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Ultrastructure of the spermatozoon of *Apus apus* (Linnaeus 1758), the common swift (Aves; Apodiformes; Apodidae), with phylogenetic implications

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Abstract

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The spermatozoon of *Apus apus* is typical of non-passerines in many respects. Features shared with palaeognaths and the Galloanserae are the conical acrosome, shorter than the nucleus; the presence of a proximal as well as distal centriole; the elongate midpiece with mitochondria grouped around an elongate distal centriole; and the presence of a fibrous or amorphous sheath around the principal piece of the axoneme. The perforatorium and endonuclear canal are lost in *A. apus* as in some other non-passerines. All non-passerines differ from palaeognaths in that the latter have a transversely ribbed fibrous sheath whereas in non-passerines it is amorphous, as in *Apus*, or absent. The absence of an annulus is an apomorphic but homoplastic feature of swift, psittaciform, gruiform and passerine spermatozoa. The long distal centriole, penetrating the entire midpiece, is a remarkably plesiomorphic feature of the swift spermatozoa, known elsewhere only in palaeognaths. The long centriole of *Apus*, if not a reversal, would be inconsistent with the former placement of the Apodiformes above the Psittaciformes from DNA–DNA hybridization. In contrast to passerines, in *A. apus* the microtubules in the spermatid are restricted to a transient single row encircling the cell. The form of the spermatozoon fully justifies the exclusion of swifts from the passerine family Hirundinidae.

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Introduction

A very brief abstract on the ultrastructure of spermiogenesis in *Apus apus*, the common swift, by Tripepi *et al.* (1984), and a brief reference to microtubules in the spermatid of *Apus melba*, the Alpine swift, by Tripepi *et al.* (1991) are the only ultrastructural works on the male gamete of the order Apodiformes. The spermatozoon was described and illustrated by light microscopy by Retzius (1911) who showed it to have a short conical acrosome, a stout, elongate fusiform nucleus, and a short, straight midpiece, followed by a free flagellum. Here we describe the ultrastructure of the late spermatid in greater detail and consider the phylogenetic position of the Apodidae as evidenced by sperm structure. In view of the fact that molecular analyses have produced widely conflicting phylogenies, as is evident if we contrast Mindrell *et al.* (1997, 1999) and Johnson (2001), on the one hand, with Braun and Kimball (2002); Sibley and Ahlquist (1981, 1990); Sibley *et al.* (1988) and Garcia Moreno *et al.* (2003), on the other, morphological characters assume special significance in testing the validity of different phylogenetic hypotheses. Spermatozoa offer a valuable set of such characters. As the structures described here are sufficiently developed to indicate definitive spermatozoal structure the cells will be referred to as spermatozoa.

Materials and Methods

Adult males of *A. apus* were captured in April. Birds were killed with chloroform and dissected; testis samples were

