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Placentation in mammals once grouped as insectivores

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ABSTRACT Interest in insectivoran grade mammals has been reawakened by taxonomic changes that place tenrecs and golden moles in a new order and separate hedgehogs from moles, shrews and solenodons. This survey of their placentation shows there is great variation even within families. As an example three subfamilies of tenrec have been examined. The interhemal region is cellular hemomonochorial in *Echinops* and *Microgale* but endotheliochorial in *Micropotamogale*. Golden moles, which are placed in the same order, have hemodichorial placentation. Many insectivores have complex arrangements for histotrophic nutrition involving columnar trophoblast cells. These range from areolae in moles through complexly folded hemophagous regions in tenrecs to the trophoblastic annulus in shrews. Of these placental characters, few offer support to current phylogenies. However, the case for placing hedgehogs and gymnures in a separate order (Erinaceomorpha) is bolstered by the presence of interstitial implantation, amniogenesis by cavitation, a hemochorial barrier and a prominent spongy zone; these features do not occur in shrews, moles or solenodons (Soricomorpha). Three insectivoran grade mammals deserve close attention as they have been selected for genome sequencing. One of these, the European hedgehog *(Erinaceus europaeus)*, has not been studied with current methodology and renewed investigation of this or the closely related genus *Atelerix* should be a priority.

KEY WORDS: *fetal membrane, golden mole, hedgehog, mole, phylogeny, placenta, shrew, solenodon, tenrec*

Introduction

Mammals appear in the fossil record at around the same time as dinosaurs (Kielan-Jaworowska *et al.*, 2004) and the earliest fossils of marsupial and placental mammals date from the Early Cretaceous of China (Ji *et al.*, 2002; Luo *et al.*, 2003). Early mammals were tiny creatures and their teeth, often all that remains, indicate an insectivorous diet. These traits are shared by a number of living mammals that accordingly were lumped together in a single order. After some initial pruning this order was given the name Lipotyphla (Simpson, 1945). It was early recognized that these insectivores had many "primitive" characters. Hubrecht (1889) chose them deliberately as the mammals most likely to preserve primitive characters of placentation. His lead was followed by other embryologists (Fig. 1). Later attention waned with relatively few studies of insectivore placentation appearing in the last half of the twentieth century.

Interest in insectivoran grade mammals has been rekindled because of the findings of molecular phylogenetics. These necessitated a complete reorganization of the group once it was recognized that the golden moles and the Malagasy tenrecs belonged to a separate lineage (Stanhope *et al.*, 1998). They were placed in their own order Afrosoricida as part of Afrotheria (Fig. 2). Afrotheria is one of four superordinal clades of mammals and includes elephants, sea cows, hyraxes, aardvarks and elephant shrews. Because these relationships had never before been suggested, it stimulated a search for morphological characters that might support such a diverse assemblage (Seiffert, 2007), including our own studies of placentation in tenrecs.

The other insectivores remained in a truncated order that was seen to belong to another superordinal clade, Laurasiatheria (Murphy *et al.*, 2001). However, the molecular data soon confirmed, as long had been suspected, that this was a heterogeneous assemblage. The most recent review of mammalian taxonomy (Fig. 2) separates out the hedgehogs and their allies in Erinaceomorpha and puts shrews, moles and solenodons in Soricomorpha. (Hutterer, 2005 a,b)

A review of placentation in insectivoran grade mammals is long

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overdue and will complement other reviews of the reproductive biology of these creatures (Bedford *et al.*, 2004; Symonds, 2005). We here summarize our studies of tenrecs and golden moles and review what is known about the other families. The emphasis is on the definitive placenta including the interhemal barrier and some fascinating arrangements for hemotrophic nutrition. Details of earlier development are given where appropriate and summarized in synoptic Tables. In the course of this project we examined Hubrecht's original material on the European hedgehog and common shrew as well as Mossman's collection of American shrews and moles.

As they did a century ago several of these species figure prominently in current attempts to further our understanding of genetics and phylogeny through comparative genomics (Springer and Murphy, 2007). Three insectivores are among the 16 mammals sequenced as part of the Mammalian Genome Project: the lesser hedgehog tenrec (*Echinops telfairi*), common shrew (*Sorex araneus*) and European hedgehog (*Erinaceus europaeus*). The full exploitation of such powerful data bases presupposes that we have a full understanding of the corresponding phenotypes including their anatomy, physiology and immunology. For that reason our aim has been not only to summarize what we know about placentation in insectivores but to highlight what still needs to be done.

