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## Ultrastructure of the Spermatozoa in the Spider Genus *Pimoa*: New Evidence for the Monophyly of Pimoidae plus Linyphiidae (Arachnida: Araneae)

PETER MICHALIK<sup>1,3</sup> AND GUSTAVO HORMIGA<sup>2,3</sup>

### ABSTRACT

The spermatozoa of spiders (Araneae) show a high structural diversity, resulting in several potential phylogenetic characters. In the present paper, we describe the spermatozoa of the spider family Pimoidae for the first time. We investigate four species of the genus *Pimoa* (*P. altiocolata*, *P. curvata*, *P. laurae*, and *P. edenticulata*) by means of light and transmission electron microscopy. The male reproductive system consists of paired testes and long convoluted paired deferent ducts. The spermatozoa are generally characterized by: (1) a cylindrical acrosomal vacuole, (2) an acrosomal filament restricted to the precentriolar part of the nucleus, (3) a nuclear canal running in the periphery but projecting towards the posterior portion of the nucleus, (4) a short postcentriolar elongation of the nucleus, (5) a 9+0 axonemal pattern, and (6) cleistospermia as transfer form. The organization of the axoneme is of particular phylogenetic interest, since a 9+0 axonemal pattern was described within spiders only for the megadiverse family Linyphiidae, the sister group of Pimoidae. We have reconstructed the evolution of the axoneme using comparative spermatozoal data for 54 orbicularian species representing 11 families. We propose that the 9+0 axonemal pattern is a new synapomorphy for Pimoidae + Linyphiidae. The phylogenetic and evolutionary implications of other potential sperm characters (e.g., length of the postcentriolar elongation of the nucleus) are discussed.

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## INTRODUCTION

Spermatozoa represent the most diverse cell type known for animals and thus a source of potential phylogenetic characters (e.g., Jamieson et al., 1999; Marotta et al., 2008). Although there are only a limited number of studies (see Alberti, 2000; e.g., Michalik et al., 2004a, 2004b; Michalik and Huber, 2006), it is known that the spermatozoa of spiders possess an astonishing structural diversity. Phylogenetic characters based on such diversity appear informative for the inference of the phylogenetic relationships among spider families (Michalik and Ramírez, unpublished cladistic data). For example, spider spermatozoa are usually characterized by an axoneme with a 9+3 microtubular pattern (Ösaki, 1969; Dallai et al., 1995), which has been suggested to be synapomorphic for Megoperculata (i.e., the lineage that includes the orders Uropygi, Amblypygi, and Araneae; Weygoldt and Paulus, 1979a, 1979b; Alberti, 1990, 2000). Until now, a different axonemal organization (a 9+0 microtubular pattern) had been described within spiders only for some linyphiids (Alberti, 1990; Michalik and Alberti, 2005). Based on the few available studies of the spermatozoa of the superfamily Araneoidea (Boissin, 1973; Alberti, 1990; Li et al., 1994; Michalik et al., 2005, 2006), Alberti (1990) and Michalik and Alberti (2005) suggested that this distinct axonemal pattern could be synapomorphic for Linyphiidae.

The sister group of the taxon-rich Linyphiidae is the relictual family Pimoidae, which includes four genera (*Pimoida*, *Nanoa*, *Putaoa* and *Weintrauboa*) and 31 extant described species (Platnick, 2009). Initially proposed by Wunderlich (1986) for the genus *Pimoida*, the monophyly of the family and its sister-group relationship to Linyphiidae has been consistently supported by several phylogenetic analyses of morphological data (e.g., Hormiga, 1994, 2000, 2003, 2008; Hormiga and Tu, 2008). The lineage that includes linyphiids plus pimoids is often known under the informal label of “linyphioids.” Li and Wunderlich (2008) recently described the monotypic family Sinopimoidae, proposing a sister relationship to Pimoidae. Such placement was questioned by Hormiga (2008), who suggested that the single known “sinopimoid”

species might instead belong to Linyphiidae (possibly Erigoninae). We will not consider *Sinopimoida* in our discussion regarding the phylogenetic implications of the obtained results.

In the present paper, we investigate the spermatozoa and the reproductive system of the family Pimoidae for the first time. The scope of this study is to answer the following questions: Is the 9+0 axonemal pattern a synapomorphy for “linyphioid” spiders? And are there any further potential phylogenetic characters in the gross morphology of the reproductive system and spermatozoa? We discuss our results with regard to “linyphioids,” but also the phylogenetic implications for orbicularian relationships.

## MATERIALS AND METHODS

### MATERIAL EXAMINED

We collected male specimens of *Pimoida alticulata* (Keyserling, 1886), *P. curvata* Chamberlin and Ivie, 1943, *P. laurae* Hormiga, 1994, and *P. edenticulata* Hormiga, 1994 (fig. 1A–D). Collection data and depositories are given in the appendix.

The male specimens were dissected in 0.1 M Sørensen phosphate buffer (Electron Microscopy Science, Hatfield, Philadelphia, USA) to which 1.8% sucrose was added (PB). The isolated genital systems were fixed in 2.5% glutaraldehyde in PB and pictures for the description of the gross morphology was photographed using a Nikon DS-Fi1 digital camera (software: NIS-Elements F 2.20) mounted on a Leica MZ16 microscope.

### ULTRASTRUCTURAL OBSERVATIONS

The samples were postfixed in PB buffered 2% OsO<sub>4</sub>. After being washed in PB, the tissue pieces were dehydrated in graded ethanols and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections (50 nm) were made with a Diatome Ultra 35° diamond knife at a Leica ultramicrotome UCT and stained with uranyl acetate and lead citrate according to Reynolds (1963). Examination was performed with a JEOL TEM-1011 electron microscope at 80 KV. Images were taken with a side-

