Sperm morphology in four species of African platypleurine cicadas (Hemiptera: Cicadomorpha: Cicadidae)

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Abstract

Mature spermatozoa from four species of platypleurine cicadas (*Albanycada albigera*, *Azanicada zuluensis*, *Platypleura capensis* and *P. hirtipennis*) were examined by light and electron microscopy. The filiform sperm have a similar ultrastructure in all species but notable variations were found in sperm dimensions. All species produce more than one discrete length of nucleated, motile sperm, a form of polymorphism termed polymegaly. Polymegaly is expressed in two ways: sperm have bi- or trimodal head and tail lengths. The anterior parts of sperm heads are embedded in an elongate homogenous matrix forming a spermatodesm. The conical acrosome is deeply invaginated posteriorly, and sits on top of the nucleus. The acrosomal contents are differentiated internally with an electron-lucent central medulla and a denser cortex. The homogenously electron-dense nucleus is pointed anteriorly and is generally cylindrical, although posteriorly there is a lateral invagination that extends part-way along the nucleus. This invagination houses fine granular material of the centriolar adjunct. Vesicle-like elements that are associated with both the posterior nucleus and the centriolar adjunct are also found within the invagination. Immediately posterior of and adjoining the centriolar adjunct is a pair of mitochondrial derivatives that are elongated and extend for almost the entire length of the tail. The absence of accessory bodies in cicada sperm suggests that within the Cicadomorpha, the families Cicadidae and Cercopidae are closely affiliated.

Keywords: Acrosome; Polymegaly; Spermatodesm; Centriolar adjunct

1. Introduction

Spermatozoa are amongst the most diverse of cell types, with an external morphology and ultrastructure that often provides reliable clues for distinguishing taxa and examining phylogenetic relationships (Jamieson, 1987, Jamieson, 1991, Jamieson et al., 1995 and Jamieson et al., 1999). It appears every animal species has spermatozoa with unique characteristics. Across the animal kingdom there are some major variations in sperm shape, size, length and ultrastructure. The greatest diversity is found in the invertebrates, especially among the insects (Sivinski, 1984 and Jamieson et al., 1999).

In most animal groups, the testes in fertile males usually produce spermatozoa of uniform structure (Parker, 1982). However, in some taxa, sperm morphology can vary within a species (e.g. Hodgson, 1999), a phenomenon known as sperm polymorphism. Sperm polymorphism can include variation in sperm length and in structure (Jamieson et al.,

1999). For example, some species of *Drosophila* (Diptera: Drosophilidae) and stalk-eyed flies (Diptera: Diopsidae) produce two discrete lengths of nucleated sperm, a form of sperm polymorphism termed polymegaly (Snook et al., 1994, Pasini et al., 1996 and Presgraves et al., 1999). Sperm polymorphism has also been reported in butterflies and moths (Lepidoptera), males producing nucleated (eupyrene) and non-nucleated (apyrene) sperm (Silberglied et al., 1984). The production of spermatozoa that vary in chromosome complement has been reported in pentatomid bugs (Schrader, 1960) and in beetles (Swallow and Wilkinson, 2002). Although the function of polymorphic sperm in insects remains uncertain, a number of authors have suggested that it may be linked to sperm competition (e.g. Snook, 1998 and Swallow and Wilkinson, 2002).

Cicadas are a diverse group of insects with over 1200 species described to date (Metcalf, 1963, Duffels and van der Laan, 1985 and Villet, 1999). The sperm structures of only three cicada species, *Cicada orni* (tribe Cicadini), *Lyristes plebejus* (tribe Lyristini) (Folliot and Maillet, 1970) and *Graptosaltria nigrofuscata* (tribe Polyneurini) (Kubo-Irie et al., 2003) have been described. Although the descriptions of sperm morphology were not that detailed, the study by Kubo-Irie et al. (2003) revealed that *G. nigrofuscata* produces two distinct sizes of spermatozoa, which they termed long and short sperm. Furthermore, they determined that fertilized eggs contained long sperm only. If sperm morphology is to be of any value in exploring systematic and phylogenetic relationships of cicadas, as well as in fertilization biology studies, comparative information from a greater number of taxa with different reproductive strategies is required.

This study presents details on the sperm structure in four species of African cicadas from the tribe Platypleurini, and is part of a wider spermatological study of the taxonomy and phylogeny of cicadas. The platypleurines are a diverse tribe of large, attractively ornate, tree-dwelling cicadas occurring throughout the Afrotropical region and southern Asia to Japan (Metcalf, 1963 and Duffels and van der Laan, 1985). This group includes several genera that show fairly high degrees of endemism (Villet, 1999 and Villet and Van Noort, 1999). They are phylogenetically compact and display interesting signalling behaviour that could be important in creating situations where males have to compete for mates. Platypleurine cicadas have a “females search” mating syndrome where males make continuous, long-range acoustical advertisements from stationary perches and females approach them (Villet and van Noort, 1999). Advertisement signals reveal the location of the signaler to predators, while searching involves travel-related exposure risk (Burk, 1982).

The aims of this study were to elucidate and compare sperm structure in four species of platypleurine cicadas [*Albanycada albigera* (Walker, 1850), *A. zuluensis* (Villet, 1989), *P. capensis* (Linnaeus, 1764) and *P. hirtipennis* (Germar, 1834)] and to determine whether they have sperm polymorphism. The four species were chosen because they have different mate-attracting behaviours that could result in different degrees of reproductive competition (Villet, 1986).

2. Materials and methods

2.1. Materials

Male cicadas were collected in the Grahamstown (33°18′ S 26°32′ E) and Kasouga (33°38′ S 26°44′ E) areas of the Eastern Cape province of South Africa.

