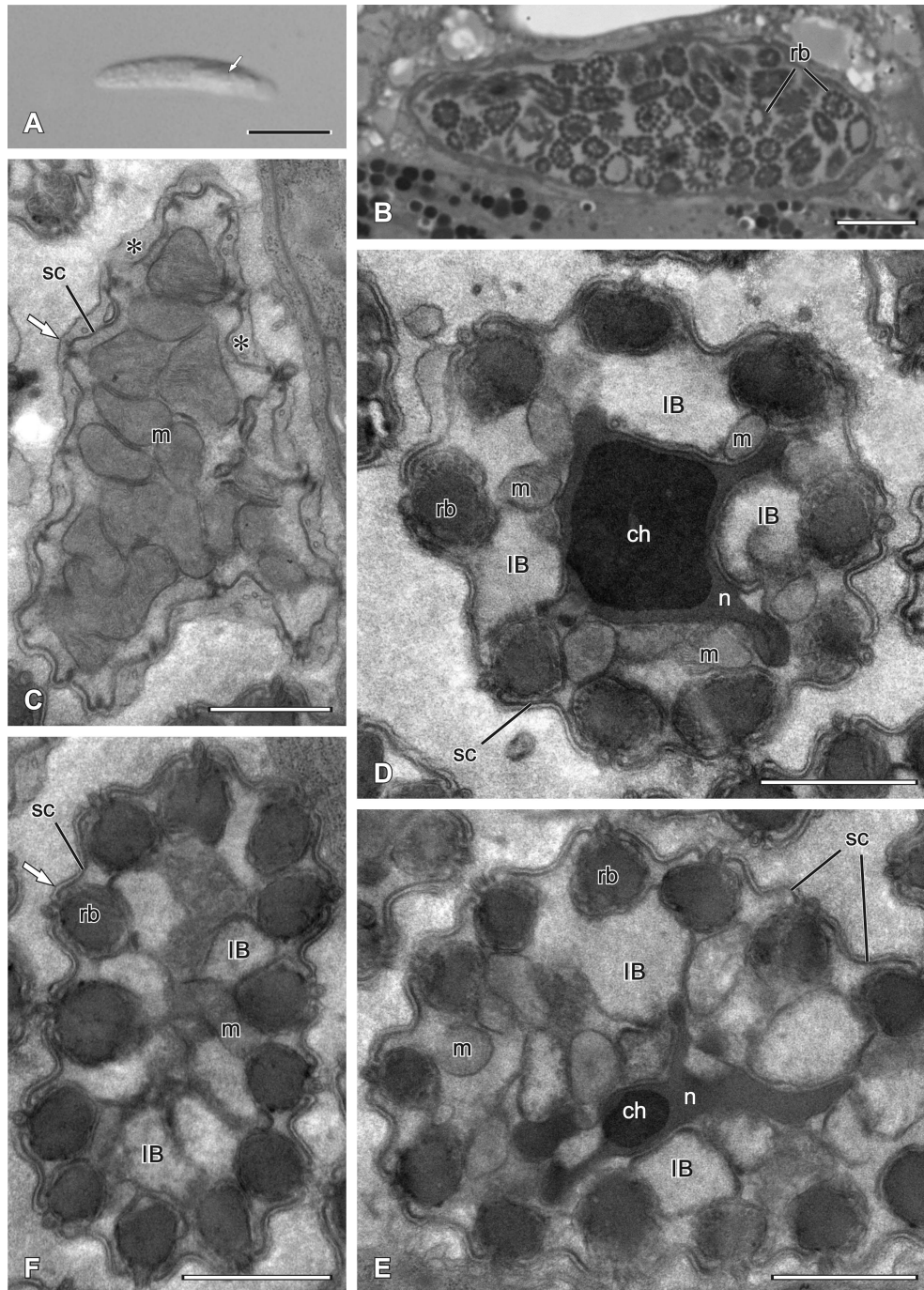


FIGURE 16: *Parasitus coleopratorum*, DIC (A), LM (B), spermatozoon cross sections in TEM (C-F). (A) Spermatozoon with anterior part containing nucleus (arrow) directed to the right. (B) Cross sections of spermatozoa filling deferent duct as visible is semithin Epon section. Dark rings of peripherally distributed ribbons (rb) are well discernible. (C) Extreme anterior end of spermatozoon packed with mitochondria (m). Note some material (asterisks) filling spaces between plasmalemma (arrow) and subplasmalemmal cisterns (sc). (D) Nuclear region showing nuclear material (n) containing a large chromatin granule (ch). Inclusion bodies (IB) with poorly discernible borders are filled with flocculent material of low electron density. (E) Section through the posterior end of nucleus (n) containing chromatin granule (ch), surrounded by inclusion bodies (IB) and mitochondria (m). (F) Postnuclear region; plasmalemma (arrow) and subplasmalemmal cisterns (sc) are in close apposition as in (D, E). Scale bars: 10 μ m in (A, B); 1 μ m in (C-F). Abbreviations: ch – chromatin, IB – inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern