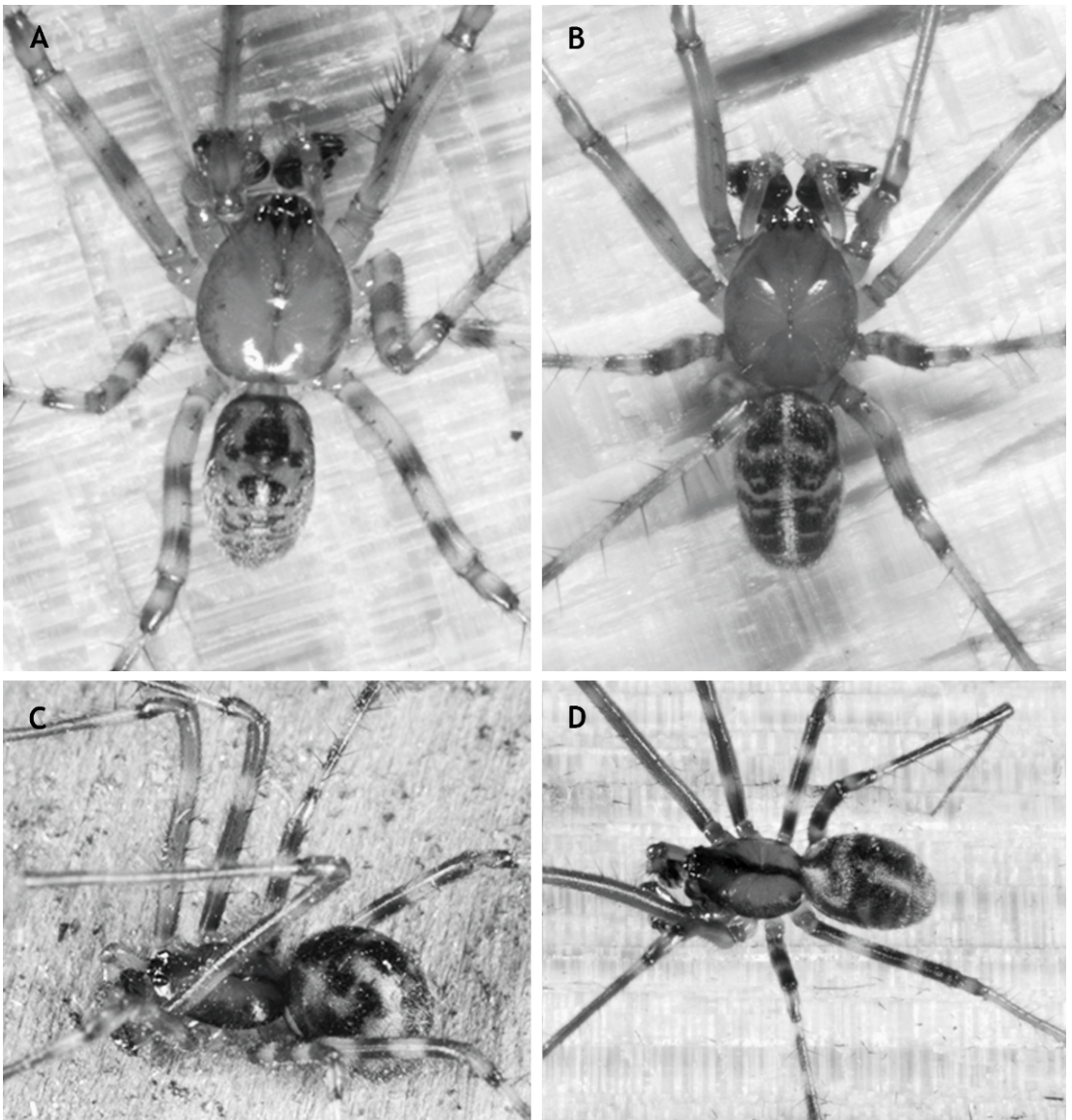


Fig. 1. Male habitus, dorsal view. A. *Pimoida altiocolata*. B. *Pimoida curvata*. C. *Pimoida laurae*. D. *Pimoida edenticulata*.

mounted Olympus MegaView III digital camera using the iTEM software.

For the observations of *Oedothorax retusus* (Westring, 1851) spermatids (Linyphiidae; G. Uhl's laboratory population, University of Bonn, Germany; vouchers are deposited in the Zoological Institute and Museum Greifswald (ZIMG), Germany) by means of scanning electron microscopy (SEM), we isolated the

male reproductive system, and opened it in a droplet of PB using thin needles on glass coverslips covered with 1% poly-L-lysine. After sedimentation (10 min), the adhering material was fixed with 2.5% glutaraldehyde in PB for 1 h at 4° C. The samples were then rinsed in PB and postfixed in buffered 2% OsO<sub>4</sub>, dehydrated in graded ethanols, dried in a BAL-TEC CPD 030 critical-point dryer using amylacetate as inter-



medium, coated with gold-palladium in a Quorum Technologies SC7620 sputtering device, and examined in a LEO DSM 940A SEM. Colors added to the digital images in Adobe® Photoshop CS4.

#### PHYLOGENETIC RECONSTRUCTION

In addition to the four *Pimoa* species mentioned above we have included comparative data on sperm ultrastructure in orbicularians taken from previous studies (Boissin, 1973; Alberti, 1990; Li et al., 1994; Michalik and Alberti, 2005; Michalik et al., 2005; Michalik, 2006; Michalik et al., 2006; voucher specimen information is provided in the original works) and unpublished data of the first author (Anapidae, Araneidae, Mimetidae, Mysmenidae, Nephilidae, Nesticidae, Synotaxidae, Theridiidae, and Uloboridae). The species studied in the aforementioned references include (in alphabetical order, by family, in bold characters): **Anapidae** (one species): *Comaroma simoni* Bertkau, 1889; **Araneidae** (seven species): *Araneus diadematus* Clerck, 1757; *Araniella cucurbitina* (Clerck, 1757); *Argiope lobata* (Pallas, 1772); *Cyclosa conica* (Pallas, 1772); *Larinioides scolopetarius* (Clerck, 1757); *Micrathena gracilis* (Walckenaer, 1806); *Zygiella x-notata* (Clerck, 1757); **Linyphiidae** (10 species): *Baryphyma trifons* (O. P.-Cambridge, 1863); *Drapetisca socialis* (Sundevall, 1833); *Erigone dentipalpis* (Wider, 1834); *Gongylidium rufipes* (Linnaeus, 1758); *Lepthyphantes* sp.; *Linyphia hortensis* Sundevall, 1830; *Linyphia triangularis* (Clerck, 1757); *Neriere clathrata* (Sundevall, 1830); *Neriere peltata* (Wider, 1834); *Oedothorax retusus* (Westring, 1851); **Mimetidae** (three species): *Ero aphana* (Walckenaer, 1802); *Gelanor* cf. *zonatus* (C. L. Koch, 1845); *Mimetis rusticus* Chickering, 1947; **Mysmenidae** (one species): *Maymena* sp.; **Nephilidae** (one species) *Nephila senegalensis* (Walckenaer, 1842); **Nesticidae** (one species): *Nesticus cellulanus* (Clerck, 1757); **Synotaxidae** (one species) *Chileotaxus sans* Platnick, 1990; **Tetragnathidae** (13 species): *Leucauge* sp.; *Metellina segmentata* (Clerck, 1757); *Pachyg-natha listeri* Sundevall, 1830; *Tetragnatha* spp. (10 species; see Michalik et al., 2006); **Theridiidae** (13 species); *Parasteatoda tabulata* (Levi, 1980); *Argyrodes argyrodes* (Walckenaer,