Early development

Events leading to establishment of the placenta are summarized in Tables 1 and 2. Implantation is superficial in most families but secondarily interstitial in the hedgehog *Erinaceus europaeus* (Hubrecht, 1889) and the moonrat *Echinosorex gymnura* (Hubrecht 1898). In the crocidurine shrews, exemplified by *Suncus murinus*, implantation occurs within a pocket of endometrium or implantation chamber (Sansom, 1937). The lips of the chamber then close over to form a decidua capsularis. This is perforated later in development and becomes an annular fold. Vestiges of the decidua capsularis have been identified in soricine shrews (Brambell and Perry, 1945). Implantation has not been observed

TABLE 1

FETAL MEMBRANES AND PLACENTATION OF INSECTIVORAN GRADE MAMMALS CURRENTLY ASSIGNED TO ORDERS ERINACEOMORPHA AND SORICOMORPHA[1]

Family (subfamily)	Erinaceidae	Soricidae (Soricinae)	Soricidae: (Crocidurinae)	Talpidae	Solenodon-tidae
Species	*Erinaceus europaeus*	*Sorex araneus*	*Suncus murinus*	*Talpa europaea*	*Solenodon paradoxus*
Implantation	Interstitial	Superficial	Superficial but with decidua capsularis	Superficial	Unknown but with decidua capsularis
Amniogenesis	Cavitation	Folding	Folding	Folding	Unknown
Bilaminar omphalopleure	Persists to term	Persists to term	Persists to term	Persists to term	Small fragment persists to term
Choriovitelline placenta	Temporary	Temporary	Temporary	Temporary	Temporary
Visceral yolk sac	Permanent; incomplete inversion	Permanent; incomplete inversion	Permanent; incomplete inversion	Permanent; incomplete inversion	Permanent; complete inversion
Trophoblastic annulus and curtain	Absent	Present	Present	Absent	Absent
Allantoic sac	Medium	Stalk only	Stalk only	Medium to large	Stalk only
Placental shape	Discoid	Discoid	Discoid	Discoid or zonary	Discoid
Pattern	Labyrinthine	Labyrinthine	Labyrinthine	Labyrinthine or villous	Labyrinthine
Interhemal membrane	Hemo-chorial	Endothelio-chorial	Endothelio-chorial	Endothelio-chorial	Hemo-chorial
Trophospongium	Present	Absent	Absent	Absent	Absent
Trophoblastic sheath	Absent	Absent	Present	Absent	Present
Areolae	Absent	Absent	Absent	Present	Absent

[1]Some authorities place these families in a single order Eulipotyphla

TABLE 2

FETAL MEMBRANES AND PLACENTATION OF INSECTIVORAN GRADE MAMMALS CURRENTLY ASSIGNED TO ORDER AFROSORICIDA

Family (subfamily)	Tenrecidae (Tenrecinae)	Tenrecidae (Oryzorictinae)	Tenrecidae (Potamogalinae)	Chrysochloridae
Species	*Echinops telfairi*	*Microgale* spp.	*Micropotamogale lammottei*	*Amblysomus hottentotus*
Implantation	Superficial	Superficial	Superficial	Superficial
Amniogenesis	Cavitation	Not known	Not known	Folding
Bilaminar omphalopleure	Blastocyst only	Temporary	Not known	Blastocyst only
Choriovitelline placenta	Temporary	Temporary	Temporary	Temporary
Visceral yolk sac	Permanent; incomplete inversion	Permanent; incomplete inversion	Permanent; incomplete inversion	Permanent; incomplete inversion
Allantoic sac	Large	Large	Large	Medium
Placental shape	Discoid	Discoid	Discoid	Discoid
Pattern	Labyrinthine	Villous early but labyrinthine later	Labyrinthine	Labyrinthine
Interhemal membrane	Hemochorial	Hemochorial	Endothelio-chorial	Hemochorial
Trophospongium	Present	Absent	Absent	Present
Areolae	Present in *Tenrec ecaudatus*	Absent	Absent	Absent
Hemophagous region	Folded, saccular central structure	Unfolded part of paraplacenta	Folded, saccular central structure	Absent

in *Solenodon* but the mid-gestation placenta is flanked by what Wislocki (1940) interpreted as the remains of a decidua capsularis.

In all these families a choriovitelline placenta is formed initially, but the yolk sac is displaced from the trophoblast adjacent to the uterine wall upon expansion of the exocelom and allantois. A partially inverted yolk sac remains to term. In the golden mole *Eremitalpa granti* it has been thought that a choriovitelline placenta is retained throughout gestation (Mossman, 1987) but this is not the case in *Amblysomus hottentotus* (Jones *et al.,* 2009).

Chorioallantoic placenta

In most insectivores the definitive placenta is sharply circumscribed and roughly discoidal in shape. The overall structure of the exchange area is labyrinthine. This pattern is broken in one subfamily of moles (Scalopinae) where the placenta of several genera is more zonary than discoidal and perhaps villous, as first described for the Eastern mole *Scalopus aquaticus* (Mossman, 1939). Some tenrecs have quite an extensive paraplacenta. In *Potamogale velox* this is probably important in gaseous exchange since the disc functions largely as a hemophagous organ (Carter

et al., 2006). There is a medium to large allantoic sac in hedgehogs, moles, tenrecs and golden moles. However, the allantoic cavity is absent in shrews and in *Solenodon paradoxus*; it is replaced by an allantoic stalk as in higher primates.