2.2. Light microscopy

Five males of each species were dissected under a binocular microscope in 2% saline solution and spermatozoa were recovered from the testes and seminal vesicles. Sperm samples were spread evenly on a microscope slide coated with gelatin and chrome alum and a drop of glycerol was added before drying in an oven overnight at 60 °C. Slides were then placed in a methanol/acetic acid (75:25, v/v) fixative for 4 min and rinsed in saline solution for 1 min. The slides were then placed in 1% bisbenzimidazole Hoechst 33258, a cell-permeable adenine–cytosine binding fluorescent dye used to stain DNA (Sakaluk and O'Day, 1984) for 1 min. Under a fluorescence microscope, nuclei and mitochondria that have been stained with Hoechst 33258 are very conspicuous and can be measured easily. The slides were then rinsed in three changes of saline before a drop of glycerol/PBS (9:1, v/v) was added to the cells and covered with a coverslip. Slides were examined with an Olympus BX-61 epifluorescence microscope at a wavelength of 343 nm. Digital images of 50–100 sperm per male were randomly captured from each species. Sperm heads were measured from these images using the analySIS Soft Imaging System programme (www.softimaging.net). Scatter diagrams were generated to show the relationship between sperm nucleus length and tail length using the Statistica 6 programme. Frequencies of different length classes of sperm within and between species were analysed by Chi-square tests. Length measurements are reported as mean ± S.E.

2.3. Scanning electron microscopy

Spermatozoa of each species were attached to gelatin-coated coverslips in 2% saline solution and fixed overnight with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7) at 4 °C, dehydrated in a graded series of acetone, critical-point dried and sputter-coated with gold. The sperm were then examined and photographed in a JEOL 840A scanning electron microscope at 12 kV.

2.4. Transmission electron microscopy

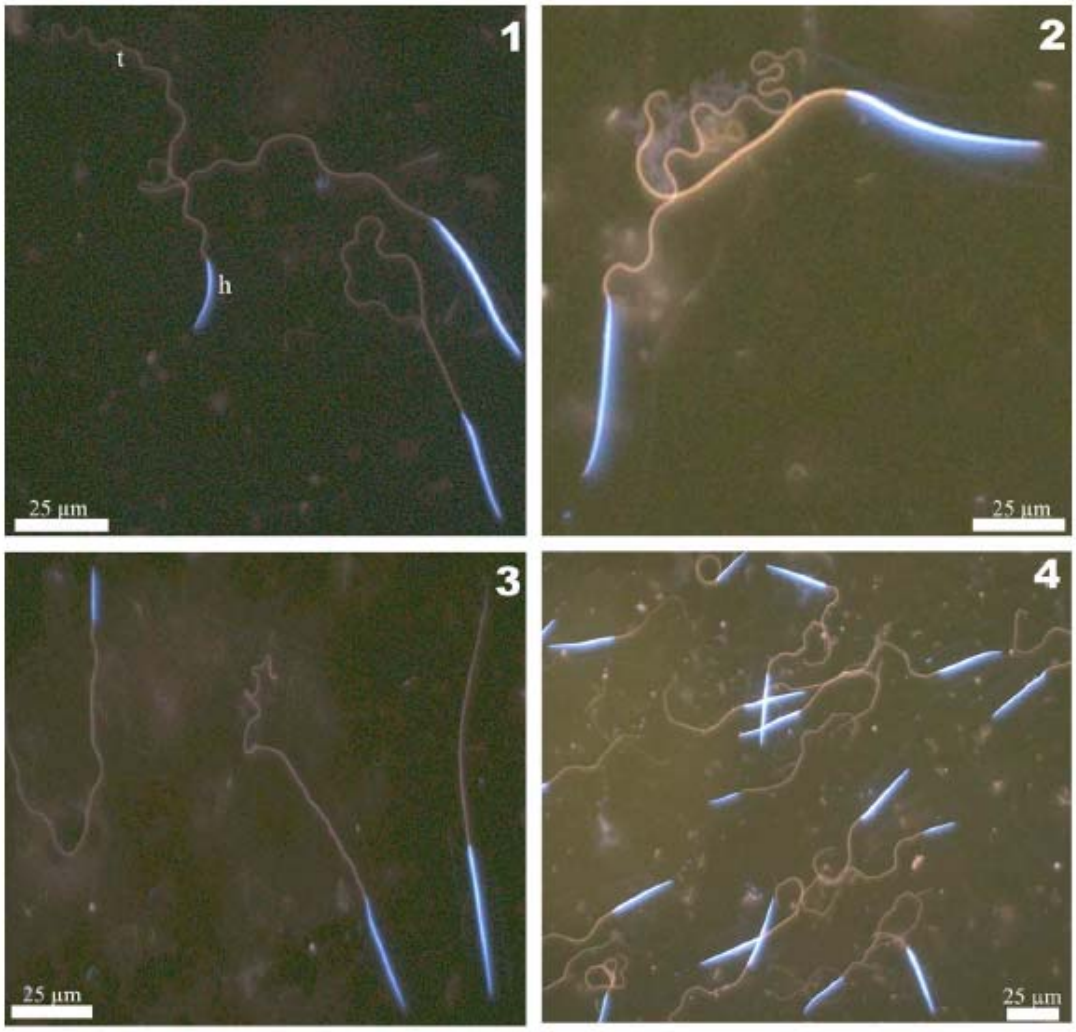
Small portions of testes and seminal vesicles were fixed overnight at 4 °C in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The material was then postfixed in 1% osmium tetroxide in phosphate buffer for 90 min at room temperature, dehydrated in ethanol and embedded in Epon 812 resin. Ultrathin sections (silver–gold) were cut using a diamond knife and collected on 300 mesh copper grids before staining with uranyl acetate and lead citrate. Sections were examined and photographed with a JEOL 1210 transmission electron microscope at 120 kV. Photographs were scanned and dimensions of the centriolar adjuncts, mitochondrial derivatives and nuclei were measured using the AnalySIS Soft Imaging System programme (www.softimaging.net).