1842); *Crustulina guttata* (Wider, 1834); *Dipoena atlantica* Chickering, 1943; *Echino-theridion gibberosum* (Kulczynski, 1899); *Enoplognatha ovata* (Clerck, 1757); *Neottiura bimaculata* (Linnaeus, 1767); *Nesticodes rufipes* (Lucas, 1846); *Steatoda bipunctata* (Linnaeus, 1758); *Steatoda grossa* (C.L. Koch, 1838); *Theridion melanurum* Hahn, 1831; *Heterotheridion nigrovariegatum* Simon, 1873; *Tidarren argo* Knoflach and van Harten, 2001; and **Uloboridae** (three species): *Hyptiotes flavidus* (Blackwall, 1862); *Philoponella fasciata* (Mello-Leitão, 1917); *Octonoba sinensis* (Simon, 1880).

We allocated the observed variation in the sperm ultrastructure into two phylogenetic characters:

CHARACTER 1. Number of outer doublets in axoneme. 0: nine; 1: 12.

CHARACTER 2. Number of central tubules in axoneme. 0: three; 1: zero.

These two characters were scored for our study taxa and their evolution reconstructed under parsimony using the modular software package Mesquite version 2.6 build 486 (Maddison and Maddison, 2009). Because no single published phylogenetic analysis includes all the orbicularian taxa that we have scored, it is not possible to use a single cladogram from the literature to attempt the phylogenetic reconstruction. Nevertheless, this problem can be overcome by building a composite cladogram (analogous to a “supertree”) for Araneoidea summarizing the published cladograms for the various groups that we have studied. In this respect our methodology is similar to that used by Hormiga et al. (2000) to reconstruct the evolution of sexual size dimorphism in orbweaving spiders or Miller (2007) to study the patterns of male sacrifice behavior in spiders. In the last decade or two there have been numerous quantitative phylogenetic analyses published that address the phylogenetic relationships of orbicularians at various hierarchical levels. We have relied only on those studies that are character matrix based and that use numerical methods of phylogenetic analysis. Most of them are based on morphological (and to a lesser extent behavioral) characters but some of the more recent ones also use nucleotide sequences or a

combination of several types of data. The araneoid interfamilial relationships are based on the analyses of Griswold et al. (1998) and Lopardo and Hormiga (2008). We have modified their results by adding Mimetidae to Araneoidea based on the works of Schütt (2000), Griswold et al. (2005), and Blackledge et al. (2009). The placement of the nephilid clade has been conservatively left as an unresolved trichotomy at the base Araneoidea due to the lack of a robust phylogenetic hypothesis (see Álvarez-Padilla et al., 2009 and references therein). Relationships within families are taken from cladistic analyses that usually include more species than those for which we have sperm data, and thus the original published optimal topologies have been pruned to match our own taxonomic sample. The relationships of Uloboridae are taken from the morphological analysis of Opell (1979; see also Coddington, 1990). The relationships within araneids, tetragnathids, and nephilids are taken from the optimal trees of Álvarez-Padilla et al. (2009) based on morphological and multigene data. We have placed the araneine *Araniella* based on the tree of Scharff and Coddington (1997: fig. 82). The theridiid topology has been taken from the multigene analysis of Arnedo et al. (2004: fig. 2). *Theridion melanurum* Hahn, 1831, has been placed based on Arnedo et al. (2007: fig. 5) and the placement of *Echinotheridion* is based on Agnarsson (2006: fig. 2). The theridiid *Heterotheridion nigrovariegatum* Simon, 1873, has not been included in the cladogram because to date no phylogenetic matrix has ever included this species, although it possesses the same axonemal characters as the other included theridiid taxa. The relationships among *Pimoa* species are taken from Hormiga (1994: fig. 442). Finally, the topology of Linyphiidae is based on the combined analysis of Arnedo et al. (2009: fig. 9) with *Gongylidium* added based on the preferred optimal trees of Hormiga (2000: fig. 38) and Miller and Hormiga (2004: fig. 3).

## RESULTS

The gross morphology of the male genital system is very similar in all observed *Pimoa* species. In general, it consists of paired testes

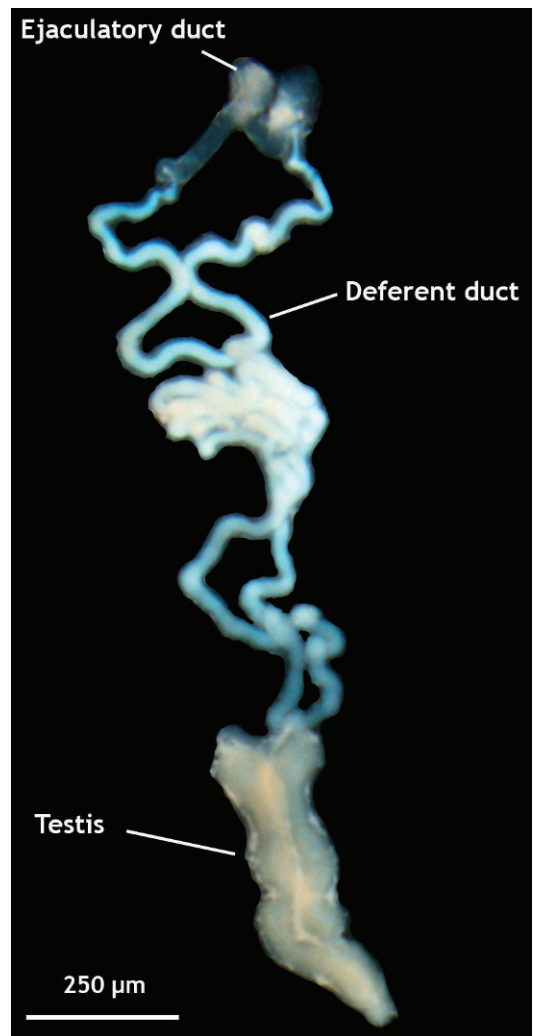


Fig. 2. *Pimoa altiocolata*. Male reproductive system, dorsal view. The highly coiled deferent ducts were partly unraveled during dissection. The testes are densely attached to each other.