As shown below most insectivoran grade mammals have endotheliochorial or hemochorial placentas. In addition to exchange across the interhemal barrier, the fetus may acquire nutrients by taking up uterine gland secretions, tissue debris or maternal blood. This is referred to as histotrophic nutrition. Several insectivore placentas have prominent structures devoted to this purpose. They all are based on a type of columnar trophoblast that has a widespread distribution among mammals (Enders and Carter, 2006). This trophoblast is highly polarized and has the necessary cellular apparatus for endocytosis and lysis of nutrients.

Placentation in shrews

The three subfamilies of shrew are Crocidurinae, Myosoricinae and Soricinae (Hutterer 2005b). Placentation in crocidurine shrews is best known from *Suncus murinus*. *Crocidura* is the most

Fig. 1 (Left). Pioneering research on insectivore placentation. (A) *An areola from the placenta of the European mole (*Talpa europaea*). The uterine gland (UG) is capped by columnar trophoblast (CTR). From Strahl (1892).* **(B)** *The trophoblastic curtain (TC) and trophoblasic annulus (TA) of the common shrew (*Sorex araneus*). Erythrocyte ingestion by the columnar trophoblast of the annulus is clearly seen. From Hubrecht (1894).*
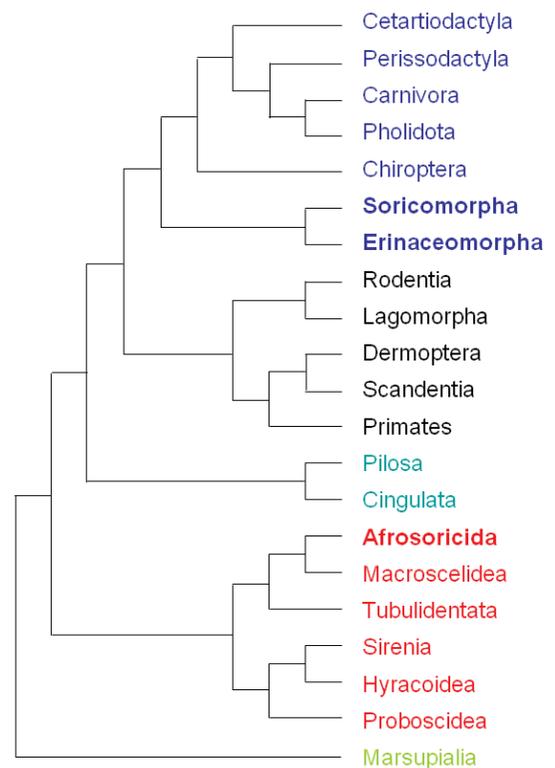
Fig. 2 (Right). Phylogeny of placental mammals. *The twenty extant orders fall into four superordinal clades named Laurasiatheria (blue), Euarchontoglires (black), Xenarthra (sky blue) and Afrotheria (red). This tree is based on an analysis of nucleotide sequences using marsupials (lime green) as an outgroup to root the tree (Murphy* et al., *2001). Insectivores are in bold type. Until quite recently the tenrecs and golden moles (Afrosoricida) were placed in the same order as hedgehogs and gymnures (Erinaceomorpha) with shrews, moles and solenodons (Soricomorpha).*

speciose genus of all mammals yet only summary accounts of its placenta are available (Starck, 1959). Nothing is known of the placenta in Myosoricinae. There are extensive studies of the soricine genera *Sorex*, *Suncus* and *Blarina*.

Interhemal barrier

In shrews, the interhemal barrier of the labyrinth has been the object of much confusion and controversy. The tissue surrounding the maternal blood spaces is a modified endothelium (Wimsatt and Wislocki, 1947), but was mistaken for syncytial trophoblast by earlier authors (Hubrecht, 1894; Sansom, 1937; Brambell and Perry, 1945). However the placenta is endotheliochorial rather than hemochorial. Owers (1960) went further and stated that the trophoblast was lost early in gestation and did not form part of the interhemal membrane in *Suncus murinus*. He proposed the term endothelio-endothelial to describe this condition. The controversy was resolved when Wimsatt *et al.* (1973) examined the placentas of *Blarina brevicauda* and *Sorex cinereus* by TEM (Fig. 3). They found that there is a continuous syncytial lamina. However, it has a unique organization, being honeycombed with interstitial spaces that open freely on both surfaces, giving the whole a sieve-like appearance. The cytoplasm is spread so thin that in places there is no room for granular endoplasmic reticulum or other organelles. It does appear as a continuous layer in semi-thin sections, but cannot so be distinguished in paraffin sections. In addition to the modified maternal epithelium and extraordinary trophoblast layer, the interhemal region of shrews features fetal endothelium without a basement membrane, but with unusual basal processes, and fetal mesenchymal cells that have hypertrophied and undergone epithelioid differentiation (Wimsatt *et al.*, 1973). Kiso *et al.* (1990) re-examined the interhemal barrier
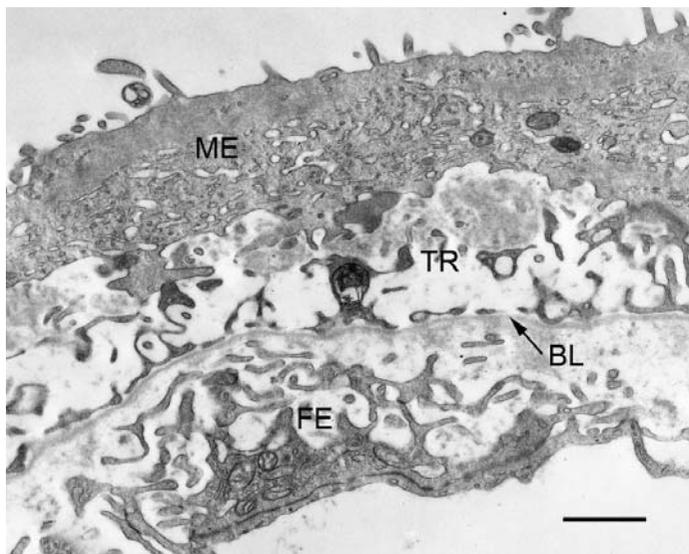