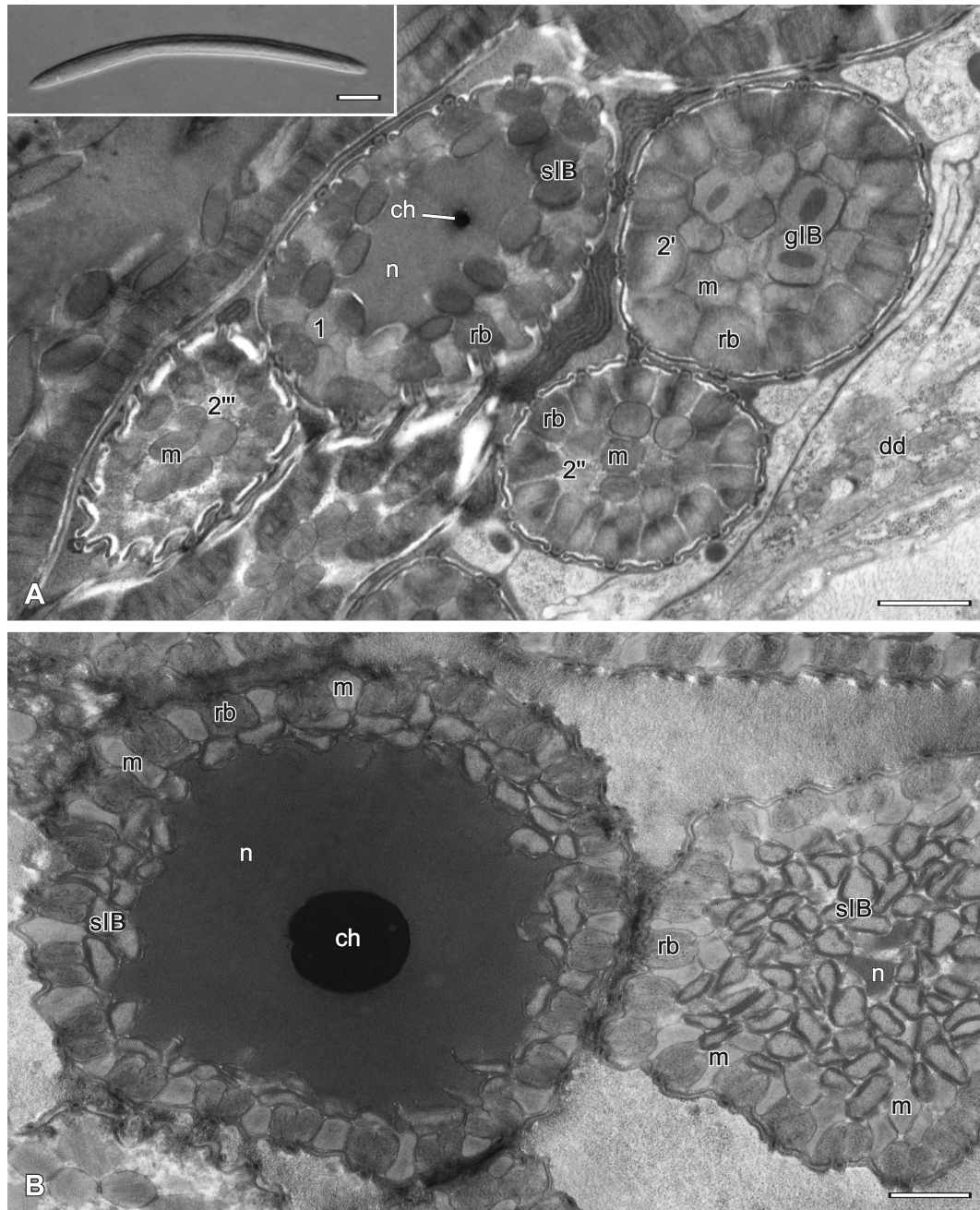


FIGURE 17: *Phorytocarpais fimetorum* (A) and *Poecilochirus carabi* (B), cross sections of deferent duct (dd) sperm, TEM. (A) sections through the nuclear region (1) and three subsequent levels of postnuclear region (2', 2'', 2'''). Inset: Spermatozoon with anterior end directed to the right, DIC. (B) Left spermatozoon is sectioned through nuclear region and presents nuclear material (n) with chromatin granule (ch), sIBs, ribbons (rb) and mitochondria (m). Right spermatozoon is sectioned through the posterior end of nucleus (n). Note a high number of sIBs. Scale bars: 1 µm in (A,B); 10 µm in (A, inset). Abbreviations: ch – chromatin, dd – deferent duct cell, gIB – granular inclusion body, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbons, sIB – striated inclusion body

