Acta Zoologica (Stockholm) **87**: 297–304 (October 2006)

Ultrastructure of the spermatozoon of *Myrmecocichla formicivora* (Vieillot, 1881) and *Philetairus socius* (Latham, 1790) (Aves; Passeriformes), with a new interpretation of the passeridan acrosome

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Keywords:
acrosome ultrastructure, birds, *Myrmecocichla*, *Philetairus*, passerine spermatozoon, phylogeny

Accepted for publication:
27 June 2006

Abstract

Jamieson, B.G.M. Hodgson, A. and Spottiswoode, C. 2006. Ultrastructure of the spermatozoon of *Myrmecocichla formicivora* (Vieillot, 1881) and *Philetairus socius* (Latham, 1790) (Aves; Passeriformes), with a new interpretation of the passeridan acrosome. — *Acta Zoologica* (Stockholm) **87**: 297–304

Passerine spermatozoa exhibit apomorphies that distinguish them from non-passerine neognaths and palaeognaths. The acrosome is longer than the nucleus (excepting the suboscines, most Corvida, and a few Passerida). A perforatorium and endonuclear canals are absent. The proximal centriole is absent (except in the suboscines). The distal centriole is secondarily short, contrasting with its elongate condition in palaeognaths and Galloanserae. In the Passerida a single mitochondrial strand winds extensively along the axoneme (restricted to the anterior axoneme in suboscines and Corvida). A fibrous, or amorphous, periaxonemal sheath, seen in palaeognaths and many non-passerines, respectively, is absent. The acrosome in *Myrmecocichla formicivora* and *Philetairus socius* is bipartite: an acrosome core is surmounted by an acrosome crest; the core is ensheathed by a layer which is a posterior extension of the crest. The acrosome helix is a lateral extension of the crest and the crest layer with (*Myrmecocichla*) or without (*Philetairus*) protrusion of material of the acrosome core into it. In *M. formicivora*, as in other muscicapoids, a fibrous helix is intertwined with at least the more proximal region of the mitochondrial helix. The fibrous helix is absent at maturity in *Philetairus* and other described passeroid spermatozoa with the possible exception of *Passer italiae*. In *Philetairus* a granular helix precedes the mitochondrial helix.

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Introduction

It has been estimated that there are 5700 species of Passeriformes (hereafter referred to as passerines), comprising approximately 60% of extant birds (Sibley and Monroe 1990). However, the ultrastructure of the spermatozoa of only some 50 passerine species has been investigated, often superficially, of which four are suboscines, 46 are oscines and, of the latter, 41 are passeridans (Jamieson 2006; for taxonomic categories see also Barker *et al.* 2004). We describe here, for the first time, the spermatozoal ultrastructure of two phylogenetically disparate species of the Passerida for its cytological interest and with a view to extracting characters of phylogenetic value. In view of the fact that molecular analyses have produced widely conflicting avian phylogenies (see References in Jamieson and Tripepi 2005), albeit with greater resolution for passerines than for non-passerines, morphological characters assume special significance when testing the validity of different phylogenetic hypotheses. Spermatozoa offer a valuable set of such characters (Jamieson and Tripepi 2005). Here data

are presented which add to this database and, combined with previously published descriptions, support recognition of the Muscicapoidea and their distinction from the Passeroidea.

Material and methods

One specimen each of *Myrmecocichla formicivora*, the southern anteater-chat (Muscicapidae) and *Philetairus socius*, the social or sociable weaver (Ploceidae), were obtained on 24 January 2005 on Benfontein Farm (28°52′S, 24°51′E), 15 km south of Kimberley, Northern Cape Province, South Africa. Samples of testes and seminal glomera were fixed for 2 h in 3% glutaraldehyde (in 0.1 M phosphate buffer) at 4 °C. They were then sent to A.H. at Rhodes University for further processing. After two washes in phosphate buffer the samples were post-fixed in 1% osmium tetroxide for 2 h and stored in buffer in a refrigerator. After dehydration through a graded ethanol series, the specimens were embedded in epon–araldite via propylene oxide, and sectioned with an ultramicrotome (RMC 7). Ultrathin sections (silver/gold interface), cut with a diamond knife and stained with uranyl acetate (20 min) and lead citrate (5 min), were observed and photographed with a Jeol 100 CXII electron microscope at 100 kv. Plates and the initial draft of the text were prepared by B.G.M.J. in Brisbane.

Results

This study of the spermatozoa of *M. formicivora* and *P. socius* has clarified hitherto imperfectly understood aspects and revealed previously unrecognized features of passerine spermatozoal ultrastructure. The filiform spermatozoa of these two species demonstrate par excellence the passeridan features of an acrosome longer than the nucleus and great prolongation of the midpiece as a single mitochondrion wound helically around much of the length of the axoneme. A new revelation is the bipartite nature of the acrosome.