Fig. 3. Interhemal region of the endotheliochorial placenta of the Northern short-tailed shrew (*Blarina brevicauda*). *The maternal endothelial cells (ME) display microvilli on the luminal surface. The basal lamina beneath them is broad and irregular. The trophoblast layer (TR) is attenuated due to apical and basal invaginations but has a continuous basal lamina (BL). There are irregular projections from the fetal endothelial cells (FE). Scale bar 1.1 μm.*

in dated pregnancies of *S. murinus*. They found the structure described by Wimsatt *et al.* (1973) up to day 24 of gestation, but in the last week of gestation the syncytiotrophoblast became discontinuous.

Histotrophic nutrition

A quite unique arrangement for histotrophic nutrition is found in shrews. Cell debris and maternal erythrocytes are found in the space between the trophoblastic curtain and annulus (Fig. 1B, 4 A-B). This material is released by the actions of the trophoblast of the curtain and taken up by the trophoblast of the annulus. Erythrocytes in various stages of degeneration can be demonstrated in the columnar trophoblast cells (Fig. 4C; King *et al.*, 1978). The breakdown products apparently cross Reichert's membrane and the endoderm lining the annulus. They are then taken up by the endoderm of the visceral yolk sac. These cells contain pigment granules that are responsible for the characteristic green appearance of the fetal membranes in shrews. It has been shown that most of the pigment is bilirubin, an iron-free breakdown product of hemoglobin (Wislocki and Wimsatt, 1947). The granules stain for iron, too, indicating that hemosiderin is present (Wislocki and Wimsatt, 1947). In addition, the presence of glycogen has been demonstrated cytochemically and by TEM (Wislocki and Wimsatt, 1947; King *et al.*, 1978). No doubt these materials are acquired by endocytosis, since the cytoplasm beneath the apical membrane contains caveolae and a variety of vesicles and tubules. The connective tissue compartment of the visceral yolk sac contains the vitelline blood vessels and hematopoietic foci. Thus acquirement of iron by endocytosis of maternal erythrocytes is spatially linked to fetal hematopoiesis. Unusually for mammals, in *Suncus murinus* yolk sac hematopoiesis continues to term (Sansom, 1937).

Trophoblastic sheath

A conspicuous feature of the crociduran placenta is the "trophoblastic sheath" that invests the allantoic vessels at the site of their attachment to the placenta. Described by Sansom (1937) for *S. murinus*, it clearly can be seen in *Crocidura russula* (Starck, 1959; Fig. 5A). The two umbilical arteries unite to form a single vessel that passes directly through the sheath. However, blood returns to the umbilical vein by way of numerous vessels that ramify in the meshwork of the trophoblastic sheath. The source of the trophoblast forming the sheath is open to discussion. Wimsatt and Wislocki (1947) make a compelling case for homology between the trophoblastic sheath of Crocidurinae and nests of cytotrophoblast found at the base of the allantoic villi in Soricinae. They argue that Sansom (1937) failed to provide direct evidence for his assumption that the sheath was derived from binucleate trophoblast cells. A third interpretation was offered by Owers (1960) who believed that the structure was derived from proliferating uterine epithelium.