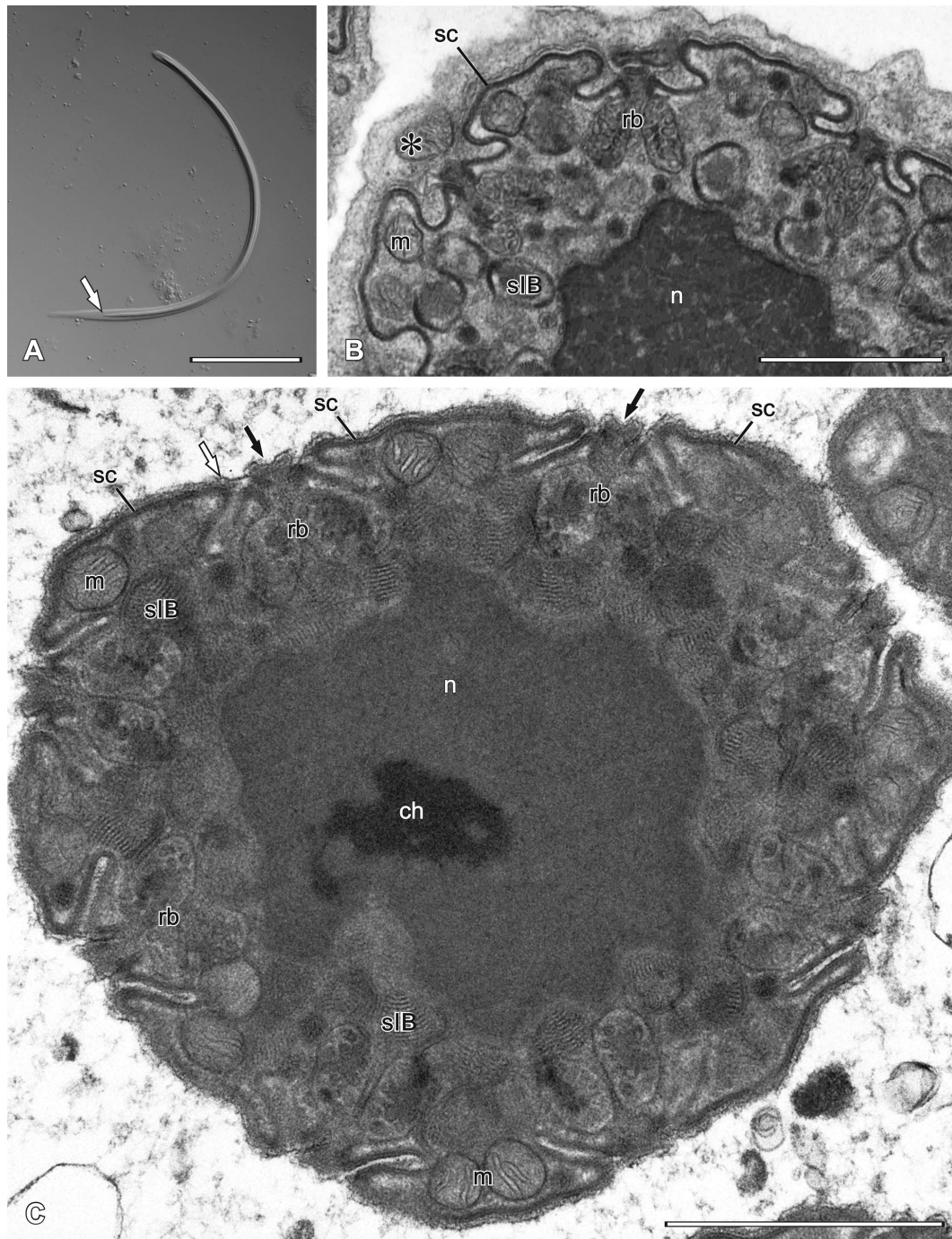


FIGURE 18: *Trachygamasus* sp., DIC (A), TEM (B, C). (A) Isolated spermatozoon with anterior end directed upward. Arrow indicates posterior end of nucleus. (B) Fragment of a late spermatid showing granular, incompletely condensed nuclear material (n), ribbons (rb), subplasmalemmal cisterns (sc), sIBs, and mitochondria (m). Asterisk marks mitochondrion in cyst cell enveloping spermatid. (C) Deferent duct spermatozoon sectioned through nucleus (n) containing chromatin (ch). Ribbons (rb), sIBs with unclear margins and mitochondria (m) are also present. Note sperm plasmalemma (white arrow), longitudinal crests (black arrows) above ribbons, as well as characteristic deep infoldings of plasmalemma and subplasmalemmal cisterns (sc) on both sides of ribbons. Scale bars: 50 μm in (A); 1 μm in (B,C). Abbreviations: ch – chromatin, m – mitochondrion, n – nucleus, rb – subplasmalemmal longitudinal ribbon, sc – subplasmalemmal cistern, sIB – striated inclusion body