Myrmecocichla formicivora

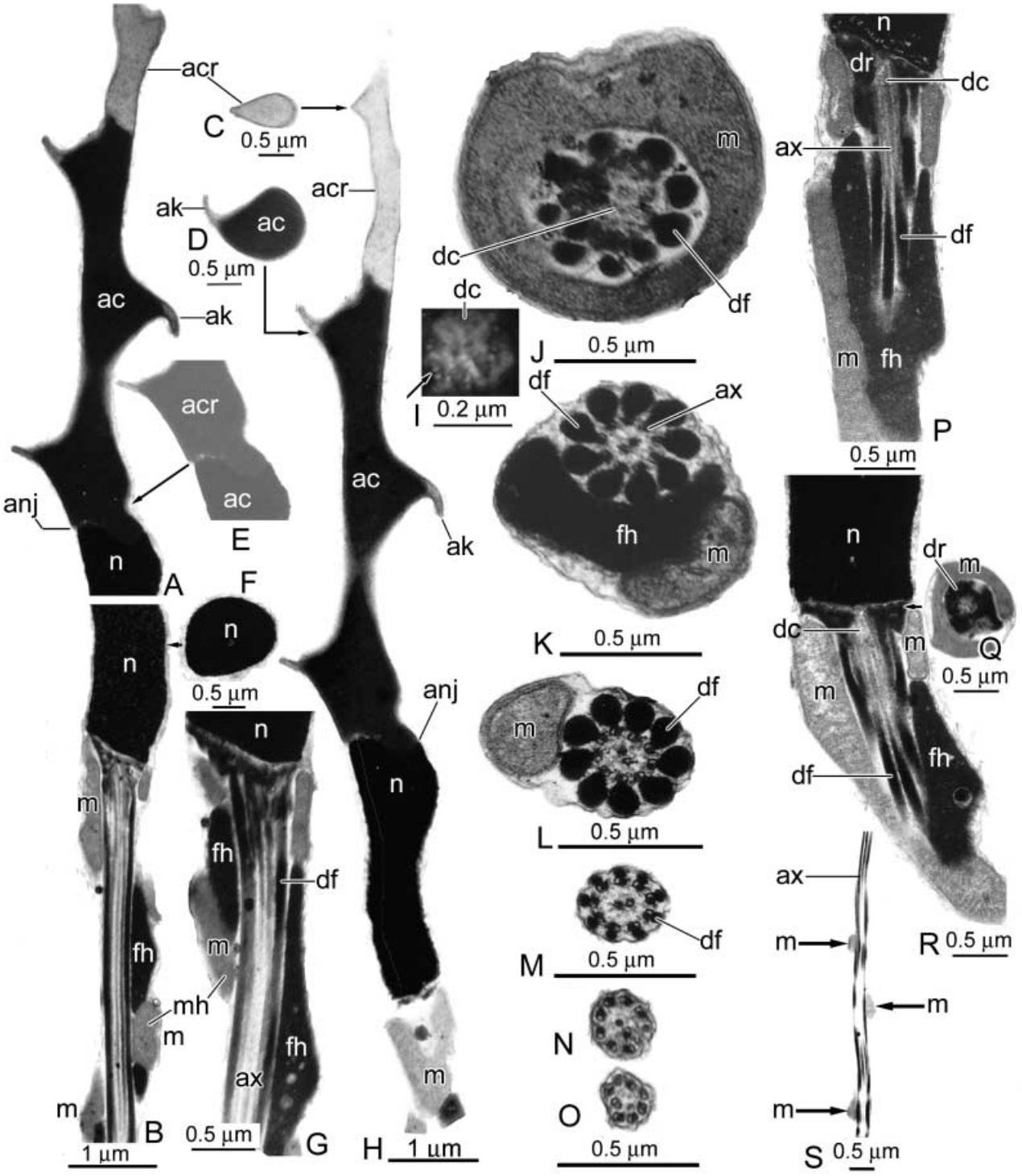
Acrosome. The acrosome, 7.9 μm long, is a helical structure 2.7 times the length of the nucleus, which it surmounts (Fig. 1H). It is inserted into the nucleus, at the acrosome–nucleus junction (Fig. 1A,E,H), in an asymmetrical fossa. The acrosome is bipartite. Its distal, longer portion consists of an electron-dense gently helical column of three gyres, here termed the acrosome core, 5.2–5.3 μm long ($n = 2$), which bears a prominent keel seen in profile as three prominent spurs. The dense core is drawn out towards the keel, as can be seen in longitudinal (Fig. 1A,E,H) and in transverse section (Fig. 1D). The proximal (anterior) portion, here termed the acrosome crest, consists of a narrower spirally angular electron-pale shaft, 3 μm long, tapering to a narrow tip (Fig. 1H). Its substance is also drawn out towards its angular projections, as seen in longitudinal (Fig. 1A,E,H) and transverse (Fig. 1C) sections. Although the acrosome keel is limited to the region of the acrosome core, it consists of electron-pale material that is continuous with that of the acrosome crest and must be considered to be part of the crest. The acrosome crest abuts on to the core at an oblique, very slightly concave junction (Fig. 1A,H). The morphological and developmental implications of the bipartite constitution are explored in the Discussion.