and two highly convoluted deferent ducts, which fuse near the genital opening forming an ejaculatory duct (fig. 2). The deferent ducts are filled with seminal fluid of white appearance consisting of spermatozoa and different secretions (e.g., fig. 3C–E; see below). In the testis of the studied species, only a few spermatogenic stages could be observed. The apical part of the somatic cells of the testis bears numerous microvilli bordering the lu-



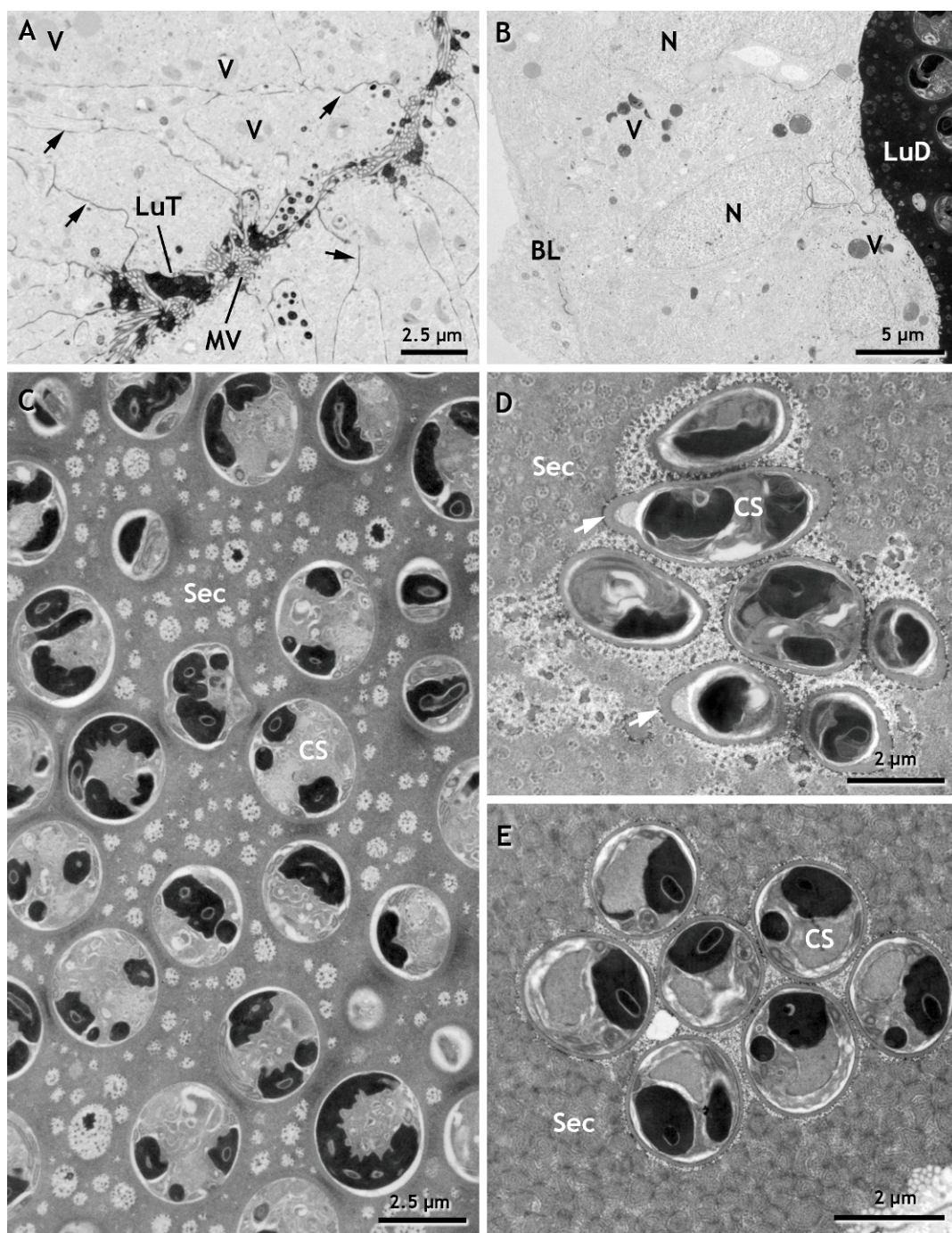


Fig. 3. **A.** *Pimoa laurae*. Detail of testis; arrows to junctions of the somatic cells. **B–C.** *Pimoa altiocolata*. **B.** Detail of deferent duct. **C.** Detail of seminal fluid. **D.** *Pimoa laurae*. Detail of seminal fluid; arrow to lateral projection of cleistospERMIA. **E.** *Pimoa curvata*. Detail of seminal fluid. Abbreviations: BL, basal lamina; CS, cleistospERMIA; LuD, lumen of deferent duct; LuT, lumen of testis; MV, microvilli; N, nucleus; Sec, secretion; V, vesicle.

men of the testis (fig. 3A). The somatic cells are connected by extensive cell junctions (fig. 3A: arrows). The deferent ducts are characterized by a thin epithelium, which contains numerous vesicles (fig. 3B). The seminal fluid present in the lumen of the deferent ducts consists of mature spermatozoa and several types of secretion (electron-dense matrix and secretion droplets of different shapes) (figs. 3B–E, 4D).

At the end of spermiogenesis the main cell components (nucleus and axoneme) of the spermatozoa coil several times within the cell (e.g., figs. 4A, B, 5A). In the deferent duct, each sperm cell obtains a thin monolayered secretion sheath representing cleistospermia (figs. 3C–E, 4D, 5A). The shape of the cleistospermia are roundish (*P. altiocularata*) to oval (*P. curvata*, *P. laurae*, *P. edenticulata*) (figs. 3C, D, 5A). In the oval-shaped cleistospermia, lateral projections may be present (figs. 3D, 4F). In general, the spermatozoa of *Pimoida* are characterized by the following features:

**ACROSOMAL COMPLEX**—acrosomal vacuole: Cylindrical (fig. 4A), irregular in cross section (figs. 4D, 5C), with a conspicuous electron-lucent part (figs. 4D, 5C); acrosomal filament: originates from narrow subacrosomal space and extends into the nuclear canal, it ends before axonemal basis as indicated by an empty nuclear canal (figs. 4A, 5A).

**NUCLEUS**: Elongated and tapering towards the anterior end (figs. 3E, 4A, B, 5B); in the oval cross section the nucleus become subtly flattened posteriorly (fig. 4D, E); deep implantation fossa (more than half the length of the precentriolar part of the nucleus) filled with an electron-dense homogenous centriolar adjunct (fig. 4B–F), the diameter of the implantation fossa is about one-third or less the diameter of the nucleus (figs. 3–5); the postcentriolar elongation (= asymmetrical elongation of the postcentriolar part of the nucleus) is flattened and relatively short (less than half the length of the precentriolar part of nucleus; extremely short in *P. altiocularata*) (fig. 4D, F); the nuclear canal runs in the periphery within the nucleus, and projects from it in the posterior portion of the precentriolar part (compare figs. 4A and 5A, C).