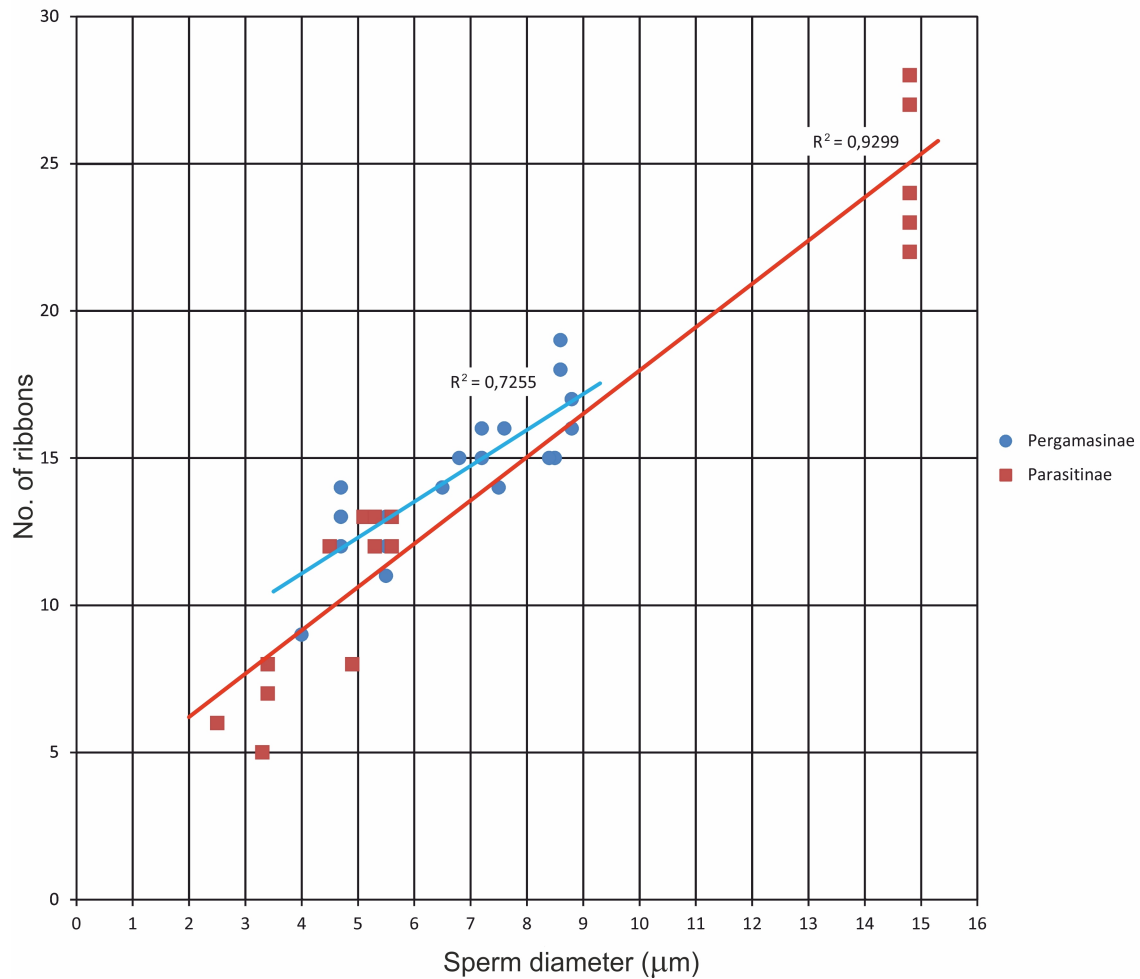


FIGURE 19: Plot generated in EXCEL OFFICE 2010 showing a correlation of average sperm diameter (nuclear region) and number of ribbons in studied species from both Parasitidae subfamilies. Number of ribbons is given either as number with modal frequency, most frequent number or numbers with the same frequencies (see Table 2). Trend lines with square of correlation coefficient (R^2) are also shown.

3B, *Aclerogamasus* – Figures 6A-C, *Paragamasus* sp. – Figures 8C-D, *Cornigamasus* – Figures 12A-B, D, *Paracarpais lunulata* – Figures 15A-B, *Trachygamasus* – Figure 18C), but in some species (e.g. *Parasitus coleoptratorum* – Figures 16D-F, *Paracarpais kraepelini* – Figures 14A, C) ribbons are visible as singular entities.