3. Results

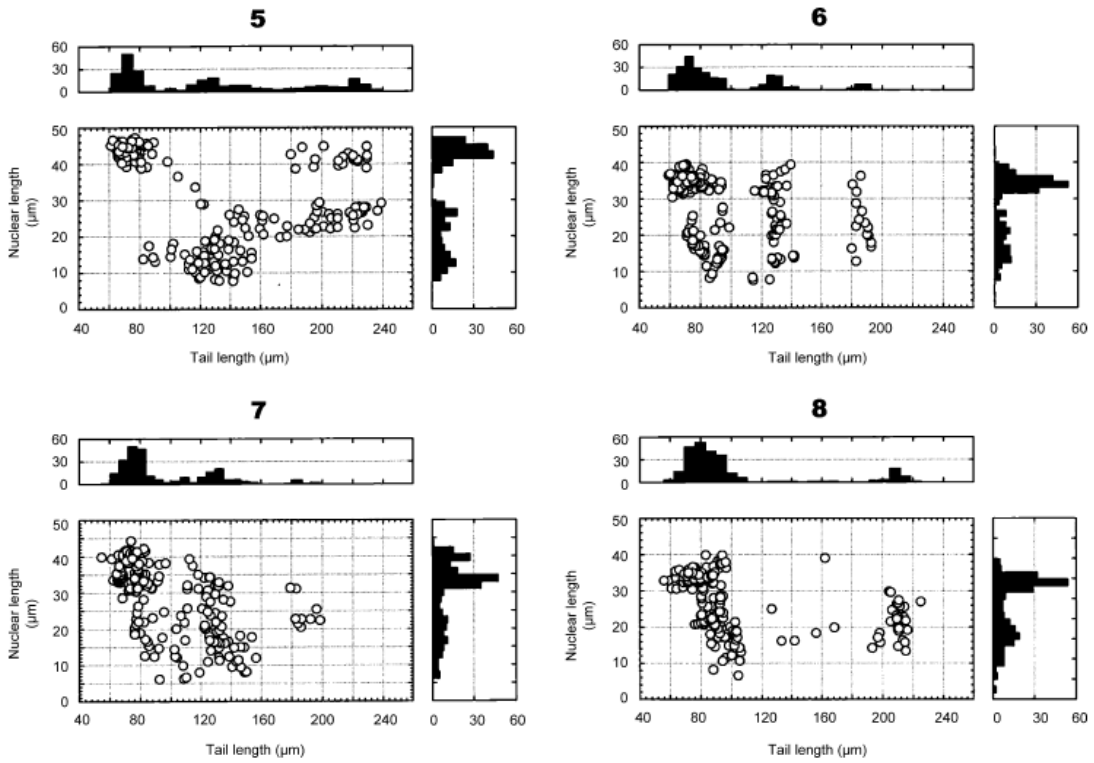
3.1. Size heteromorphism

Each of the four species of cicadas has motile spermatozoa that are elongated and thread-like, with slender, needle-like heads, and long tails that taper posteriorly (Figs. 1–4). Total sperm lengths vary considerably within and between species. This variation is due to the different lengths of the sperm nuclei and tails (Figs. 5–8). In all species except *A. zuluensis* there is evidence of three different modes of sperm nuclear and tail lengths. These have been categorized as short, intermediate and long. The majority of sperm have

long heads and short tails (Figs. 5–8). There is no statistically significant correlation ($P > 0.05$) between nuclear and flagellum length in any species.



Figs. 1–4. Fig. 1. Sperm (*Albanycada albigera*) with long and short heads stained with Hoechst 33258 and examined with a fluorescence microscope at 342 nm. h: head; t: tail. Fig. 2. Sperm (*Platypleura capensis*) with long heads stained with Hoechst 33258. Fig. 3. Sperm (*Azanicada zuluensis*) with intermediate and short heads stained with Hoechst 33258. Fig. 4. Sperm (*Platypleura hirtipennis*) with long and short heads stained with Hoechst 33258.



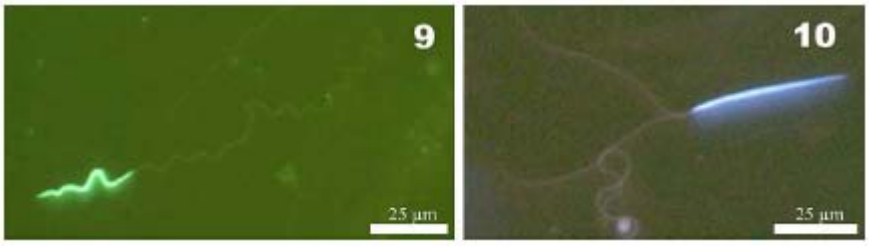
Figs. 5–8. Scatter-plots of nuclear lengths vs. tail lengths and size-frequency histograms of nuclear lengths and tail lengths in four species of cicadas. Fig. 5. *Albanycada albigera*. Fig. 6. *Platypleura capensis*. Fig. 7. *P. hirtipennis*. Fig. 8. *Azanicada zuluensis*.

Three modes of sperm tail lengths are found in *A. albigera*; these are 74, 130 and 220 μm. Nuclei dimensions have a trimodal distribution too, with modal lengths of 43, 27 and 13 μm (Fig. 5). Sixty percent of sperm heads have longer nuclei, whilst sperm with short and intermediate nuclei are found in similar abundances. The tail length distributions of *P. capensis*, with three modal classes of 74, 130 and 182 μm, are similar to those in *P. hirtipennis*. However, nuclear lengths in both species are bimodal, with modes of 33 and 14 μm in *P. capensis* and 33 and 18 μm in *P. hirtipennis*. In *A. zuluensis*, both nuclear and tail lengths have a bimodal distribution pattern (nuclear modes = 32 and 17 μm; tail modes = 80 and 210 μm). Tail lengths, unlike the nuclear lengths, are quite discrete (Fig. 8). Sperm with intermediate tail lengths are almost absent in this cicada. Chi-square tests revealed that observed frequencies of sperm dimensions from all individual cicadas within a species were homogenous for there were no significant differences between observed and expected frequencies. In *A. zuluensis*, *P. capensis* and *P. hirtipennis* the majority of sperm (60–80%) have short tails (Figs. 6–8). Tail lengths are generally longer in *A. albigera* than in the other three species. In *P. capensis* and *P. hirtipennis* sperm have shorter tails than those of *A. albigera* and *A. zuluensis*; about 6–8% of sperm in the last two species have long nuclei and long tails. Such sperm have the longest dimensions, often exceeding 250 μm in total length.