**AXONEME**: All species possess a 9+0 axonemal pattern (figs. 4D, 5); the centrioles are arranged in tandem position (fig. 4C); the length of the axoneme ranges from 1.5 to 2.5 coils in the cleistospermia (figs. 4E, 5A–C); the axoneme may be flattened (figs. 4E, 5A–D).

**CELL INCLUSIONS**: The cytoplasm of the coiled spermatozoa contains extensive membrane cisternae (unordered or organized in stacks) and large amounts of glycogen (figs. 4D, E, 5A–C).

The parsimony reconstruction implies a single origin of the 9+0 pattern in Pimoidae + Linyphiidae (fig. 6). The loss of the central tubules in the axoneme has occurred independently in “linyphioids” and in the synotaxid *Chileotaxus sans* (Michalik, 2006).

## DISCUSSION

The reproductive system of the investigated *Pimoida* species resembles the general organization reported in spiders, with paired testes and deferent ducts (see Michalik, 2009). Modified parts of the reproductive system, as is the case of the seminal vesicle known from cobweb spiders (Theridiidae; Knoflach, 1998; Michalik, 2009), could not be observed. Similar to linyphiid spiders, the deferent ducts of the *Pimoida* species are convoluted and very long compared to the length of the testes (Michalik, 2009), in contrast to the male reproductive system of other orbicularian spiders reported so far (Michalik, 2009; Michalik et al., 2010, and further own unpublished observations). The gross morphology of the male reproductive system in spiders is insufficiently studied (Bertkau, 1875; Crome, 1951; Michalik, 2009) and therefore it remains unclear how this system has evolved within spiders. The same is also true for the secretion in seminal fluid, which varies enormously in spiders (for details see Michalik, 2009). The urgent need of data is more evident when one considers recent studies, such as Burger and Michalik (2010), who described an unpaired testis in goblin spiders (Oonopidae)—the first clear empirical evidence for the monophyly of this family.

Knowledge about the fine structure of the spermatozoa within Orbiculariae is limited to



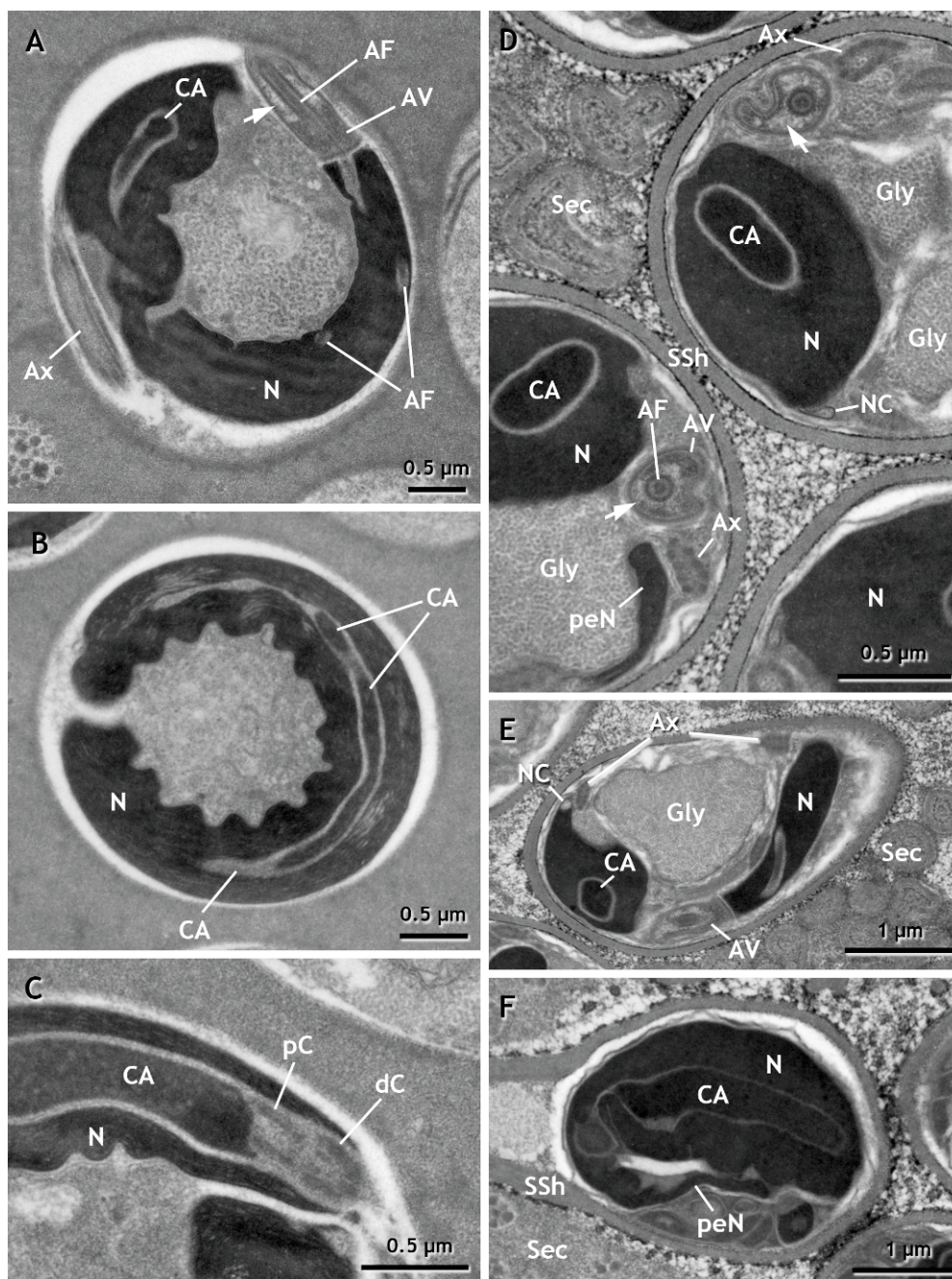


Fig. 4. A–C. *Pimoid altiocolata*. Coiled sperm cells in lumen of the testis; arrow to electron-lucent part of the acrosomal vacuole (see also fig. 4D). D–E. *Pimoid curvata*. CleistospERMIA in lumen of the deferent duct; arrows to electron-lucent part of the acrosomal vacuole. F. *Pimoid laurae*. CleistospERMIA in lumen of the deferent duct. Abbreviations: AF, acrosomal filament; AV, acrosomal vacuole; Ax, axoneme; CA, centriolar adjunct; dC, distal centriole; Gly, glycogen; pC, proximal centriole; peN, postcentriolar elongation of the nucleus; N, nucleus; NC, nuclear canal; Sec, secretion; SSh, secretion sheath.