Subplasmalemmal cisterns are invariably located between ribbons and run parallel to the plasmalemma (Figures 3, 4, 6C, 12E, 14A, 15B, 16C-F, 18B-C). They extend from the anterior to nearly posterior tip of the spermatozoon where the ribbons

are absent (Figures 3A, C, 5B, 6A, 16C). Both the plasmalemma and cistern are located close to each other. Only in *Cornigamasus lunaris* and *Paracarpais kraepelini* are the spaces between plasmalemma and cisterns wider; the spaces are filled with some granular material (Figures 12A, E, 14A), showing regularly arranged strands in *Cornigamasus* (Figure 12E). As observed in most species, cisterns are moderately arcuate on cross sections, but in *Trachygamasus* their marginal portions are deeply infolded (Figure 18B-C).

Between ribbons, as well as between gIBs in the

postnuclear region, there are many more or less crista-type mitochondria, spherical or somewhat elongated (e.g. Figures 7, 8C-D, 12A-E, 14, 15B, 17A-B, 18B-C), but irregular in shape in *Aclerogamasus* (Figures 6A-C). The anterior tip of the spermatozoon in *Parasitus coleoptratorum* is tightly packed with mitochondria (Figure 16C).

DISCUSSION

Spermatozoa of parasitid mites are classified as ribbon-type in contrary to the vacuolated sperm type present in Opilioacarida, Holothyrida, Ixodida and in the basal part of Gamasida (Alberti 1980, 1995; Alberti and Coons 1999; Alberti and Klompen 2002; Alberti and Seeman 2004; Reiher *et al.* 2006) and are unusual in their internal structure (Witaliński 1975; Alberti 1980). The same sperm type but modified in details is also present in Dermanyssina (Alberti 1980; Alberti and Hänel 1986; Alberti and Di Palma 2007; Alberti *et al.* 2013), a Parasitina-related but most recently evolved Gamasida group (Klompen *et al.* 2007). Differences in sperm morphology in both sister taxa could be the result of different modes of insemination (Di Palma *et al.* 2009): in Parasitina (Parasitidae) sperm is packed into a spermatophore-like vesicle and transferred into the only, primary genital orifice (oviporus) of the female by means of modified male chelicerae functioning as gonopods. Thus, sperm is directly deposited in the ultrastructurally complicated spermatheca (Alberti *et al.* 1999a; Witaliński and Borsuk 2002) located just behind the genital orifice (in a process called neotocospermy acc. to Alberti 2002). In Dermanyssina (e.g. Ameroseiidae, Dermanyssidae, Phytoseiidae, Varroidae, Veigaiidae), neopodosperry (Alberti 2002) occurs; in this mode of insemination, spermatozoa either closed or unenveloped within the spermatophore are introduced into solenostomes, i.e. two inseminatory secondary openings located near the coxae of the last leg pairs in the female (Di Palma and Alberti 2001; Alberti *et al.* 2009; Di Palma *et al.* 2012). Male chelicerae are more modified than in parasitid mites and show a prominent protrusion (spermatodactyl) of the movable digit, used for sperm insertion into the solenostome (Evans 1992; Alberti and Coons

1999; Di Palma *et al.* 2006; Di Palma *et al.* 2009). Both left and right solenostomes are connected with spermatheca via a complicated tubular inseminatory system (for further details and references concerning parasitid *versus* dermanyssid reproductive systems see Alberti and Coons 1999; Alberti *et al.* 1999a,b; Di Palma and Alberti 2001; Alberti and Di Palma 2002; Di Palma *et al.* 2012; Alberti *et al.* 2013).