3.2. Shape dimorphism

Although the majority of sperm heads have rectilinear nuclei, small proportions (approximately 6%) of heads in all four species have twisted nuclei (Fig. 9). In addition a

small proportion of sperm in all species (approximately 10%) have two tails instead of the conventional single flagellum (Fig. 10). Such biflagellate sperm are all uninucleate.

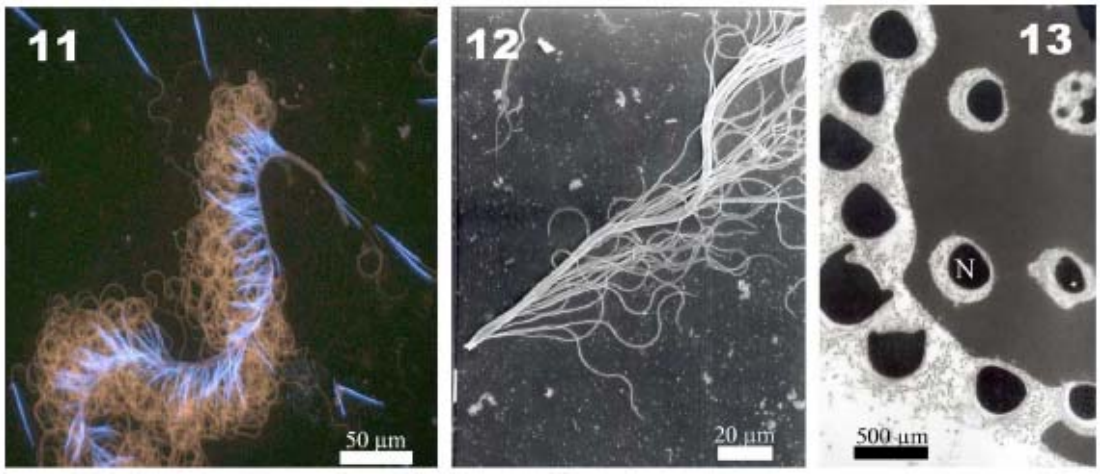


Figs. 9–10. Fig. 9. *Platypleura capensis* spermatozoon. Fluorescence image of a sperm cell with a twisted nucleus. Fig. 10. *Platypleura hirtipennis* spermatozoon. Fluorescence image of biflagellate sperm. All such sperm were uninucleate.

3.3. Ultrastructure

Because it was not possible to distinguish between sperm of different lengths within or between species with the transmission electron microscope, only a single ultrastructural description follows.

Spermatozoa are released in bundles with their anterior ends embedded in an elongate homogenous electron-dense matrix forming a spermatodesm (Figs. 11–13).

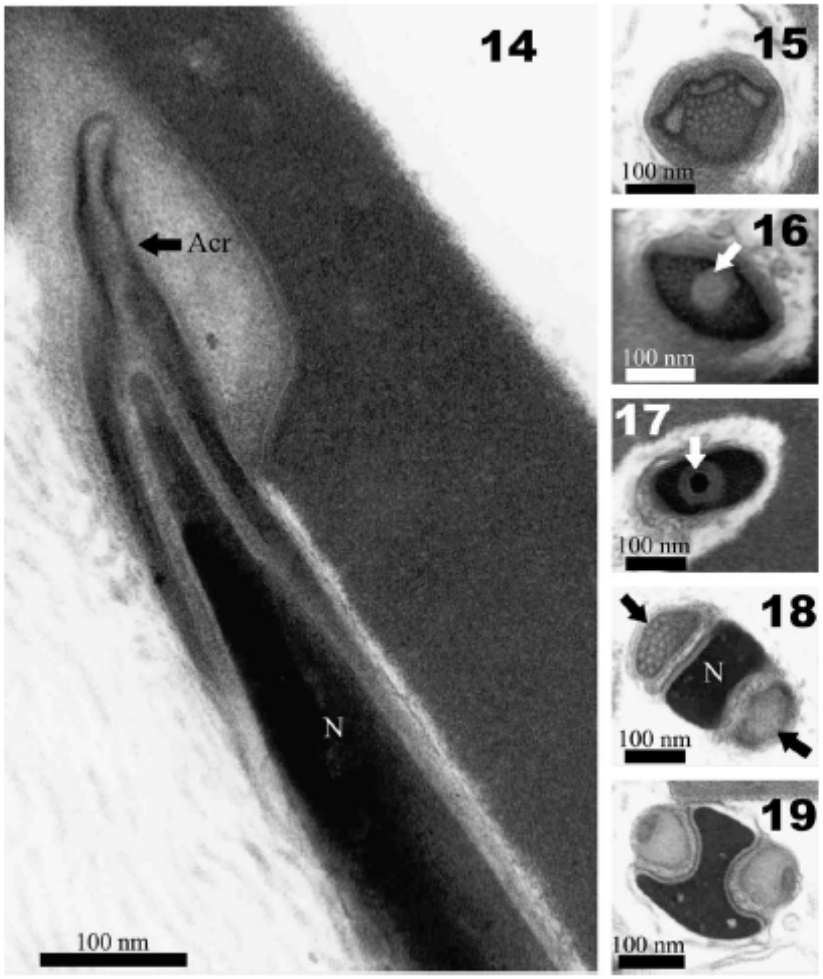


Figs. 11–13. Fig. 11. Fluorescence image (wavelength 343 nm) of a sperm bundle in *A. zuluensis* stained with Hoechst 33258. Spermatozoa are released in bundles with their anterior ends embedded in a matrix forming a spermatodesm. Fig. 12. Example of a sperm bundle in *A. zuluensis*, shown by scanning electron microscopy. Fig. 13. Transverse section through a sperm bundle in *P. capensis* showing the nuclei (N) and the homogenous electron-dense matrix.