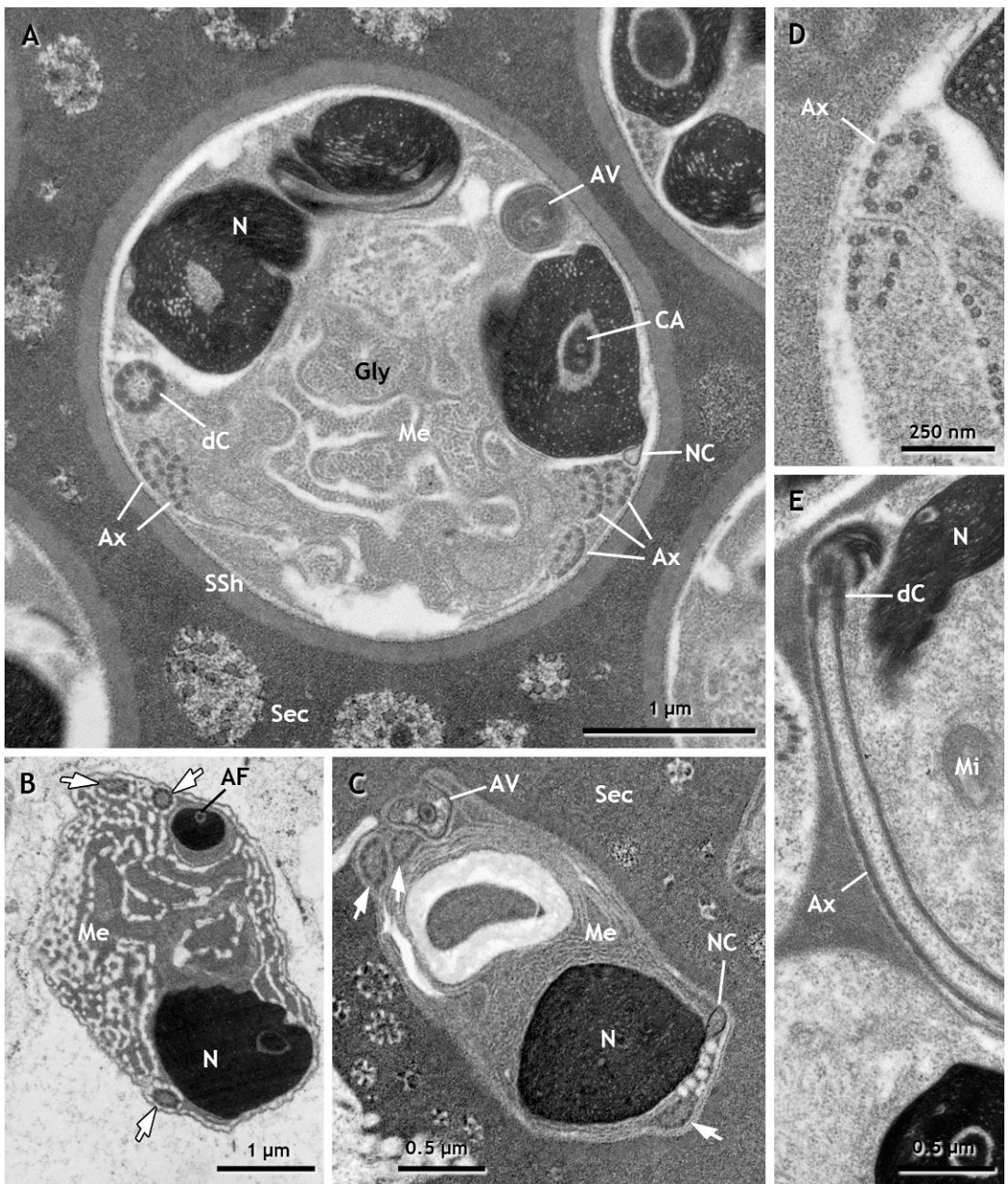


Fig. 5. **A.** *Pimoida altiocularata*. Cleistospermium in lumen of the deferent duct. **B.** *Pimoida edenticulata*. Coiled sperm cell in testis; arrows to the axoneme. **C.** *Pimoida laurae*. Coiled sperm cell in lumen of the testis; arrows to axoneme. **D–E.** *Pimoida altiocularata*. Detail of the axoneme (D, cross section; E, longitudinal section). Abbreviations: AF, acrosomal filament; AV, acrosomal vacuole; Ax, axoneme; CA, centriolar adjunct; dC, distal centriole; Gly, glycogen; Me, membrane cisternae; Mi, mitochondria; N, nucleus; NC, nuclear canal; Sec, secretion; SSh, secretion sheath.