Placentation in solenodons

Solenodons are a relict confined to the Greater Antilles. Both extant species are endangered and we are fortunate in having a description of the definitive placenta by Wislocki (1940). He found that *Solenodon paradoxus* has a typical hemochorial
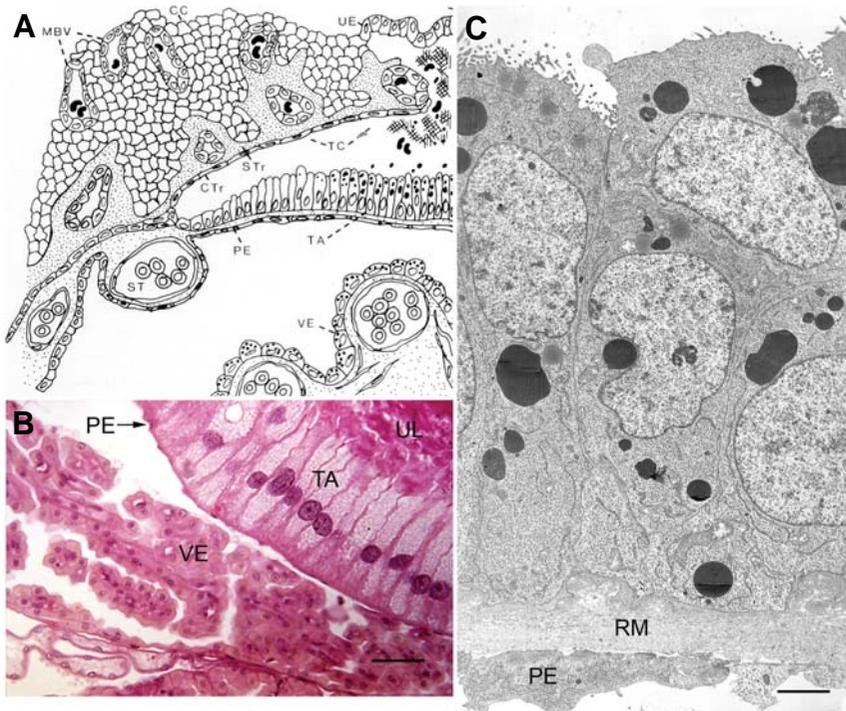
Fig. 4. Histiotrophic nutrition in the placenta of a shrew (*B. brevicauda*). (A) *Diagram showing the relation between the uterine trophoblastic curtain (TC) and the trophoblastic annulus of the yolk sac. Syncytiotrophoblast (S Tr) surrounds maternal capillaries (MBV) and has an outer covering of cytotrophoblast (C Tr). In places this is lost and masses of syncytium, cellular debris and maternal erythrocytes are sloughed off into the lumen. This material is phagocytosed by the tall columnar cells of the trophoblastic annulus (TA). This columnar trophoblast is separated from the parietal endodermal cells (PE) by Reichert's membrane. The visceral endodermal cells (VE) border the yolk sac cavity and contain granules of green pigment. From King* et al. *(1978). Copyright © 1978. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.* **(B)** *Trophoblastic annulus and visceral yolk sac in* Crocidura *sp. UL is uterine lumen with debris; other labels as in panel (A). Courtesy of the Harland W. Mossman Collection, University of Wisconsin Zoological Museum.* **(C)** *Columnar trophoblast cells of the trophoblastic annulus in* B. brevicauda. *The apical membrane facing the uterine lumen has microvilli. The lateral borders are closely apposed. Within the cytoplasm are phagocytosed erythrocytes or erythrocyte fragments. Beneath the tropho-blast is Reichert's membrane (RM). Part of a parietal endodermal cell (PE) is seen. Reproduced from King* et al. *(1978). Copyright © 1978. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Scale bars 37 (B), 2.85 (C) µm.*

labyrinth. On the fetal side there is a core of fetal capillaries and mesenchyme clothed by a delicate sheath of syncytiotrophoblast.

A trophoblastic sheath surrounds the principal allantoic vessels of *Solenodon* (Fig. 5B). It is made up of cords of trophoblast enclosing lacunar spaces. The apparent similarity to crociduran shrews is striking, the more so since nothing approaching these structures is known from any other mammal. According to Wislocki (1940), however, the spaces communicate with maternal blood lacunae in the labyrinth. The small amount of blood contained within them is maternal in origin and occurs together with granular debris. The fetal venous channels seen in the trophoblastic sheath of crociduran shrews are absent.

Two other interesting features in S. paradoxus (Fig. 5B) are the decidua capsularis and differentiation of the visceral yolk sac above the placenta into a series of richly branching villi that likely function to absorb histiotrophe (Wislocki, 1940). Whilst unusual among insectivores, elongated villi are seen in hystricomorph rodents such as the agouti (*Dasyprocta leporina*; Miglino *et al.*, 2002).

Placentation in moles

Moles are fossorial or subfossorial mammals of which three subfamilies cur-

rently are recognized (Hutterer, 2005b). Scalopinae includes three genera with a diffuse placenta and *Condylura* with a more discoid placenta (Mossman, 1989). Talpinae includes moles and desmans. Nothing is known about placentation in the Asiatic shrew moles, subfamily Uropsilinae.