3.3.1. Head region

The head region consists of a nucleus and an acrosome. The anteriorly positioned acrosome (Fig. 14) is conical in shape, deeply invaginated posteriorly and sits on top of the nucleus. Apically the acrosome is attenuated with a small narrow furrow on one face. The acrosome completely surrounds the anterior tip of the nucleus but posteriorly it

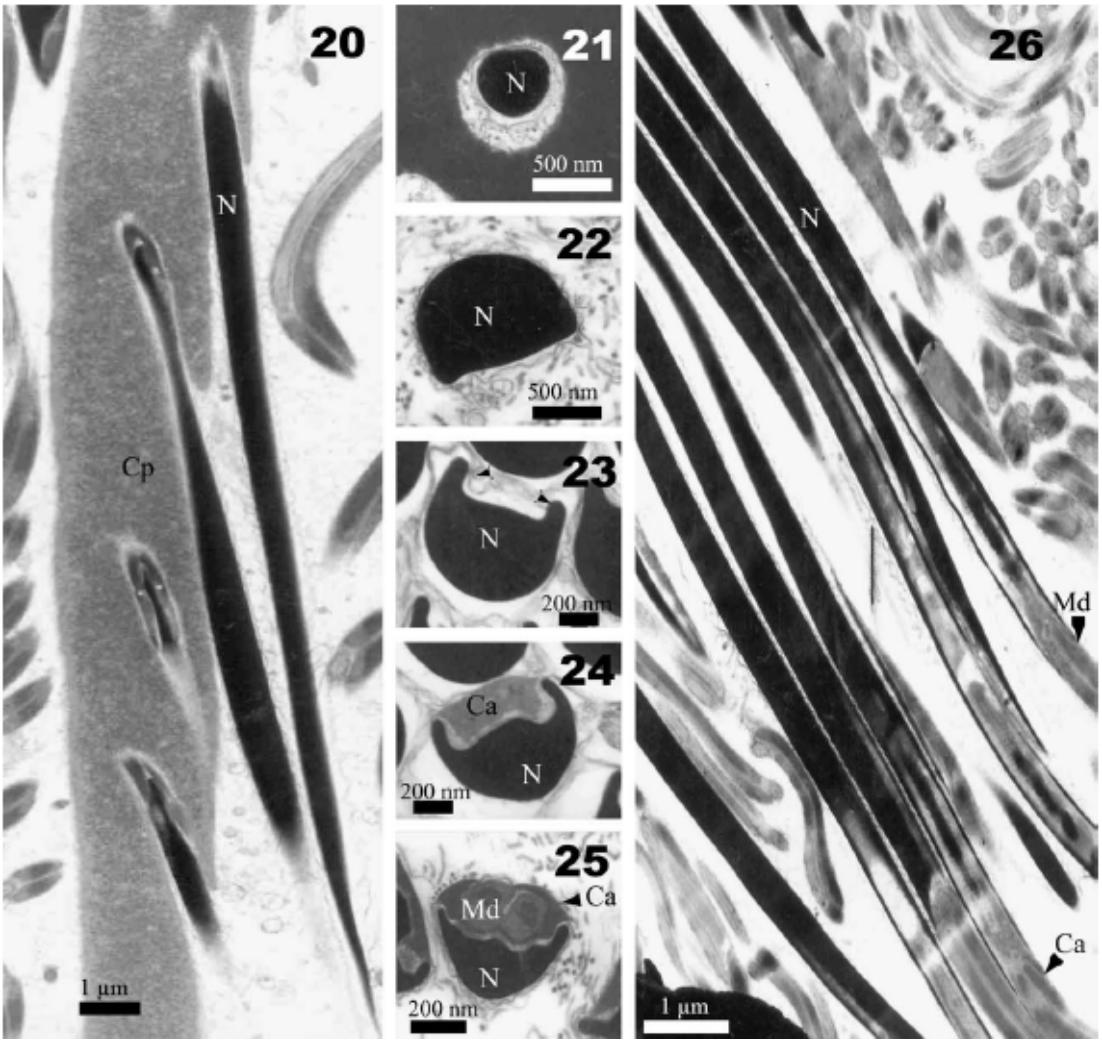
widens gradually with its extensions sitting on either side of the anterior section of the nucleus (Figs. 14, 17, 18, and 19). The acrosomal contents are differentiated internally; anteriorly the contents appear to be vesicular (Fig. 15); posteriorly there are distinct electron-lucent and electron-dense areas (Figs. 16–19). Dimensions of the tip width, base width and length of the acrosomes of the four species ranged between 71 and 81, 432 and 487 and 919 and 998 nm, respectively.