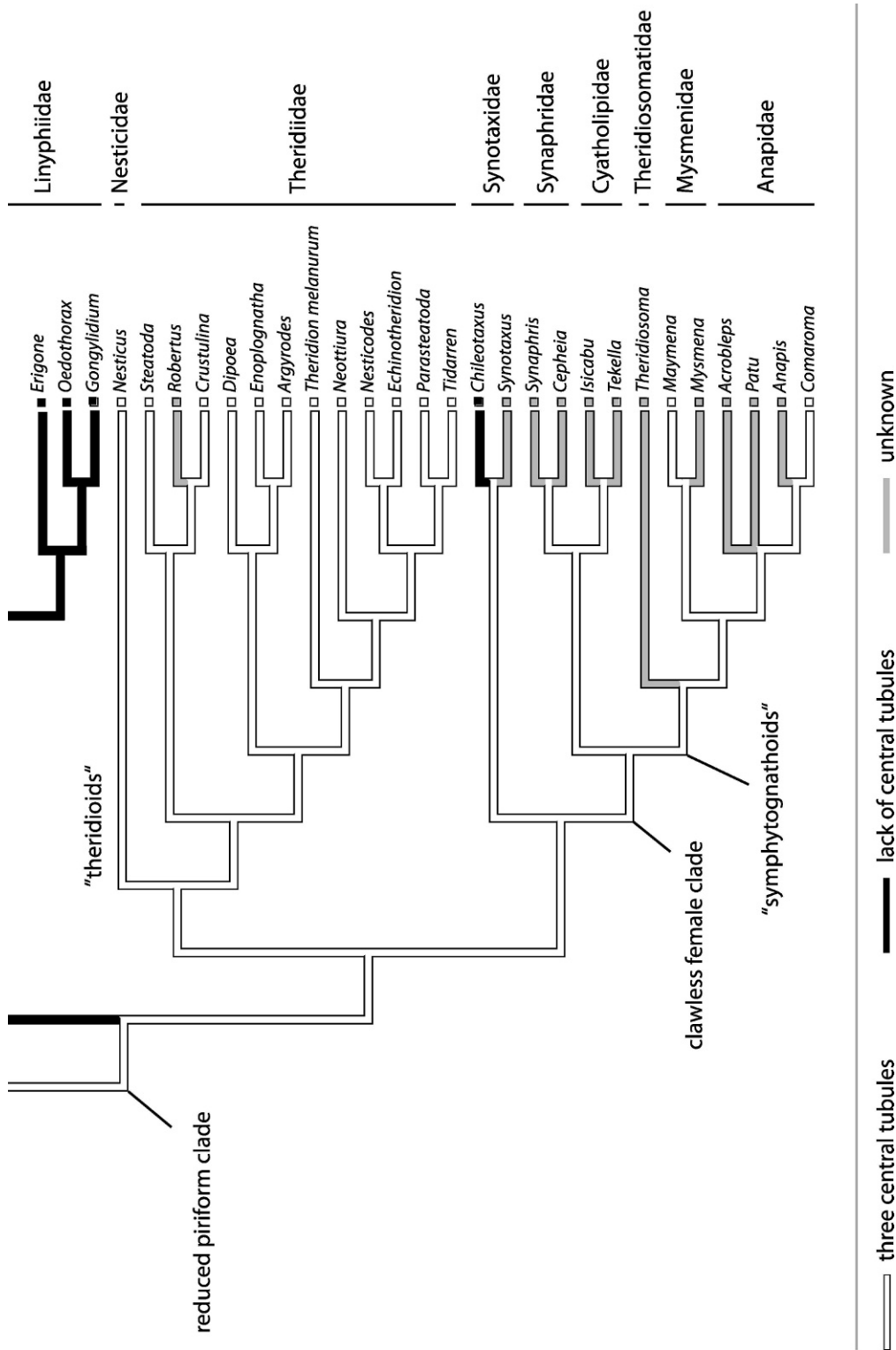


Fig. 6. Interfamilial phylogenetic relationships of orbicularian spiders (based on Griswold et al. 1998 and Lopardo and Hormiga, 2008; see text for additional information and for sources of intrafamilial relationships). The optimization of the character 2 describing the axonemal pattern (three versus no central tubules) is reconstructed using parsimony. Data of the organization of the axoneme based on: Boissin, 1973; Alberti, 1990; Li et al., 1994; Michalik and Alberti, 2005; Michalik et al., 2005; Michalik, 2006; Michalik et al., 2006; and further own unpublished observations (see text for additional details).



a few relatively detailed studies on Uloboridae (one species; Li et al., 1994), Araneidae (one species; Alberti, 1990), Tetragnathidae (13 species; Boissin, 1973; Alberti, 1990; Michalik et al., 2006), Linyphiidae (eight species; Alberti, 1990; Michalik and Alberti, 2005) and Theridiidae (two species; Alberti, 1990; Michalik et al., 2005). As indicated by those studies, the organization of the spermatozoa differs remarkably between these families. For example, theridiid spiders (*Theridion melanurum* and *Tidarren argo*) possess globules in the implantation fossa, whereas in other families (as well as in the *Pimoida* species studied herein) an electron-dense centriolar adjunct is present. To date, the spermatozoa of Linyphiidae, the sister group of Pimoidae, are only known in terms of their axonemal pattern (Alberti, 1990; Michalik and Alberti, 2005). Thus, comparison to linyphiids is restricted to this character (see below). Nevertheless, when compared with other spider families, the spermatozoa of the investigated *Pimoida* species are characterized by several structures: (1) A cylindrical acrosomal vacuole, with an electron-lucent part. The shape of the acrosomal vacuole can differ remarkably across spider species, although a comparative analysis is still lacking. The acrosomal vacuole can vary from conical, as in *Scytodes* (Alberti and Weinmann, 1985), to extreme forms, such as the corkscrew-shaped acrosomal vacuole in *Tetragnatha* species (Michalik et al., 2006). (2) An acrosomal filament restricted to the precentriolar part of the nucleus. This is clearly apomorphic since an acrosomal filament extending to the end of the nuclear canal has been reported only from Mesothelae (Ōsaki, 1969; Michalik, 2007) and Filistatidae (Alberti and Weinmann, 1985; Michalik et al., 2003). (3) A nuclear canal running in the periphery but projecting towards the posterior portion of the nucleus. This organization is widespread, found in all main spider clades (e.g., Lopez and Boissin, 1976; Alberti et al., 1986). (4) A nucleus with a thin (and deep) implantation fossa. A thin fossa might also represent a derived feature. The implantation fossa is small in Mesothelae (Ōsaki, 1969; Michalik, 2007) and Mygalomorphae (Alberti et al., 1986) and rather diverse in Araneomorphae (e.g., Alberti and Weinmann, 1985; Michalik

et al., 2005), often depending on the shape of the nucleus. (5) A short postcentriolar elongation of the nucleus. The length of the nuclear elongation varies enormously compared to the length of the precentriolar part of the nucleus (Alberti, 2000). Such a short elongation as observed in *Pimoida* and Linyphiidae (Michalik and Alberti, 2005: fig. 7) has not been reported from other orbicularian families so far. (6) Cleistospermia with a monolayered secretion sheath. This transfer form has been reported from all araneomorph clades and seems to be a synapomorphy for that spider group (see Alberti, 2000; Michalik et al., 2004b).

The typical 9+3 axonemal pattern has been reported in representatives of all spider families studied so far (but see below) (e.g., Ōsaki, 1969; Juberthie et al., 1981; Alberti and Weinmann, 1985; Alberti et al., 1986; Alberti and Coyle, 1991; Michalik et al., 2006). Strikingly, Orbiculariae is to date the only spider group in which a change to a 9+0 pattern has occurred in some of its members (i.e., in the Linyphiidae; Alberti, 1990, 2000). The 9+0 microtubular pattern was also observed in the *Pimoida* species studied herein (see fig. 6), suggesting that this pattern might be a synapomorphy of the clade comprising Linyphiidae plus Pimoidae (i.e., the “linyphioids” sensu Hormiga, 2003 and Arnedo et al., 2009). The presence of a 9+0 pattern in *Pimoida* species and in several lineages of Linyphiidae suggests that the remaining pimoid genera (*Weintrauboa*, *Nanoa*, and *Putaoa*) will also exhibit the 9+0 microtubular pattern. The loss of microtubules is also present in the only synotaxid studied to date, *Chileotaxus sans*, but in contrast to “linyphioids” the axoneme of this species consists of 12 outer doublets and thus represent a 12+0 pattern (Michalik, 2006).