Relatively simple insemination in Parasitidae is complemented with further events preceding fertilization (Alberti *et al.* 1999a,b; Alberti *et al.* 2000): spermatozoa leak from a spermatheca-inserted spermatophore into the cuticle lining the vaginal duct then uterus; they purportedly penetrate the uterine wall to migrate via hemocoelic spaces to eventually reach the ovarian germinal layer, hosting the oocytes, from the hemocoelic side. Scarce and fragmentary observations of capacitation changes in sperm morphology during their route to the fertilization place (the ovary) (Alberti *et al.* 2000) do not explain much of the function of the peculiar structures of the sperm. This scarce functional background makes evolutionary considerations of sperm structure especially weakly founded and speculative. Except for spermatozoa length, which vary between 21–27 μm (*Parasitus coleoptratorum*) and 322–335 μm (*Cornigamasus lunaris*), the most strikingly variable feature is the number of ribbons. It ranges from 5–7 in *Parasitus berlesei* and *Paracarpais loricatus*, up to 30 in *Paracarpais lunulata*. In most species, the number of ribbons is between 10 and 20. There is a rough correlation between cell diameter and number of ribbons (Figure 19), but from an evolutionary perspective it is unclear which factor (sperm diameter or ribbon number) was most important for selection. Moreover, there is no evident relation of sperm cell diameter/ribbon number and ecological or behavioral aspects of the genus: free-living litter predators (all studied Pergamasinae genera) and predatory inhabitants of decaying hay stacks (*Cornigamasus*, *Gamasodes*) have ca. 10–20 ribbons, whereas the litter inhabiting predator *Paracarpais lunulata* has as many as 30 ribbons. In the predatory ectoparasitic genus *Poecilochirus* (in fact being an ectoparasite as deutonymphs only: Baker and Schwarz 1997), the number of ribbons is

up to 25. The most surprising result was obtained in the genus *Paracarpais* Athias-Henriot, 1978. In three species currently studied, the full range of ribbon numbers was represented: the lowest (5 ribbons) in (*Paracarpais* (= *Parasitus*) *loricatus*, a medium number (7-9 ribbons) in *Paracarpais* (= *Vulgarogamasus*) *kraepelini* and the highest (30 ribbons) in *Paracarpais* (= *Porrhostaspis*) *lunulata*. Thus, the lowest and the highest number of ribbons can be represented within the same genus (but see Introduction for notes on taxonomy at generic/subgeneric level in Parasitidae). This suggests that the source of sperm modifications in reproductive physiology should be studied in detail at the cellular level.