Nucleus. The nucleus, like the acrosome core, is strongly electron-dense. It forms a very slightly sinuous stout cylinder (Fig. 1H), 2.9 μm long, of subcircular transverse section (Fig. 1F). Its greatest width, 0.9 μm, is shortly below the acrosomal nuclear junction. Its base closely abuts the pericentriolar dense ring (Fig. 1B,G,H,P,R) against which it forms a slight convexity or shallow, slightly protuberant double concavity.

Centriolar complex. There is no proximal centriole. The distal centriole is surrounded by and fused with a dense ring outside which is a ring formed by the proximal end of the mitochondrion of the midpiece (Fig. 1Q). At least some of the centriolar microtubules have been shown to form triplets (Fig. 1I). It is short and it appears to be penetrated by the two central axonemal singlets or material continuous with these. At its junction with the axoneme, the dense ring has given way to nine large, dense fibres, but it is still encircled by the mitochondrial ring (Fig. 1J).

Helical components of the axonemal region. Longitudinal sections of the base of the nucleus and adjacent centriolar complex and midpiece reveal two components spiralled around the axoneme: a very elongate mitochondrion, which proximally forms the continuous mitochondrial ring, and a strongly electron-dense component here termed the fibrous helix. Dense fibres are also seen in glancing longitudinal profiles,

Fig. 1—*Myrmecocichla formicivora*. Transmission electron microscopy of spermatozoon. —**A**. Longitudinal section (LS) of the nucleus surmounted by the acrosome. The acrosome is bipartite, consisting of a long, electron-dense acrosome core, which bears a prominent keel, and a distal spirally angular acrosome crest. The acrosome core fits into an oblique fossa at the tip of the nucleus, more clearly seen in **E**. —**B**, **G**, **P**, **R**. LS of the base of the nucleus and adjacent centriolar complex, midpiece and anterior axoneme. Note two components spiralled around the axoneme: a very elongate mitochondrion that proximally forms a continuous ring and a strongly electron-dense component here termed the fibrous helix. Dense fibres are also seen encircling the axoneme, —**C**. Transverse section (TS) of the acrosome crest, —**D**. TS acrosome core through helical keel of the acrosome, —**F**. TS nucleus, —**H**. LS of the entire length of the acrosome crest and core and of the short nucleus, followed by a glancing section of the mitochondrial circlet, —**I**. Detail of Q, showing the triplets of the distal centriole, —**J**. TS mitochondrial ring encircling junction of distal centriole and axoneme, with large dense fibres, —**K**. TS near proximal end



of axoneme, showing 9 + 2 pattern with nine dense fibres, crescentic section of fibrous helix and, external to this, the mitochondrion, —**L**. TS axoneme with dense fibres and section of the mitochondrial helix, —**M**. TS distal region of the flagellum with no mitochondrial helix and reduced dense fibres, —**N**. TS end-piece of flagellum lacking dense fibres and mitochondrial helix, —**O**. Extreme posterior end of end-piece with disrupted arrangement of doublets and singlets, —**Q**. TS of the distal (and only) centriole, surrounded by a dense ring and the mitochondrial ring, —**S**. LS of a portion of the longest region of the flagellum around which spirals the mitochondrial helix. acr, acrosome crest; ac, acrosome core; ak, acrosome keel; anj, acrosome–nucleus junction; ax, axoneme; dc, distal (only) centriole; df, dense fibre; dr, dense ring around centriole; fh, fibrous helix; m, mitochondrion; mh, mitochondrial helix; n, nucleus.

encircling the axoneme (Fig. 1B,G,P,R). Further distally, for the greater length of the axoneme, the only helical component is the mitochondrial helix (Fig. 1S).

Mitochondria. As noted, a mitochondrial ring encircles the junction of the distal centriole and the axoneme together with its large dense fibres (Fig. 1J). This mitochondrial ring is continuous with the single, extremely elongate mitochondrion, which spirals along the axoneme for the greater part of the length of the latter. The course of this mitochondrial helix is seen in longitudinal section in Fig. 1(B,G,P,R,S). In transverse section the mitochondrion is seen to accompany the portion of the axoneme, which has large dense fibres where initially it lies external to the helical fibre (Fig. 1K). More distally, in the absence of the helical fibre, it is in direct contact with the dense fibres (Fig. 1L).

Fibrous helix. A well-developed electron-dense helix intervenes between the mitochondrial helix and the axoneme in the proximal region of the latter, as seen in longitudinal (Fig. 1B,G,P,R) and transverse section, in which its crescentic form is seen (Fig. 1K).