Another potential synapomorphic feature for the “linyphioids,” in striking contrast to other orbicularians and all spiders observed so far, is the length of the axoneme (flagellum). Detailed studies of this feature are lacking, but we found compelling evidence that the axoneme of “linyphioids” is short when compared to other spider taxa. As indicated in figure 7, the axoneme in linyphiids is not markedly longer than the nucleus. A similar situation is

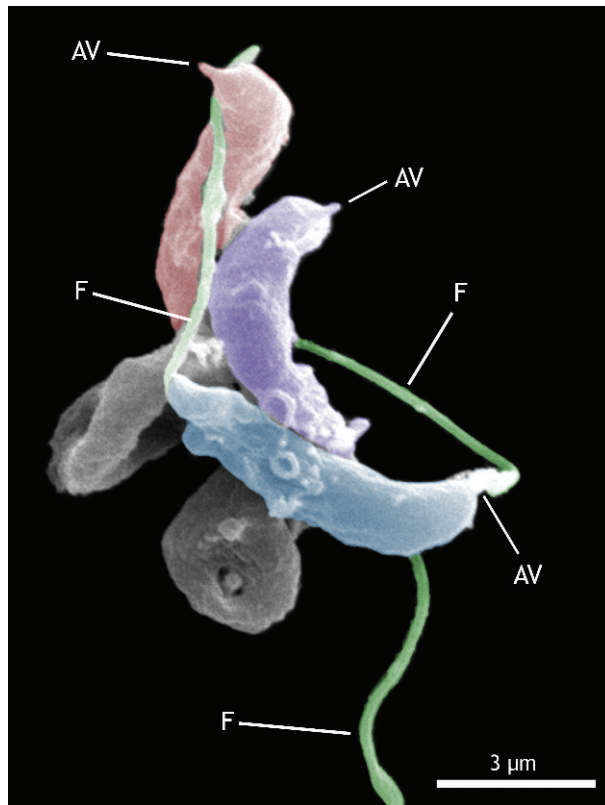


Fig. 7. Late spermatids of *Oedothorax retusus* (Linyphiidae). SEM. Colors (red, purple, blue) indicate three different spermatids. Abbreviations: AV, acrosomal vacuole; F, flagellum.

depicted in sections of sperm cells. For example, the depicted coiled spermatozoon of *Linyphia hortensis* in Michalik and Alberti (2005) possesses an axoneme that coils  $1\frac{1}{2}$  times. This situation was also found here for most *Pimoa* species (with the exception of *P. altiocolata*). As mentioned above, this situation is in contrast to all other spider species observed to this point, where the axoneme coils at least  $2\frac{1}{2}$  times (e.g., Alberti and Weinmann, 1985; Alberti et al., 1986; Michalik et al., 2003; Michalik and Huber, 2006; Costa-Ayub and Faraco, 2007; Michalik, 2007). Future comparative studies within orbicularians using morphometric methods based on SEM studies and 3D reconstructions of the spermatozoa are needed to clarify the consistency of this character.

The driving forces for the evolution of a different axonemal type within “linyphioids”

remain unclear. It could be hypothesized that a different microtubular pattern (as well as its length) results in differences in the spermatozoa movements, as has been shown for other chelicerates (Ishijima et al., 1988; Michalik and Mercati, 2010). Since sperm competition has a strong influence on the sperm phenotype (see Pizzari and Parker, 2009), it could likewise be hypothesized that a different motility performance is required to maximize paternity success. Still, observations on sexual selection and sperm competition of “linyphioids” are restricted to only a few linyphiid taxa (e.g., Watson, 1990; Gunnarsson and Andersson, 1996; Watson, 1998; Uhl and Gunnarsson, 2001; Gunnarsson et al., 2004). Due to variability in the patterns, no clear pattern is obvious. As summarized by Uhl (2002), differences in the organization of the female genitalia can lead to a shift in sperm

priority patterns. Nevertheless, given the present state of knowledge, no conclusion can be made regarding the evolution of the axonemal pattern in spiders, especially when comparative studies testing relationships between changes in sperm phenotypes and female genitalia are still lacking.

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## APPENDIX 1

### *Pimoa* specimens studied

Vouchers of the spermatozoal study are deposited in the American Museum of Natural History (AMNH, New York, USA). Additional specimens of the study species (females and males), often from the same locality, are deposited at the California Academy of Sciences (San Francisco) and the Museum of Comparative Zoology (Cambridge).

#### *Pimoa altiocularata*

USA: Washington: Klickitat Co., 1 km NE B Z Corner, Gilmer Creek. N45° 51' 56.1": W121° 29' 54.2", elev. 490 m, small, shaded stream in forest, 15 August 2008. G. Hormiga, F. Álvarez Padilla, A. Carmichael, D. Dimitrov, C. Griswold, and A. Saucedo collectors; subadult male, molted 28 August 2008.

#### *Pimoa curvata*

USA: Washington: Klickitat Co., 2.69 km NNW Husum. N45° 48' 49.6": W121° 30' 43.1", elev. 52 m, forest with open understory, 15 August 2008. G. Hormiga, F. Álvarez Padilla, A. Carmichael, D. Dimitrov, C. Griswold, and A. Saucedo collectors; 2 subadult males, molted 12 and 13 September 2008.

#### *Pimoa edenticulata*

USA: California: Contra Costa Co., Charles Lee Tilden Regional Park. N37° 54' 41.65": W122° 16' 4.93", elev. 158 m., 22 August 2008, G. Hormiga and D. Dimitrov; subadult male, molted 24 September 2008.

#### *Pimoa laurae*

USA: California: Placer Co., Lake Tahoe, Bear Creek nr. Interstc. of Alpine Meadows Rd. and Rt. 89. N39° 10' 43.3": W120° 13' 49.0", elev. 2155 m., open mixed conifer forest, 21 August 2008, G. Hormiga, F. Álvarez Padilla, and D. Dimitrov; subadult male, molted 21 September 2008.