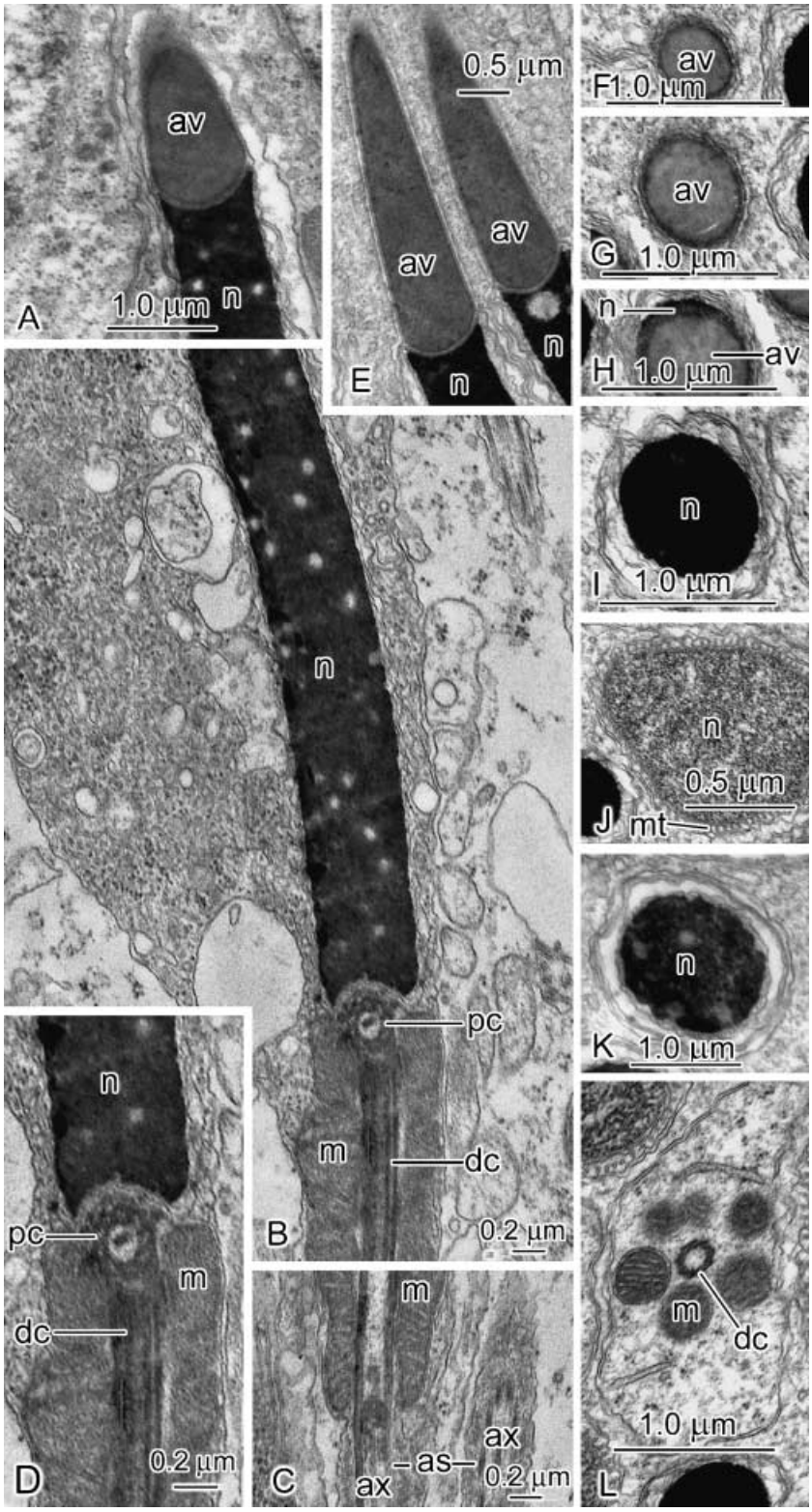

Fig. 1—*Apus apus*, common swift; transmission electron micrographs of spermatids. —**A**. Oblique longitudinal section (LS) of acrosome on tip of nucleus. —**B**. LS late spermatid showing elongate nucleus, with scattered uncondensed areas, and anterior portion of midpiece with enclosed proximal and distal centrioles. —**C**. Same cell, showing posterior end of midpiece and anterior portion of principal piece. —**D**. Detail from (C). —**E**. LS of two acrosomes. —**F**–**H**. Progressively posterior transverse sections (TS) of acrosome vesicle, —**H**. at the level of the anterior nuclear fossa. —**I**. TS of an advanced nucleus with strongly condensed chromatin and lacking peripheral microtubules. —**J**. TS nucleus of younger spermatid with uncondensed granular chromatin and peripheral single layer of microtubules. —**K**. TS nucleus at intermediate stage still showing pale uncondensed areas. —**L**. TS immature midpiece, showing six mitochondria surrounding the distal centriole. as = amorphous sheath; av = acrosome vesicle; ax = axoneme; dc = distal centriole; m = mitochondrion; mt = microtubules; n = nucleus; pc = proximal centriole.

fixed for 2 h in 3% glutaraldehyde (in 0.1 phosphate buffer). After two washes in phosphate buffer the samples were postfixed in 1% osmium tetroxide for 2 h. After dehydration through a graded ethanol series, the specimens, prestained in uranyl acetate, were embedded in epon-araldite and sectioned with an ultramicrotome (Leica Ultracut UCT). Ultrathin sections, stained with lead citrate, were observed and photographed using a Zeiss EM 900 electron microscope.

Results

General morphology

The spermatozoon of *A. apus* is a filiform cell consisting of a head region containing the acrosome and nucleus; a midpiece; and a tail region. The tail, posterior to the midpiece, consists of the axoneme surrounded by an amorphous fibrous sheath, the latter defining the principal piece, and an endpiece lacking the sheath.