Axoneme. The axoneme has the conventional 9 + 2 arrangement of microtubules. For much of its length each doublet is accompanied by a dense fibre which is circular in cross-section except for a small prolongation which joins each A microtubule near the junction of the latter with the B subtubule (Fig. 1K,L). The dense fibres greatly reduce in size distally (Fig. 1M). Judging from the small number of transverse sections, the end-piece of the flagellum, lacking dense fibres and mitochondrial helix, is short (Fig. 1N). The extreme posterior end of the end-piece has a disrupted arrangement of doublets and singlets (Fig. 1O).

Philetairus socius

Fixation of the sperm of *P. socius* in difficult conditions was less favourable than that of the previous species and only a brief description will be given here. The spermatozoon conforms to all of the passeridan features described for *M. formicivora*.

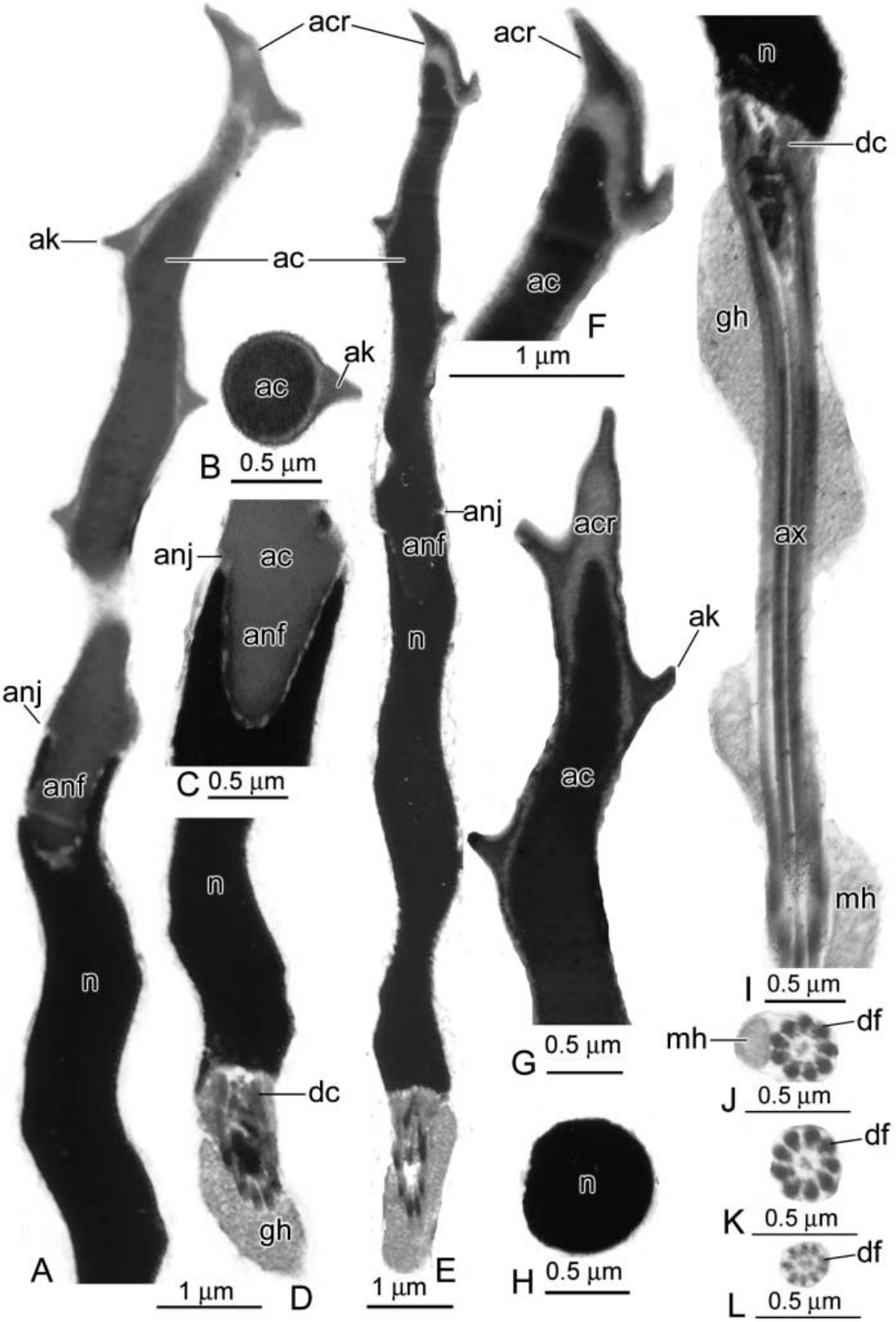
Acrosome. The acrosome, 6.9 μm long, is a helical structure 1.2 times the length of the nucleus, which it surmounts (Fig. 2E). It is inserted into the nucleus, at the acrosome–nucleus junction (Fig. 2A,C,E), in an approximately symmetrical V-shaped fossa. The acrosome is bipartite. Its distal, much longer, portion consists of an electron-dense gently helical column of three gyres, the acrosome core, 6.2 μm long, which bears a prominent keel seen in profile as three prominent spurs. Unlike *M. formicivora*, the dense core is not drawn out towards the keel, which is formed solely from the crest layer that ensheathes the core, as seen in longitudinal (Fig. 2A,E,G) and transverse section (Fig. 2B). The proximal (anterior) portion of the acrosome, the acrosome crest, as seen in longitudinal section, consists of a short, electron-pale shaft, the pre-core portion of which is 1.2 μm long, tapering to a narrow tip (Fig. 2G) and bearing near the level of the core, a single spur representing the keel. However, the crest extends posteriorly as the crest layer or sleeve to the base of the acrosome and bears along its length three spurs representing the continuation of the acrosome keel (seen in cross-section in Fig. 2B). In longitudinal sections approximately at right angles to this (Fig. 2A,E,F), the short acrosome crest has the form of an inverted shoe, of which the first spur forms the heel. The tip of the acrosome core fits into a deep asymmetrical fossa formed by the base of the crest (Fig. 2A,E,F).