The structure of ribbons in Parasitidae sperm is less variable than their number. These occur as a double rows of saccular infoldings of plasmalemma (not present close to both ends of spermatozoon) which invaginate secondarily forming ramified tubules penetrating the matrix of each sacculus (Witaliński 1975, 1976, 1979; Alberti 1980). In most studied species, the ribbons are more electron-dense in the anterior (i.e. nuclear) than posterior (postnuclear) part of sperm and their internal structure is more clearly visible in the posterior, postnuclear region. This is most striking in *Holoparasitus calcaratus*, *Leptogamasus* and *Anidogamasus* species, whereas in *Paracarpais kraepelini* saccular bodies of ribbons are electron-lucent and similar in appearance along the spermatozoon. Similar but more electron-dense ribbons are also present in *Parasitus coleoptratorum*; in this species saccular bodies seems to coalesce, thus the double nature of the ribbons on cross sections is doubtful. Ribbons in *Paracarpais lunulata* are also similar in appearance along the sperm length but saccular bodies penetrate unusually deep into the cell.

Above each ribbon, the plasmalemma forms a crest running along the sperm cell; each crest contains a canaliculus opening near the end of the spermatozoon (Witaliński 1975; Alberti 1980). The last element of the superficial complex of structures includes subplasmalemmal cisterns extending laterally from one ribbon to the other. Subplasmalemmal cisterns are usually arcuate in cross-section, but in *Trachygamasus* their marginal portions fol-

lowed by plasmalemma are deeply folded. Ribbons, crests and subplasmalemmal cisterns form a characteristic, compact cylindrical assemblage of subplasmalemmal organelles. As sperm penetrate female tissue towards the ovary, the plasmalemma and subplasmalemmal assemblage of organelles undergo morphological changes and secretion of some material from ribbons into a subplasmalemmal space has been observed (Alberti *et al.* 2000). This could lead to the growth of the subplasmalemmal space which eventually is quite large in sperm close to the ovary. In such sperm cells two distinct compartments are present: the peripheral or subplasmalemmal compartment delimited by plasmalemma, and a more central compartment formed by an organelle assemblage (central cylinder acc. to Alberti *et al.* 2000). Interestingly, close to the previtellogenic oocyte, the sperm plasmalemma forms many protrusions probably involved in cell movement towards the oolemma whereas the unchanged central cylinder remains compact and meanders within the dilated peripheral compartment. The possible role of ribbon secretion and alterations of inclusion bodies just before fertilization are discussed in detail by Alberti and co-workers (Alberti *et al.* 2000).