Acrosome

The anterior-most region of the head consists of the acrosome, which is composed of an acrosome vesicle with an enveloping plasma membrane. Longitudinal (Fig. 1A,E) and transverse sections (Fig. 1F–H), reveal that a perforatorium is absent. The acrosome vesicle forms a slender, smooth, pointed cone. In a longitudinal section (Fig. 1E) which is sagittal, or nearly so, the length of the vesicle is 3 μm and the greatest width, at its base, is 0.75 μm. The base of the cone is rounded and closely fits a depression of the anterior end of the nucleus, though separated from the latter by the acrosomal and nuclear membranes.

Nucleus

The nucleus is an elongate cylinder (Fig. 1A,B) tapering only slightly towards its tip. Its full length has not been determined but it exceeds 8 μm, with a basal width of 0.6 μm. In young spermatids the chromatin is finely granular and the nuclear membrane is surrounded by microtubules, in single file, which lie under the plasma membrane (Fig. 1J). In the more mature nucleus (Fig. 1K) the chromatin forms dark clumps interspersed sporadically throughout its length with pale areas and few microtubules remain. Some of the pale areas impinge on the surface of the nucleus beneath its investing membrane (Fig. 1B). A larger body of this type is seen to occupy the central third at the anterior end of one nucleus (Fig. 1E, right). In the mature nucleus the chromatin is electron dense and almost homogeneous and microtubules are absent (Fig. 1I). The nuclear surface is almost smooth. The anterior nuclear fossa is matched by a concave posterior fossa, the implantation fossa (Fig. 1B,D).

Midpiece and centrioles

The elongate cylindrical midpiece in which the mitochondria are located is wider, at 1.1 μm, than the nucleus. Its length is approximately 3.5 μm (Fig. 2A). Its central axis is occupied by the proximal centriole, which lies partly within the implantation fossa, and by the distal centriole. The proximal centriole is short, with its longitudinal axis perpendicular to the sperm axis. It shows the usual nine triplets of microtubules (Figs 1B,D and 2D–E) but its central space is occupied by a structure which is annular in transverse section (Fig. 2C–E). The distal centriole, perpendicular to the proximal centriole and in the long axis of the cell, also shows a triplet configuration (Figs 1L and 2B). It extends for the whole length of the midpiece. Its axis is empty except for intrusion of the central singlets of the axoneme a very short distance into its base (Figs 1B,C and 2A).

The mitochondria form a circle around the distal centriole, numbering five or six in a transverse section of the cell (Figs 1L and 2B). There are six or seven in longitudinal sequence (Fig. 2A,F) but some of these may be partly conjoined (Fig. 2A). Posteriorly the midpiece narrows slightly but is not demarcated by a recognizable annulus (Figs 1G and 2A,F). Each mitochondrion has several cristae which appear transverse in cross-section and oblique in longitudinal section of the midpiece. The mitochondria are initially subspherical (Fig. 2F) but become more elongate nearer maturity (Figs 1B–D and 2A).

Axoneme

Immediately behind the midpiece, the axoneme commences as indicated by the presence of central singlets. A moderately electron-dense mass at the anterior end of these protrudes a little into the midpiece (Figs 1D,C and 2A). This configuration confirms that the distal centriole occupies the entire length of the midpiece. An amorphous sheath (Figs 1C and 2A,F) surrounds the axoneme behind the midpiece and the long ensheathed region constitutes the principal piece. A presumed endpiece, with axoneme lacking the amorphous sheath, has been observed but is surrounded by a transient cytoplasmic canal and sheath during development (Fig. 2H). Some transverse sections appear transitional between principal piece and endpiece (Fig. 2G).

Discussion

The spermatozoon of *A. apus* is typical of non-passerines in many respects. Features shared with palaeognaths and the Galloanserae (e.g. rooster and duck) are the conical acrosome, shorter than the nucleus; presence of a proximal centriole (excepting perhaps guinea fowl, Thurston *et al.* 1982; Thurston and Hess 1987) as well as a distal centriole; the elongate midpiece with mitochondria grouped around an elongate distal centriole; and presence of a fibrous or