Nucleus. The nucleus, like the acrosome core, is strongly electron-dense. It forms a very slightly sinuous stout cylinder (Fig. 2A,D,E), 6.1 μm long, of subcircular transverse section (Fig. 2H). Its greatest width, 0.8 μm, is basal but there is little variation throughout its length. Its base closely abuts the centriolar region (Fig. 2D,E).

Centriolar complex. There is no proximal centriole. A dense ring surrounding the distal centriole, seen in *M. formicivora*, has not been found and there is no mitochondrial ring. However, dense convoluted masses are present within the lumen of this centriole and the proximal region of the axoneme (Fig. 2I).

Helical components of the axonemal region. A longitudinal section of the base of the nucleus and adjacent centriolar complex and midpiece (Fig. 2I) reveals a very elongated mitochondrion wound helically around the axoneme but preceded anteriorly by a granular helix (see Discussion). No fibrous helix, seen in *M. formicivora*, is present. Glancing sections of nine dense fibres are visible between the mitochondrion and the axonemal doublets, as confirmed from transverse sections (Fig. 2J).

Fig. 2—*Philetairus socius*. —**A**. Longitudinal section (LS) of acrosome and anterior nucleus, —**B**. Transverse section (TS) of acrosome core through the helical keel, —**C**. Acrosome–nucleus junction showing anterior nuclear fossa receiving the base of the acrosome core, —**D**. LS base of nucleus and the centriolar region, —**E**. LS of the entire acrosome, nucleus and centriolar region, —**F**. Detail of acrosome crest and anterior acrosome core, —**G**. LS acrosome crest and anterior acrosome core at right angles to F. —**H**. TS nucleus, —**I**. LS base of nucleus, and centriolar and anterior axonemal region surrounded by the granular helix preceding the mitochondrial helix, —**J**. TS axoneme and mitochondrial helix, showing nine dense fibres associated with the axonemal doublets, —**K**. TS axoneme posterior to the mitochondrial helix, —**L**. TS posterior region of axoneme with reduced dense fibres. acr, acrosome crest; ac, acrosome core; ak, acrosome keel; anj, acrosome-nuclear junction; ax, axoneme; dc, distal (only) centriole; df, dense fibre; gh, granular helix; mh, mitochondrial helix; n, nucleus.



Axoneme. The axoneme has the conventional 9 + 2 arrangement of microtubules. For much of its length each doublet is accompanied by a dense fibre which is circular in cross-section except for a small prolongation which joins each A microtubule near the junction of the latter with the B subtubule, at the level of the mitochondrial helix (Fig. 2J) and posterior to this (Fig. 2K). The dense fibres greatly reduce in size distally (Fig. 2L). Sections of the end-piece have not been obtained.

Discussion

The spermatozoa of *M. formicivora* and *P. socius* show the following characteristics, all of which are apomorphic, are typical of passerines (with exceptions noted), and distinguish them from non-passerines and palaeognaths. The acrosome is longer than the nucleus (excepting the poorly known suboscines, most Corvida, and a few Passerida). A perforatorium and an endonuclear canal are absent (but see below). A proximal centriole is absent (excepting suboscines) and the distal centriole is always short. Although the short condition of the distal centriole is plesiomorphic for the Animalia, in passerines it appears to be secondary as the centriole is elongate in palaeognaths and Galloanserae (see review by Jamieson 2006). In the Passerida a single mitochondrial strand is wound for a great distance along the axoneme. In the Corvida (e.g. Retzius 1909) and apparently in the little known suboscines (e.g. McFarlane 1971 cited in Koehler 1995), the mitochondrial helix is restricted to the anterior region of the axoneme. A fibrous, or amorphous, periaxonemal sheath, seen in palaeognaths and many non-passerines, respectively, is absent. Passerines differ further from non-passerines in possessing, in the spermatid, a 'helical membrane', consisting of multiple microtubules forming a thick strand helically coiled around at least the flagellum, though the condition in suboscines is imperfectly known (see Jamieson 2006).