Interhemal barrier

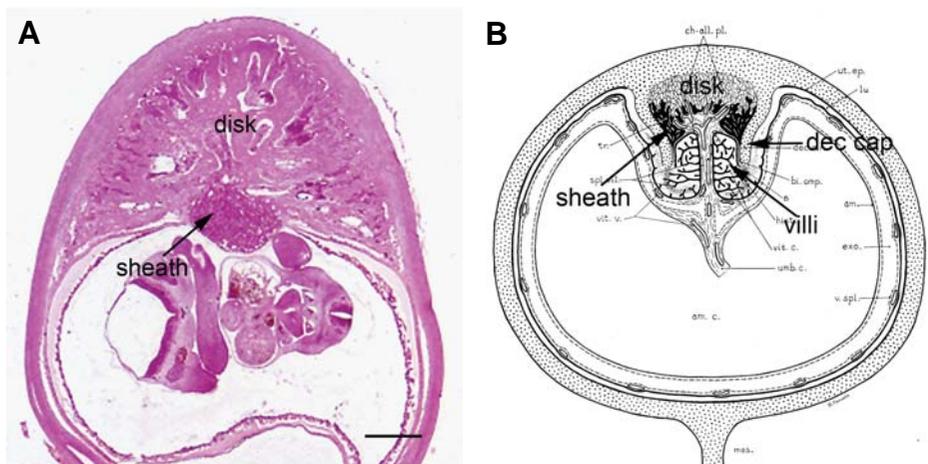The interhemal barrier of the European mole *Talpa europaea*

Fig. 5. The trophoblastic sheath of shrew and solenodon placentas. (A) *In the Asian house shrew* (Suncus murinus) *the sheath is seen at the fetal surface of the placental disk. Courtesy of the Harland W. Mossman Collection, University of Wisconsin Zoological Museum.* **(B)** *In the Hispaniolan solenodon (*Solenodon paradoxus*) a remarkably similar sheath appears in the same position. Note also the presence of a decidua capsularis (dec cap) and the elaborately branched villi of the visceral yolk sac (villi). From Wislocki (1940). Copyright © 1940. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Scale bar, 317 µm.*
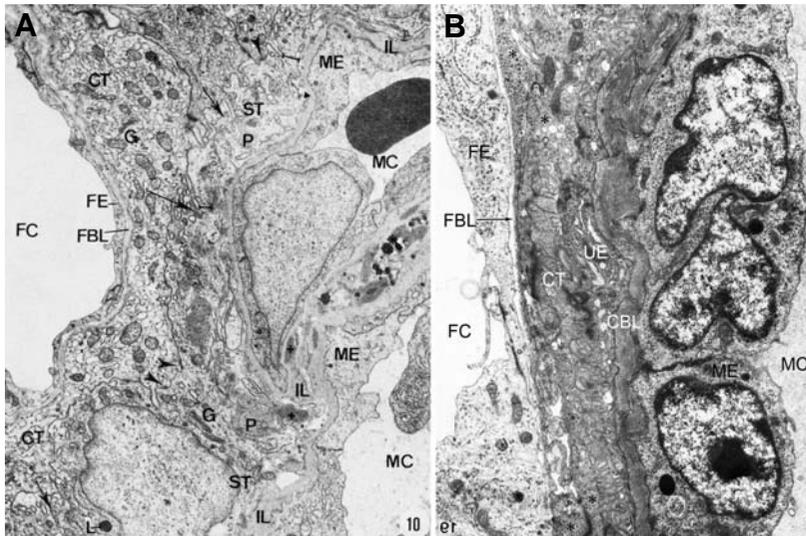
Fig. 6. The interhemal region of the placenta in moles. (A) *The placenta of the European mole as interpreted by Malassiné & Leiser is endotheliochorial. MC, maternal capillary; ME, maternal endothelium; IL, interstitial layer; ST, syncytiotrophoblast; CT, cytotrophoblast; FBL, fetal basal lamina; FE, fetal endothelium; FC, fetal capillary. From Malassiné & Leiser (1984) copyright © 1984 with permission from Elsevier.* **(B)** *The placenta of the Eastern mole as interpreted by Prasad* et al. *is epitheliochorial. UE, uterine epithelium; CBL, complex layer composed of basal laminae of endothelium and uterine epithelium; other labels as above. Adapted from Prasad* et al. *(1979). Copyright © 1979. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.*

is endotheliochorial (Malassiné and Leiser, 1984; Fig. 6A). As frequently is the case in placentas of this type, the maternal endothelial cells are enlarged. They rest on a thick interstitial membrane. Beneath this are two layers of trophoblast, the outer syncytial and the inner cellular, then the fetal capillary endothelium with its basal lamina. The two layers of trophoblast are connected by interdigitation of their membranes as well as by desmosomes and gap junctions.

Mossman (1939) declared that the placenta of the Eastern mole, *Scalopus aquaticus*, was epitheliochorial. His graduate student later found that the uterine epithelium disappeared during establishment of the yolk sac placenta, only to reappear in the chorioallantoic placenta (Prasad, 1958; see Carter, 2005). Prasad *et al*. (1979) established by TEM that the placenta was epitheliochorial at the single stage available for study (Fig. 6B). It will be seen that the number of layers in the interhemal membrane of *Scalopus* is the same as in *Talpa*. In both the maternal endothelium is underlain by a thick interstitial membrane. According to Prasad *et al*. (1979), in *Scalopus* this is formed by fusion of

the basal laminae of the endothelium and the uterine epithelium. The layer identified as epithelium has widely scattered nuclei and the intervening regions are very thin. No cell junctions can be seen, so this layer is syncytial. Beyond it is a more substantial layer of cytotrophoblast. There is interdigitation between microvilli on the trophoblast cells and processes from the syncytial layer. Desmosomes occur at the junction between the two layers. It will be seen that the characteristics of the putative epithelial layer in *Scalopus* match those of the syncytiotrophoblast in *Talpa*. The final component of the interhemal membrane is the fetal capillary endothelium.