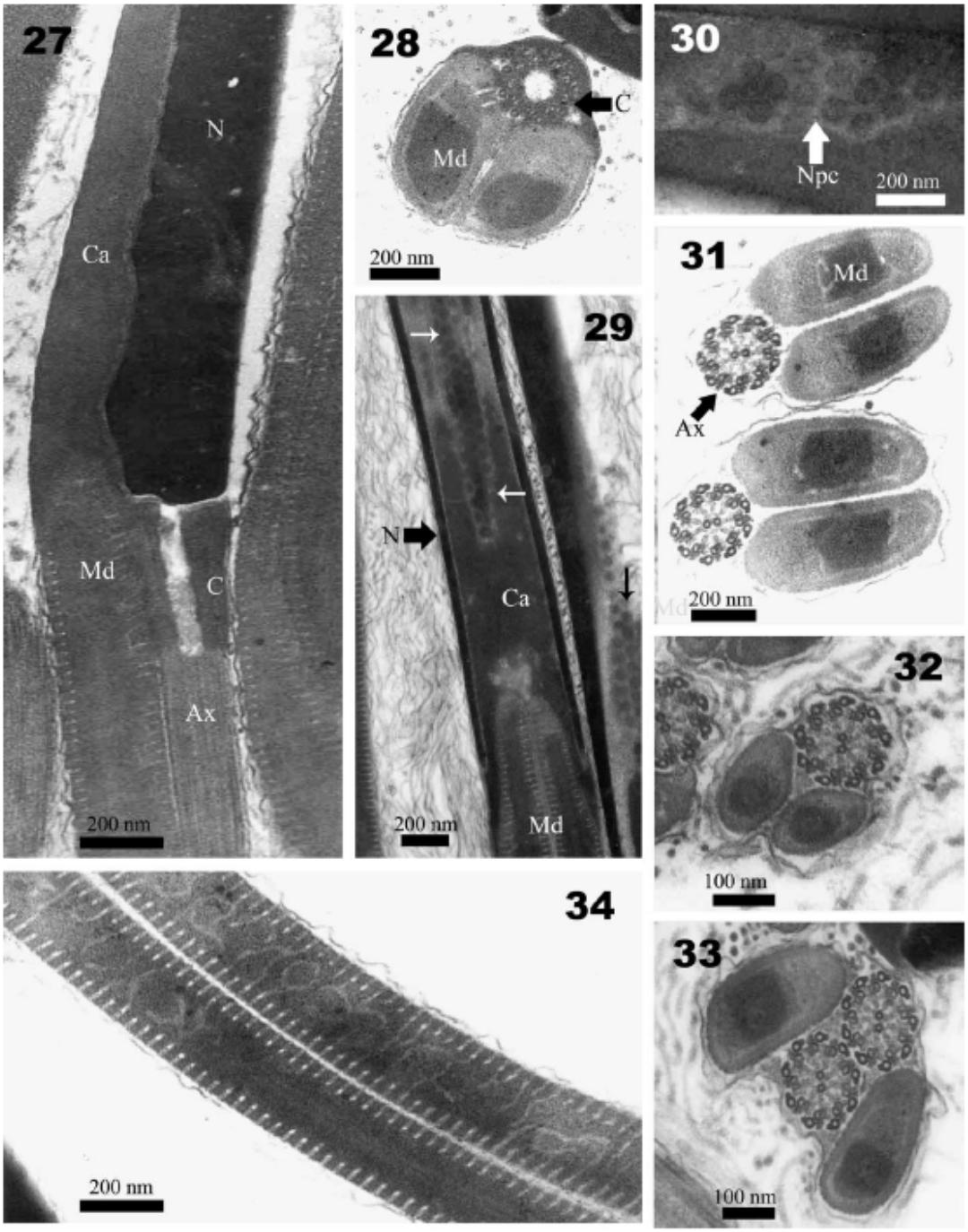
Figs. 14–19. Fig. 14. Spermatozoon of *A. albigera*. Longitudinal section through the acrosome and nucleus. The conical acrosome (Acr) completely encloses the anterior tip of the nucleus (N). Fig. 15. Transverse section through the anterior tip of the acrosome in *A. albigera* showing its vesicular appearance. Fig. 16. Spermatozoon of *A. albigera*. Transverse section through the acrosome showing the acrosomal vesicle and the central sub-acrosomal space (arrow). Fig. 17. Spermatozoon of *A. albigera*. Transverse section through the acrosome showing the anterior tip of the nucleus (arrow) and sub-acrosomal space surrounding it. Fig. 18. Transverse section towards the base of the acrosome in *A. albigera*. The nucleus (N) is bilaterally concave and is now flanked laterally by extensions of the acrosome (arrows). Note the vesicular appearance of the acrosome. Fig. 19. Transverse section at the posterior level of the acrosome in *A. albigera*. The concavity of the nucleus is more pronounced forming two lateral grooves into which the acrosomal extensions project. Note the electron-dense and electron-lucent regions of the acrosome.

In all species the homogenously electron-dense nucleus (Figs. 20 and 26) is pointed anteriorly, with a mid-nuclear diameter ranging from 522 to 614 nm. Anteriorly the nucleus forms a slender cone and is circular in cross-section (Fig. 21); towards the base

of the acrosome the nucleus becomes bilaterally concave (Figs. 18 and 19), the degree of concavity gradually increasing posteriorly. The lateral extensions of the acrosome are located in these concavities (Figs. 18 and 19). Posteriorly the nucleus becomes laterally flattened and then laterally invaginated (Figs. 21–25). The lateral invagination ranges in width from 428 to 484 nm and in depth from 157 to 173 nm. The posterior segment of the invagination houses fine granular material of the centriolar adjunct (Figs. 24–26). The centriolar adjunct (Fig. 27) is located anterior to the mitochondrial derivatives to which it adjoins. In longitudinal sections, the centriolar adjunct can be seen to be elongated and composed of electron-dense material. In addition vesicular-like structures of about 88 nm in diameter (Figs. 29 and 30) can be seen in some sections. These structures are located between the centriolar adjunct and the nucleus.