The perforatorium and endonuclear canal are typically present in palaeognaths (e.g. Soley 1993, 1999) and the Galloanserae (e.g. Aire and Soley 2003; Jamieson 2006), and are basic to non-passerines. These are lost, homoplastically with passerines, in the emu, *Dromaius novahollandiae* (Baccetti *et al.* 1991), Piciformes (?) (Henley *et al.* 1978), Apodiformes (Jamieson and Tripepi 2005); *Charadriiformes* (*Jacana*, Saita *et al.* 1983) and Columbiformes (Mattei *et al.* 1972; Jamieson 1995, 1999, 2006), as in the Passeriformes.

In lacking an appreciable annulus, which in reptiles terminates the midpiece, the sperm of passerines (e.g. Asa and Phillips 1987; Jamieson 1999), like those of Psittaciformes (Jamieson *et al.* 1995; Jamieson 1999), Gruiformes (*Grus vipio*, Phillips *et al.* 1987) and Apodiformes (Jamieson and Tripepi 2005), differ from those of palaeognaths (e.g. Baccetti *et al.* 1991) and lower non-passerines including the mallard duck (Humphreys 1972; Maretta 1975), the turkey, chicken, guinea fowl (Thurston and Hess 1987; Jamieson 2006) and *Charadriiformes* as represented by *Jacana* (Saita *et al.* 1983). An annulus is basal to palaeognaths and these non-passerines. Absence of the annulus is therefore an apomorphic feature of passerine spermatozoa, but in view of other differences it must be considered homoplastic relative to other orders lacking the annulus.

A major finding of the present study pertains to the structure of the passeridan acrosome, for which a new interpretation is here advanced. The passeridan acrosome has been regarded as a helical structure with or without an encompassing helical keel. The distinctness of this keel from the mitochondrial helix has often been unrecognized, the two, with or without a nuclear keel, being termed the 'helical membrane'. A helical keel ('helical membrane') on the acrosome but not extending onto the nucleus has been described, or is apparent from illustrations, in ultrastructural works for *Piranga rubra*, summer tanager (Thraupidae); *Tachycineta thalassina*, violet-green swallow (Hirundinidae); *Turdus migratorius*, American robin (Turdidae) (McFarlane 1971 *fide* Koehler 1995); *Turdus merula*, blackbird (Turdidae) and *Passer italiae*, Italian sparrow (Passeridae) (Furieri 1961); *Lonchura striata*, 'lovebird' or Bengales finch (Kondo *et al.* 1988); *Passer domesticus*, house sparrow (Passeridae) and *Parus bicolor*, tufted titmouse (Paridae) (Koehler 1995). In *Sturnus vulgaris*, starling (Sturnidae), scanning electron microscopy similarly appears to show that the nuclear region, though spiral, is not keeled while the acrosome is (Vernon and Woolley 1999); the structure of the mitochondrial helix in this species is discussed below. Furieri (1961) clearly depicts the acrosomal keel of *T. merula* and *Passer italiae* as a lateral projection, differing in constitution from what is here called the acrosome core, as shown in the present study for *M. formicivora* and *P. socius*.

What has become clear from the present study is that the acrosome, in passeridan species as far apart phylogenetically as *M. formicivora* (a member of the Muscicapoidea) and *P. socius* (a member of the Passeroidea), is bipartite in nature. An acrosome crest surmounts the acrosome core like the scion on a stock, and the core is invested by a layer that is a posterior extension of the crest, here termed the crest layer or sleeve. The acrosome helix is a lateral extension of the crest and the crest layer with (*Myrmecocichla*) or without (*Philetairus*) protrusion of material of the acrosome core into it. Although the crest is longer in *M. formicivora* than in *P. socius*, in both species the helix of the crest proper consists of only a single lateral extension or spur whereas the acrosome core enveloped by the crest layer bears three extensions when viewed in longitudinal section. The acrosome keel thus has only one full gyre on the crest and three gyres on the core. It would be of great interest to investigate development during spermiogenesis of the crest and the spur. It is possible that the crest and its layer surrounding the core form the true acrosome vesicle and that the core is subacrosomal or perforatorial material, in which case loss of the perforatorium would not be an apomorphy of passeridans. However, the crest and its posterior layer may merely be a modification of the surface

layer of an acrosome vesicle represented by the core. These alternative possibilities would easily be resolved by study of the spermatid. In either interpretation, the anterior nuclear fossa, into which the acrosome core is inserted, might be considered the homologue of the endonuclear canal.