Histotrophic nutrition

The placental areolae of moles appear at the stage of yolk sac placentation and are retained through the chorioallantoic stage, as first described by Strahl (1892; Fig. 1A). The uterine glands persist in the region of the placenta and a dome-like structure of columnar trophoblast covers the opening of each gland. A PAS-positive substance fills the lumen of the glands and can be found within the trophoblast cells (Morris, 1958; Prasad *et al*., 1979). There is some extravasation of blood and erythrocytes are taken up by the trophoblast in *Talpa* although not in *Scalopus*. The glandular epithelium immediately below the areolae shows signs of differentiation, but the epithelium in the neck of the gland appears to be trophoblast-resistant (Malassiné and Leiser, 1984).
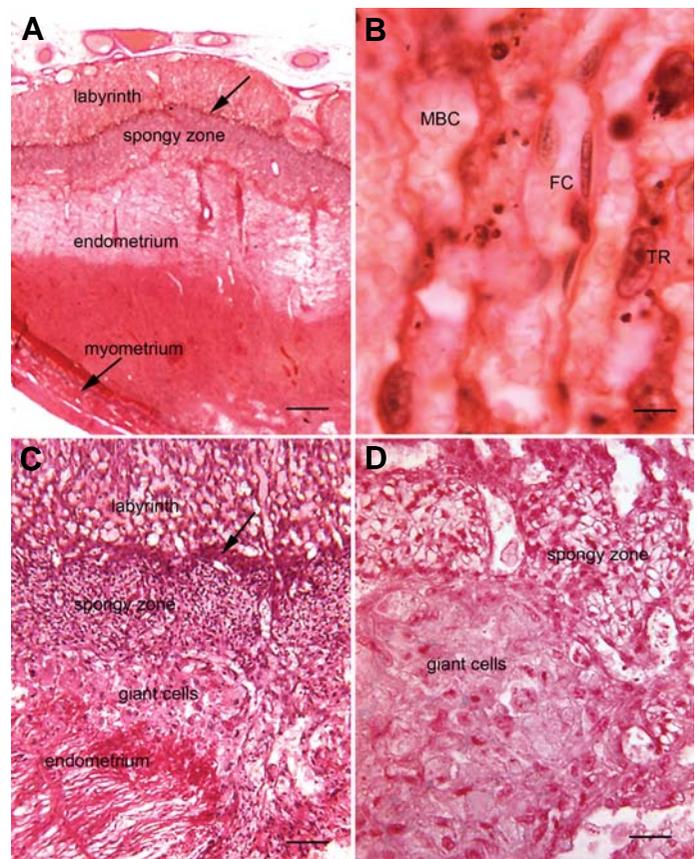

Fig. 7. Placentation in the Southern African hedgehog *(Atelerix frontalis)*. (A) *Overview of a midgestation placenta. The arrow indicates a layer of small cells situated between the labyrinth and spongy zone.* **(B)** *Detail of the labyrinth showing maternal blood channels (MBC), fetal capillaries (FC) and trophoblast (TR).* **(C)** *The giant cell layer is interposed between the spongy zone and the unmodified connective tissue of the endometrium. Arrow as in panel (A).* **(D)** *Note that most cells in the spongy zone are vacuolated and there is a large mass of giant cells beneath them. (A-D) Courtesy of the Harland W. Mossman Collection, University of Wisconsin Zoological Museum. Scale bars 800 (A), 13 (B), 88 (C) and 35 (D) μm.*

In *Scalopus*, too, the glandular epithelium below the areolae breaks down and the resultant debris is phago-cytosed by the areolar trophoblast. In the hairy-tailed mole *Parascalops breweri* a striking feature is highly altered glandular cells with large nuclei indicative of polyploidy that sometimes form multinucleate masses (Enders and Carter, 2006).

Placentation in hedgehogs and gymnures

Hedgehogs (Erinaceinae) are instantly recogniz-able by their spiny pelage. Gymnures or moonrats (Galericinae) have coarse hair and outwardly resemble opossums. There is a growing consensus that hedge-hogs and gymnures should be placed in a separate order Erinaceomorpha (Hutterer, 2005a). Because there are no recent studies of placentation in these animals, our account relies heavily on the paper by Hubrecht (1889). We have examined the original slides and, in addition, those of the South African hedgehog *Atelerix frontalis* found at the Harland W. Mossman Collection (Fig. 7 A-D).

Although studied only by light microscopy, there is little doubt that the hedgehog placenta is hemochorial. Even before the chorioallantoic placenta has been established, maternal blood is found in lacunae lined by trophoblast (Hubrecht, 1889). Further study is needed to tell whether the trophoblast lining the blood spaces (Fig 7B) is cellular or syncytial. There is an inner layer of small basophilic cells that appears as a dark band in stained sections (Fig. 7 A,C).