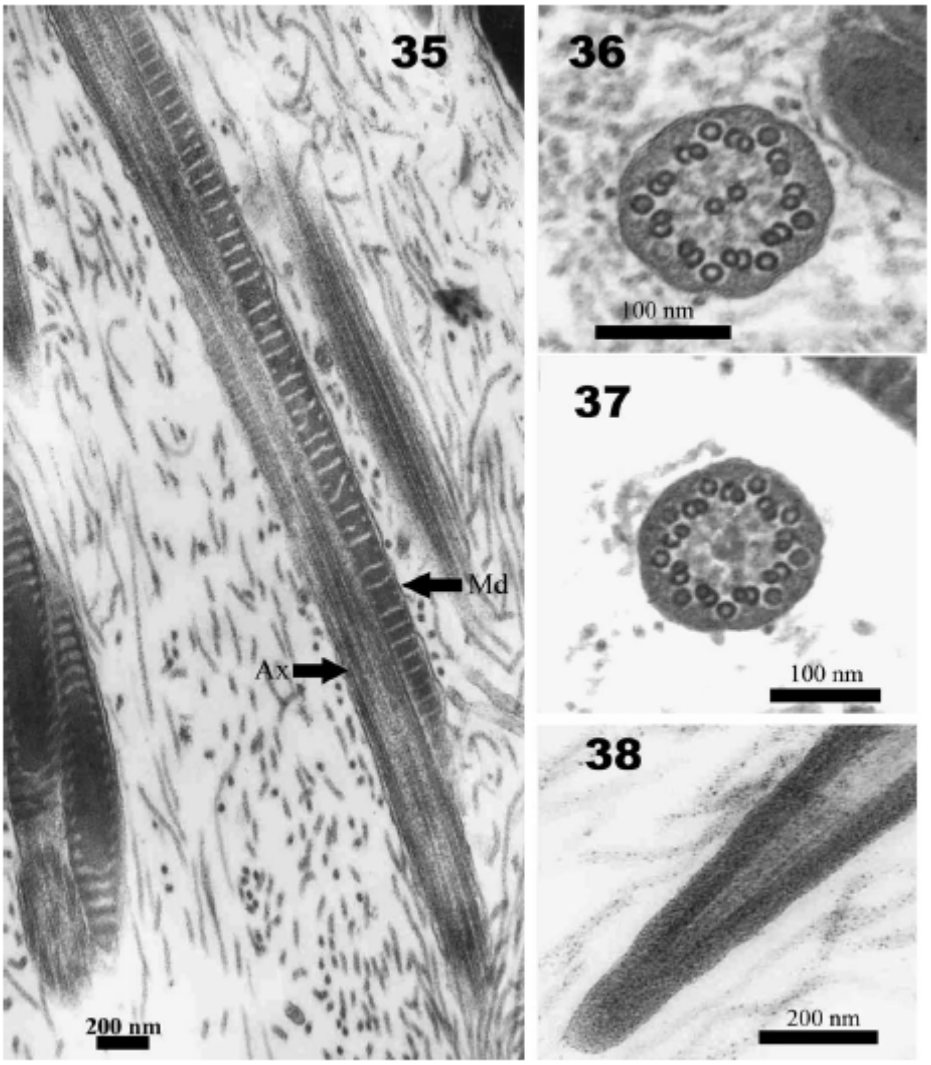
Figs. 20–26. Fig. 20. Spermatozoon of *P. hirtipennis*. Longitudinal section through the anterior part of the nucleus. The nucleus (N) is elongate and the entire acrosome together with a small portion of the nucleus is embedded in a homogenous matrix. Fig. 21. Transverse section of the nucleus at a region just posterior to the acrosome in *P. hirtipennis*. The nucleus (N) has a cylindrical shape. Fig. 22. Spermatozoon of *P. hirtipennis*. Transverse section of the nucleus at a region further from the posterior end of the acrosome. The cylindrical nucleus is now shaped like a semi-circle. Fig. 23. Transverse section of the nucleus through part of the sperm mid-piece in *P. hirtipennis*. The nucleus now has a lateral groove (arrows), which is devoid of material. Fig. 24. Transverse section of a more posterior region of the nucleus of *P. hirtipennis* showing the centriolar adjunct (Ca) housed in the nuclear groove. Fig. 25. Transverse section at the nuclear base in *P. hirtipennis*. The anterior ends of the mitochondrial derivatives (Md) protrude into the material of the centriolar adjunct (Ca). The nuclear diameter has decreased. Fig. 26. Longitudinal section through the posterior part of the sperm nucleus and anterior mid-piece in *P. hirtipennis* showing the nuclear grooves housing the centriolar adjunct and the anterior ends of the two mitochondrial derivatives.



Figs. 27–34. Fig. 27. Longitudinal section through the nuclear-flagellum transition in *P. hirtipennis*. The centriole (C) lies posterior to the nucleus and the axoneme (Ax) emerges from it. The two mitochondrial derivatives (Md) are positioned lateral to the axoneme and posterior to the elongated centriolar adjunct (Ca). Fig. 28. Transverse section through the neck region of the sperm in *P. hirtipennis*. The centriole (C) consists of a ring of nine microtubular triplets and no central microtubules. Md: mitochondrial derivative. Fig. 29. Spermatozoon of *P. hirtipennis*. Longitudinal section through part of the nuclear-flagellum region showing the centriolar adjunct (Ca), nucleus (N) and the mitochondrial derivatives (Md). The centriolar adjunct is composed of electron-dense material. Note the vesicular-like structures located between the posterior nucleus and the centriolar adjunct. Fig. 30. Longitudinal section through the sperm mid-piece in *P. hirtipennis* showing the vesicular-like structures. Fig. 31. Transverse section through the sperm tail in *P. hirtipennis* showing the 9 + 9 + 2 axoneme (Ax) flanked by the two mitochondrial derivatives (Md). Fig. 32. Cross-section at the posterior level of the sperm in *P. hirtipennis*. The mitochondrial derivatives have decreased in diameter. Fig. 33. Spermatozoon of *P. hirtipennis*. Biflagellate sperm with two axonemes. Fig. 34. Spermatozoon of *P. capensis*. Longitudinal section through a portion of the sperm tail showing the mitochondrial derivatives (Md). The peripheral cristae in each derivative are arranged in regular patterns perpendicular to the longitudinal axis of the derivative.

3.3.2. Mid-piece region

The neck region of the mid-piece contains a single centriole (Figs. 27 and 28), about 254 nm in diameter and approximately 662 nm in length that lies posterior to the nucleus. It consists of a ring of nine microtubular triplets and no central microtubules. The axoneme (Figs. 27 and 31), which extends for the entire length of the tail, emerges from the centriole and has a 9 (peripheral singlets), +9 (intermediate doublets), +2 (central singlets) pattern of microtubules. The diameter of the axoneme, about 254 nm, was similar in all species. A pair of mitochondrial derivatives, which extend along most of the tail, are positioned lateral to the axoneme and posterior to the centriolar adjunct (Figs. 27 and 32). Each derivative contains an electron-dense crystallized region and cristae at the periphery (Figs. 31 and 34). The maximum anterior cross-sectional width of the derivatives measured was 241 nm. Posteriorly the derivatives decrease in diameter (Figs. 32 and 33). In longitudinal sections (Figs. 34 and 35) the derivative has a striated appearance, the striations having a periodicity of approximately 43 nm. In all species, a small percentage of sperm (10%) were uninucleate and biflagellate (Fig. 33).



Figs. 35–38. Fig. 35. Spermatozoon of *A. zuluensis*, sectioned longitudinally along part of the sperm tail. The two mitochondrial derivatives (Md) do not flank the axoneme (Ax) along the entire length of the sperm tail. Fig. 36. Cross-section at the posterior level of the sperm tail in *A. zuluensis* showing the axoneme. Fig. 37. Cross-section at the terminal end of the sperm in *A. albigera*. The central microtubules of the axoneme have been lost. Fig. 38. Spermatozoon of *A. albigera*. Cross-section at the terminal end of the sperm tail showing the narrowing of the axoneme.