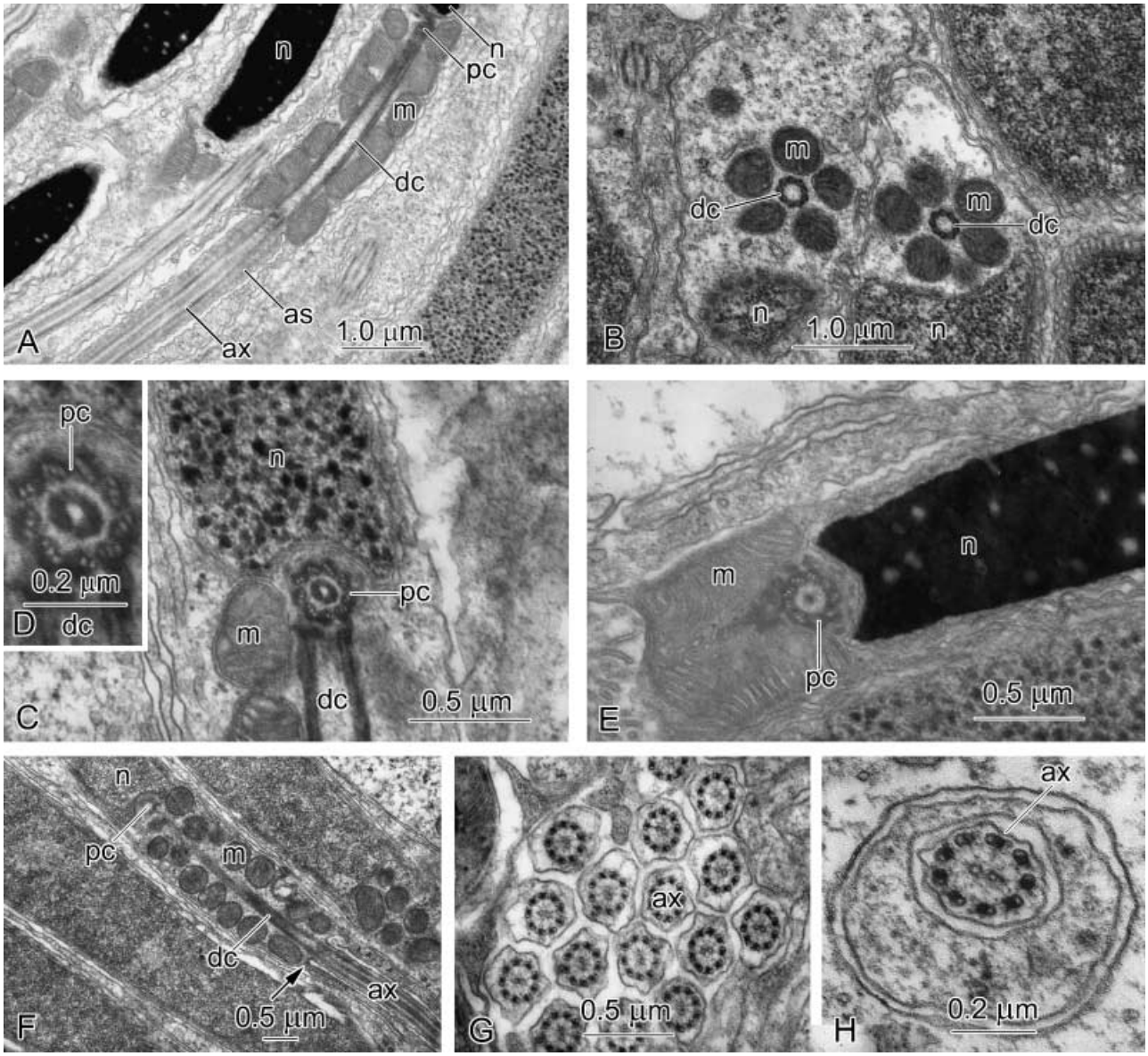

Fig. 2—*Apus apus*, common swift; transmission electron micrographs of spermatids. —**A**. Longitudinal section (LS) of an advanced spermatid through the entire length of the midpiece and through the principal piece. —**B**. Transverse sections (TS) through two midpieces. —**C, D**. TS proximal centriole and adjacent midpiece, (C) being detail of (D), showing the central structure of unknown significance. —**E**. TS proximal centriole and adjacent nucleus and midpiece. —**F**. LS of the midpiece of a younger spermatid in which mitochondria are subspherical. Arrow indicates absence of an annulus. —**G**. TS axonemes which appear to be transitional between the principal piece, possessing an amorphous sheath, and endpiece, lacking this. —**H**. TS endpiece still surrounded by a transient cytoplasmic canal and cytoplasmic sheath.
as = amorphous sheath; ax = axoneme; dc = distal centriole; m = mitochondrion; n = nucleus; pc = proximal centriole.