The portion of the so-called helical membrane surrounding the axoneme is what is here termed the mitochondrial helix in many passeridan species. However, in *M. formicivora*, unlike *P. socius*, a second helix intertwines with at least the more proximal region of the mitochondrial helix. This has here been termed the fibrous helix. It is clearly the smaller structure described for *Sturnus vulgaris* (also a muscicapoid) as 'an extra helical structure' and labelled 'x' by Vernon and Woolley (1999), the larger structure being the single, helical chain of (fused) mitochondria. It was earlier described by Furieri (1961) as a small crest (i.e. keel) in *T. merula* and by Henley *et al.* (1978), as a fibrous component, in *T. migratorius* (both also muscicapoids). That the fibrous keel is derived from helical microtubules present in the spermatid is uncertain. Certainly these microtubules and any fibrous structure are lost by maturity of the sperm of some if not all non-muscicapoid passeridans (Jamieson 2006). Thus the fibrous keel is absent at maturity not only in *Philetairus* but also in *Taeniopygia* (= *Poephila*) *guttata*, the zebra finch (Fawcett *et al.* 1971) and *Lonchura striata* (Kondo *et al.* 1988) and is not reported for many other described passeroid spermatozoa with the exception, requiring confirmation, of *Passer italiae* where there is said to be a large fibre helically surrounding the anterior region of the axoneme in addition to the mitochondrial helix (Furieri 1961).

A helical granular structure spatially preceding the mitochondrial helix in some passeridan spermatozoa has been recognized (Humphreys 1972; Tripepi and Perrotta 1991; Koehler 1995). This is recognized here for the passeroid *P. socius* but not for the muscicapoid *M. formicivora*. It was considered diagnostic of the Passeroidea by Tripepi and Perrotta (1991) but it has not been demonstrated, for instance, in the estrildid *Taeniopygia guttata* and is present in the treecreeper, *Certhia brachydactyla* (Tripepi and Perrotta 1991), now in the Certhioidea, and in the muscicapoid *Sturnus vulgaris* (Koehler 1995). It thus occurs in some members of these three passeridan superfamilies (for further details see Jamieson 2006).

Harshman (2006) has reviewed molecular evidence that supports monophyly of the Muscicapoidea. Their monophyly and distinction from the Passeroidea was supported by Barker *et al.* (2004). It would be pertinent to investigate whether the fibrous helix, here shown to characterize the few investigated species of the Muscicapoidea, in families as diverse as the Sturnidae (starlings), Turdidae (thrushes) and their supposed sister-group the Muscicapidae (*sensu stricto* – Old World flycatchers) is present in all muscicapoid species. The most difficult question (Harshman 2006) is whether Bombycillidae (waxwings, silky flycatchers, palmchat) belong to this superfamily, and the presence or absence of a fibrous helix would aid in testing this proposition.

Acknowledgements

We are grateful to Marvin Randall (Electron Microscope Unit, Rhodes University) for his technical assistance, to the Northern Cape Department of Tourism, Environment & Conservation, South Africa, for collecting permits and to De Beers Consolidated Mines for permission to conduct research on their property. The School of Integrative Biology, University of Queensland provided facilities. The work was partly funded by a Rhodes University research grant to A.N.H.

References

Aire, T. A. and Soley, J. T. 2003. The guinea fowl centriolar complex: a morphological deviation for a non-passerine bird. – *Proceedings of the Microscopy Society of Southern Africa* **33**: 75.

Asa, C. S. and Phillips, D. M. 1987. Ultrastructure of avian spermatozoa: a short review. In Mohri, H. (Ed.): *New Horizons in Sperm Cell Research*, pp. 365–373. Japan Scientific Societies Press, Tokyo; and Gordon and Breach Scientific Publishers, New York.

Baccetti, B., Burrini, A. G. and Falchetti, E. 1991. Spermatozoa and relationships in paleognath birds. – *Biology of the Cell (Paris)* **71** (1–2): 209–216.

Barker, F. K., Cibois, A., Schikler, P., Feinstein, J. and Cracraft, J. 2004. Phylogeny and diversification of the largest avian radiation. – *Proceedings of the National Academy of Sciences of the USA* **101**: 11040–11045.

Fawcett, D. W., Anderson, W. A. and Phillips, D. M. 1971. Morphogenetic factors influencing the shape of the sperm head. – *Developmental Biology* **26**: 220–251.

Furieri, P. 1961. Caratteri ultrastrutturali di spermi flagellati di anfibi e uccelli. Studio al microscopio elettronico. – *Archivio Zoologico Italiano (Napoli)* **46**: 123–147.