A large, elongated nucleus in parasitid sperm is another peculiarity since DNA is limited to several chromatin granules located in a small area in the center of the nucleus; the rest of the nucleus is filled with a proteinaceous material at least in *Pergamasus barbarus* (Witaliński 1975). In most species, the nucleus occupies slightly more than the anterior half of the spermatozoon, but in *Anidogamasus teutonicus*, *Paragamasus* sp., *Paracarpais kraepelini*, *Phorytocarpais fimetorum* and *Gamasodes spiniger* its length is ca. $\frac{3}{4}$ of the cell, whereas in *Cornigamasus lunaris* and *Trachygamasus* sp. the nucleus extends nearly from the anterior to the posterior end of the spermatozoon. The only sperm with a helically coiled nucleus is in *Paracarpais loricatus*. The functional reasons for such variability of nucleus length and shape are unknown.

The cytoplasm around and behind the nucleus contains inclusion bodies in two forms: striated inclusion bodies (sIBs) and granular inclusion bodies

(gIBs). In most cases, sIBs occur around the nucleus, whereas the gIBs are localized around the posterior part of the nucleus and in the postnuclear region of the spermatozoon. Typically, sIBs are filled with a paracrystalline striated core; in gIBs this core is limited to the inclusion body center and is surrounded with less electron-dense granular material. In *Paracarpais kraepelini*, however, sIBs have not been found and gIBs located in the postnuclear region are filled with granular material; striated cores are absent. Inclusion bodies in *Parasitus coleoptratorum* are unusual since they are filled with electron-lucent flocculent material and their limiting membranes in some places are not discernible; moreover, both striated cores and granular material are absent, meaning that distinguishing between sIBs and gIBs is impossible.

In terms of the function of IBs, there is a scarce pool of information on their modifications after insemination (Alberti *et al.* 2000). The first changes in IB structure can be noted not earlier than in spermatozoa within the haemocoel, in which IBs lose their ovoid shape and transform into dense stacks of membranes forming myelin-like structures. In spermatozoa in contact with the ovary, stacks of membranes reorganize to form concentric structures without a myelin-like appearance. The participation of transformed IB material in the secretory process by ribbon saccular bodies has been suggested by Alberti and co-workers (Alberti *et al.* 2000).

Mitochondria in Parasitidae spermatozoa are numerous and of crista-type; they are distributed between ribbons, but also fill the postnuclear region together with gIBs. Their unchanged typical structure suggests a function as energy donors during and after insemination. It is likely that the spermatozoon population of mitochondria is the same as the spermatocyte population involved in synthesis of IBs, since segregation of mitochondria during spermatogenesis in and out of spermatids (e.g. into residual cytoplasm) has not been observed (Witaliński 1976; Alberti 1980).

There is no evident correlation of variable sperm morphology with either reproduction, ecology of species or taxonomic position at the genus or sub-

family levels in parasitid mites. It should be noted that details of reproduction and reproductive behavior are known in outline only (Micherdziński 1969; Lindquist *et al.* 2009) and our knowledge is too modest to form conclusions that are not speculative. Furthermore, the systematics of Parasitidae, even at the generic level, is not clear, nor is it supported with any molecular studies. It is likely that new achievements of molecular taxonomy will modify the pattern of genera within Parasitidae and show real evolutionary traits compatible with sperm structure making the conclusions on mechanisms and trends in spermatozoa evolution in this family possible.

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