amorphous sheath around the axoneme. Most of these features characterize non-passerines in general. In addition a perforatorium and endonuclear canal are typically present in palaeognaths and the Galloanserae and are basic to non-passerines. The perforatorium and the endonuclear canal are lost, as in *A. apus* (not examined in *A. melba*), in the emu, *Dromaius novahollandiae* (Baccetti *et al.* 1991), Piciformes (?) (Henley *et al.* 1978), Ciconiiformes (*Jacana*, Saita *et al.* 1983) and Columbiformes (Jamieson *et al.* 1995, Mattei *et al.* 1972; Jamieson 1999, 2006), as also in the Passeriformes. The distal centriole in *Apus* is longer than the 1.8 µm recorded for *Gallus*, 1.3 µm for *Tragopan*, and 2 µm for *Anas* and resembles that of palaeognaths in extending for the complete length of the midpiece. All non-passerines differ from

palaeognaths in that the latter have a transversely ribbed fibrous sheath whereas in non-passerines it is amorphous, as in *Apus*, or absent.

In lacking an appreciable annulus, which in reptiles terminates the midpiece, the spermatozoa of the swift, like those of Psittaciformes (Jamieson 1999; Jamieson *et al.* 1995), Gruiformes (*Grus vipio*, Phillips *et al.* 1987) and passerines (e.g. Asa & Phillips 1987; Jamieson 1999) 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 Ciconiiformes 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 swift spermatozoa, but in view of other differences it must be considered homoplastic relative to other orders lacking the annulus. Despite absence of the annulus, the midpiece–tail junction in the spermatozoa of swifts and parrots is clearly demarcated, because at this point there is a rather abrupt narrowing of the spermatozoon.

The long distal centriole is a remarkably plesiomorphic feature of the swift spermatozoon, being seen only in palaeognaths, as it is somewhat shortened even in the Galloanserae. Palaeognaths differ, however, in penetration of the distal centriole by the two central axonemal singlets (Phillips and Asa 1989; Baccetti *et al.* 1991; Soley 1993, 1999), though these reach only about halfway into the centriole in tinamou (see Fig. 1 of Asa and Phillips 1987). As shown above, many other basic features of avian spermatozoa are also retained by *Apus*. On the other hand, loss of the perforatorium is a notable apomorphic departure from the palaeognaths and Galloanserae.

The midpiece-length distal centriole of *A. apus*, unless a reversal, could be considered to place the apodids very basally in the avian phylogenetic tree but the loss of the perforatorium is derived relative to the Galloanserae. The long centriole of *A. apus*, if not a reversal, would be inconsistent with placement of the Apodiformes above the Psittaciformes from DNA–DNA hybridization by Sibley and Ahlquist (1990), despite the general agreement of sperm ultrastructure with their system. The *Apus* spermatozoa appear closest ultrastructurally to those of the Piciformes and Cuculiformes, both little evolved relative to the Galloanserae. However, the spermatozoa of the woodpecker, *Melanerpes* (Henley *et al.* 1978) is undescribed with regard to the perforatorium, centrioles and annulus. The spermatozoon of *Cuculus canorus*, the European cuckoo, illustrated by Ballowitz (1888) (his Fig. 110) is straight, non-helical, with a pointed acrosome much shorter than the stout cylindrical nucleus, a midpiece shorter than the nucleus, and a long free flagellum. In *Crotophaga ani*, the smooth-billed ani (Saita *et al.* 1982; Tripepi and Jamieson, unpublished results), it has been further shown that a perforatorium and subacrosomal space are absent; the short, conical acrosome rests in a shallow concavity of the tip of the long cylindrical nucleus and the midpiece has five mitochondria encircling the axoneme. An amorphous sheath is present.

In contrast with the spermatozoa of palaeognaths and non-passerines, passerine spermatozoa have the acrosome longer than the nucleus; lack a proximal centriole; have a short distal centriole; a single mitochondrial strand along the anterior region (Corvida) or wound for a great distance along the axoneme (Passerida); and lack the fibrous, or amorphous, sheath. Passerines differ further from non-passerines in possessing, in the spermatid, an 'helical membrane', consisting of multiple microtubules forming a thick strand helically coiled around at least the flagellum (e.g. Asa and Phillips 1987; Jamieson 2006). In contrast, in *A. apus*, microtubules in the spermatid are restricted to a transient single row encircling the cell, though longitudinal microtubules are also present in the Sertoli cell which invests the spermatid, as also seen in *A. melba* (Tripepi *et al.* 1991).

The form of the spermatozoon fully justifies the late nineteenth century removal of swifts from the passerine family Hirundinidae, the spermatozoa of which have been described optically by Ballowitz (1888) and McFarlane (1963). Hirundinidae have typical passerine sperm morphology.

Acknowledgements

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