Harshman, J. 2006. Classification and phylogeny. In Jamieson, B. G. M. (Ed.): *Avian Spermatozoa: Structure and Phylogeny*. *Reproductive Biology and Phylogeny of Birds*. – Science Publishers, Enfield New Hampshire, USA. Plymouth, UK. (in press).

Henley, C., Feduccia, A. and Costello, D. P. 1978. Oscine spermatozoa: a light and electron-microscopy study. – *Condor* **80**: 41–48.

Humphreys, P. N. 1972. Brief observations on the semen and spermatozoa of certain passerine and non-passserine birds. – *Journal of Reproduction and Fertility* **29**: 327–336.

Jamieson, B. G. M. 1995. Evolution of tetrapod spermatozoa with particular reference to amniotes. – *Memoires Du Muséum National d'Histoire Naturelle* **166**: 343–358.

Jamieson, B. G. M. 1999. Spermatozoal phylogeny of the Vertebrata. In Gagnon, C. (Ed.): *The Male Gamete. From Basic Science to Clinical Applications*, pp. 303–331. Cache River Press, Vienna, USA.

Jamieson, B. G. M. 2006. Avian spermatozoa: structure and phylogeny. In Jamieson, B. G. M. (Ed.): *Reproductive Biology and Phylogeny of Birds*. – Science Publishers, Enfield New Hampshire, USA. Plymouth, UK. (in press).

Jamieson, B. G. M., Koehler, L. and Todd, B. J. 1995. Spermatozoal ultrastructure in three species of parrots (Aves, Psittaciformes) and its phylogenetic implications. – *Anatomical Record* **241** (4): 461–468.

Jamieson, B. G. M. and Tripepi, S. 2005. Ultrastructure of the spermatozoon of *Apus apus*, the common swift (Aves; Apodiformes, Apodidae), with phylogenetic implications. – *Acta Zoologica* **88**: 239–244.

Koehler, L. D. 1995. Diversity of avian spermatozoa ultrastructure with emphasis on the members of the order Passeriformes. – *Mémoires Du Muséum National d'Histoire Naturelle* **166**: 437–444.

Kondo, T., Hasegawa, K. and Uchida, T. A. 1988. Formation of the microtubule bundle and helical shaping of the spermatid in the common finch *Lonchura-Striata*-Var-*Domestica*. *Journal of Ultrastructure and Molecular Structure Research* **98** (2): 158–168.

Maretta, M. 1975. The ultrastructure of the spermatozoon of the drake. II. Tail. – *Acta Veterinaria Academiae Scientiarum Hungarica* **25** (1): 53–60.

Mattei, C., Mattei, X. and Manfredi, J.-L. 1972. Electron microscope study of the spermiogenesis of *Streptopelia roseogrisea*. – *Journal of Submicroscopic Cytology* **4**: 57–73.

McFarlane, R. W. 1971. *The Ultrastructure and Phylogenetic Significance of Avian Spermatozoa*. – Unpublished PhD Thesis. University of Florida, U.S.A.

Phillips, D. M., Asa, C. S. and Stover, J. 1987. Ultrastructure of spermatozoa of the White-Naped Crane. – *Journal of Submicroscopic Cytology* **19** (3): 489–494.

Retzius, G. 1909. Die Spermien der Voegel. – *Biologische Untersuchungen, Neue Folge* **14** (10): 89–122.

Saita, A., Longo, O. M. and Tripepi, S. 1983. Osservazioni comparative sulla spermiogenesi. III. Aspetti ultrastrutturali della spermiogenesi di *Jacana jacana* (Charadriformes). – *Accademia Nazionale Dei Lincei. (Rendiconti Della Classe Di Scienze Fisiche, Matematiche E Naturali)* **74**: 417–430.

Sibley, C. G. and Monroe, B. L. Jr. 1990. *Distribution and Taxonomy of Birds of the World*. Yale University Press, New Haven CT.

Soley, J. 1999. Reproduction. In Deeming, D. C. (Ed.): *The Ostrich: Biology, Production and Health*, pp. 129–158. CAB International, Wallingford, Oxon, UK.

Soley, J. T. 1993. Ultrastructure of ostrich (*Struthio camelus*) spermatozoa: I. Transmission electron microscopy. – *Onderstepoort Journal of Veterinary Research* **60** (2): 119–130.

Thurston, R. J. and Hess, R. A. 1987. Ultrastructure of spermatozoa from domesticated birds comparative study of turkey chicken and guinea fowl. – *Scanning Microscopy* **1** (4): 1829–1838.

Tripepi, S. and Perrotta, E. 1991. Spermiogenesis and sperm of passerine birds. In Baccetti, B. (Ed.): *Comparative Spermatology 20 years after*, pp. 75, 1021–1023. Serono Symposia Publications, Ravel Press, Rome.

Vernon, G. G. and Woolley, D. M. 1999. Three-dimensional motion of avian spermatozoa. – *Cell Motility and the Cytoskeleton* **42**: 149–161.