3.3.3. Endpiece region

Approximately 900 nm of axoneme extends beyond the posterior limits of the two mitochondrial derivatives, narrows to a point at the terminal end, and disrupts the normal 9 + 9 + 2 pattern of microtubules (Figs. 36 and 38).

4. Discussion

The sperm of the platypleurine cicadas described in this study have a number of morphological features that are similar to the Cicadomorpha (Cicadidae, Cercopidae and Cicadellidae) and Fulgoromorpha described to date (Folliot and Maillet, 1970, Jamieson et al., 1999 and Kubo-Irie et al., 2003). Similarities include a cylindrical, bilaterally symmetrical nucleus; absence of a perforatorium; a single axoneme with a 9 + 9 + 2 arrangement of microtubules; two crystalline mitochondrial derivatives that are lateral in position and extend along the axoneme, a centriolar adjunct that is located in front of the mitochondrial derivatives and sperm that are grouped together to form a spermatodesm. The sperm mid-pieces of the Cicadidae and Cercopidae lack accessory bodies, suggesting that, within the Cicadomorpha, these two families are more closely related. This relationship between the cicadas and spit bugs is supported by current phylogeny (Campbell et al., 1995 and Bourgoin and Campbell, 2002).

It is difficult to compare the results of the present study with those of previous investigations on cicadas and cercopids (Folliot and Maillet, 1970 and Kubo-Irie et al., 2003) because sperm morphologies in these insects were not described completely. For example, these studies lacked details of the internal structure of the acrosome, axoneme and the centriolar adjunct. However, according to Folliot and Maillet (1970) and Kubo-Irie et al. (2003) the acrosomes in *L. plebejus*, *C. orni* and *G. nigrofuscata* whilst anterior in position, are not located at the very tip of the nucleus because there is an anteacrosomal bleb that is as long as the acrosome itself. In this study the acrosomes of the four species of platypleurine cicadas were positioned at the tip of the nucleus and the anteacrosomal bleb was absent. The presence of nuclear pores and their complexes at the base of the nucleus has been reported by Folliot and Maillet (1970). From this study it is not clear whether the vesicular structures housed within the nuclear invaginations and associated with both the nucleus and centriolar adjunct are indeed nuclear pore complexes. Further detailed structural work is needed to determine that they are.

Two previously undescribed features, the presence of some biflagellate sperm and some sperm heads with twisted (rather than rectilinear nuclei) were found in platypleurine cicadas. Biflagellate and binucleate sperm have been described in the primitive zygentoman insect *Tricholepidion gertshii* (Dallai et al., 2001). The sperm cells of this insect are released as individuals from the testes; pairing occurs in the deferent ducts. The spermatozoon thus formed has two acrosomes, two nuclei and two separate tails (Dallai et al., 2001). There is no evidence of two nuclei or two acrosomes in the four cicada species studied. Hence, there is no sperm pairing. The small proportion of biflagellate, uninucleate sperm in the four cicada species studied suggests that such sperm are abnormal, and a result of infrequent aberrations during spermatogenesis.

Kubo-Irie et al. (2003) have previously described two distinct sizes of spermatozoa in the cicada *G. nigrofuscata*. They found a significant correlation between nuclear length and total sperm length. In the present study, there were no significant correlations ($P > 0.05$) between these parameters (Figs. 5–8). Sperm with long nuclei could have either short or long tails, although the majority possessed long heads with short tails. The higher

frequency of long nuclei might indicate that such sperm are favoured in fertilizations. The dimensions of sperm heads and tails in *G. nigrofuscata* are considerably shorter than those from the four platypleurine cicadas examined in this study. Sperm head length therefore might be a useful taxonomic character at the generic level in cicadas.

The functional significance of sperm polymorphism remains unclear (Snook, 1998 and Swallow and Wilkinson, 2002). Variation in total sperm length has been of particular interest, as comparative studies on diverse taxa have found positive relationships between sperm size and the risk of sperm competition (Pitnick et al., 2003). Patterns of correlated evolution between sperm length and certain dimensions of the female reproductive tract have been identified in ptiliid beetles (Dybas and Dybas, 1981), fleas (Rothschild, 1991), *Drosophila* (Hihara and Kurukawa, 1987, Pitnick and Markow, 1994 and Pitnick et al., 1999), stalk-eyed flies (Presgraves et al., 1999) and birds (Briskie et al., 1997). Collectively these studies strongly implicate postcopulatory sexual selection mediated by a component of female cryptic choice.

In *Drosophila obscura* (Snook et al., 1994) and in *G. nigrofuscata* (Kubo-Irie et al., 2003), sperm possessing long nuclei and long tails fertilized all eggs. Unfortunately, nothing was mentioned about the role of short sperm that are also produced in abundance. Such a high proportion of the short sperm might indicate that they have functional significance and are not aberrations such as sperm with helical nuclei or those that are biflagellate. Variation in sperm size may represent different provisioning of gametes by males as a form of parental investment. Sperm with longer tails have longer mitochondrial derivatives. These mitochondrial derivatives may be important as nutritive resources to the zygote or may function in cytoplasmic inheritance (Perotti, 1973, Afzelius et al., 1976 and Sivinski, 1984). Studies on *D. melanogaster* have shown that the sperm tail is sequestered into the egg capsule during fertilization where it continues to persist well into embryonic development (Karr, 1991). The high percentage of sperm with long heads and short tails in the four species of cicadas studied probably indicate that nuclear length may be more critical to successful fertilization. Theoretically, sperm with short tails might also provide these nutritive resources, although the quantity would probably be less. Such investments by the male may increase future fecundity. This would be important to insects like cicadas that have a short adult life span and yet spend years underground as nymphs. There is need for further studies to determine the specific roles of these sperm morphs and the spermatodesm.

In conclusion, sperm nuclear length appears to be an important morphological character within the Cicadidae. Further spermatological comparisons with cicadas from different tribes would be useful to fully understand sperm structure in all tribes and the functional significance of polymegaly.

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