A well developed spongy zone sits beneath the labyrinth and this is also present in the moonrat *Echinosorex gymnura* (Meister and Davis, 1953). The major cell type in this region is a highly vacuolated cell (Fig. 7 C-D). In this respect the spongy zone of hedgehogs differs from that found in hedgehog tenrecs (Carter *et al.*, 2004) and golden moles (Gabie, 1960). Mononuclear giant cells form a distinct layer between the spongy zone and en-dometrium of the midgestation hedgehog placenta (Fig. 7 C-D). These cells are likely trophoblastic in origin since they are under-lain by unmodified endometrial connective tissue. Hubrecht (1889) called them "deciduofracts." As a matter of fact he thought even the spongy zone was maternal in origin and coined the reciprocal terms "trophoblast" and "trophospongium" to denote what he regarded as the fetal and maternal components involved in embryonic nutrition. In *Atelerix* the spongy zone cells extend into maternal blood vessels. In *Echinosorex* mononuclear giant cells are found in clusters, some of which occupy and largely occlude maternal venous channels (Meister and Davis, 1953).

Placentation in golden moles

The golden moles are placed in the same order as the tenrecs. A revision of golden mole taxonomy is pending but currently two subfamilies are recognized (Bronner and Jenkins, 2005). Placen-tation has been studied in two species of Chrysochlorinae, *Chrysochloris asiatica* (De Lange, 1919) and *Eremitalpa granti* (van der Horst, 1948; Gabie, 1959, 1960) and one of Amblysominae, *Amblysomus hottentotus* (Jones *et al.*, 2009).
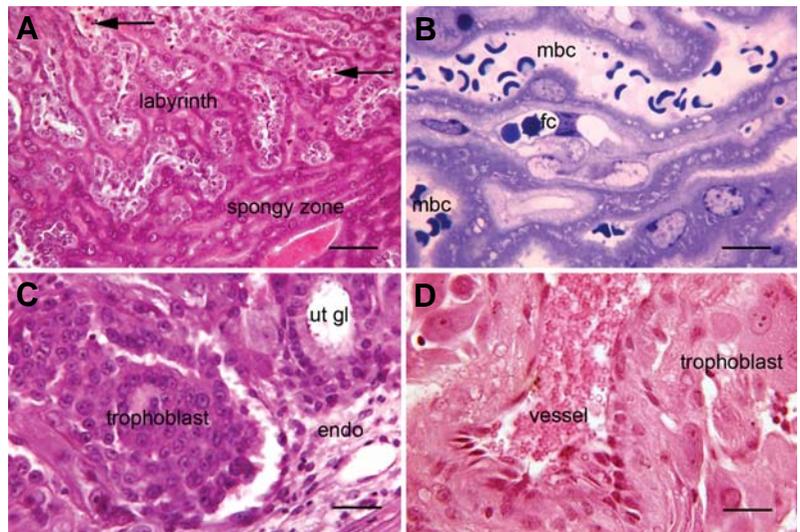

Fig. 8. Placentation in golden moles. (A) *An extensive labyrinth with two layers of trophoblast melds into a spongy zone with maternal blood vessels lined by synctiotrophoblast* (Eremitalpa granti). *The fetal capillaries (arrows) contain nucle-ated erythrocytes. Courtesy of the Harland W. Mossman Collection, University of Wisconsin Zoological Museum.* **(B)** *Interhemal region of placenta of* Amblysomus hottentotus *in late gestation. Fetal capillaries (fc) are separated from maternal blood channels by two layers of syncytiotrophoblast.* **(C)** *Multinucleate masses of tropho-blast in a fetal vessel within the endometrium (endo) at the placental site.* E. granti. *Courtesy of the Harland W. Mossman Collection, University of Wisconsin Zoological Museum.* **(D)** *Large trophoblast cells intrude on a maternal vessel.* A. hottentotus. *Scale bars 88 (A), 13 (B), 33 (C) and 22 (D) μm.*

The placental disc of the golden mole comprises a labyrinth, which makes up two-thirds of the thickness, and a spongy zone (Fig. 8A). Unlike in tenrecs there are no hemophagous regions and no secondary placenta. The interhemal area (Fig 8B) is hemodichorial with two layers of syncytiotrophoblast, the outer-most being continuous with the spongy zone and in contact with the maternal blood. The inner layer, which faces the stroma and fetal blood vessels, is cellular initially but forms a syncytium in later pregnancy, and there is an interdigitating microvillous inter-face between the two layers. The outer trophoblast also has a microvillous apical surface facing the maternal blood.

Beneath the inner trophoblast there is a fine basal lamina and sparse stroma within which lies the fetal capillaries which are small and often difficult to detect; their basal lamina may be continuous in places with that of the inner trophoblast. In the spongy layer, there are maternal vascular spaces edged by the microvillous border of the outer syncytiotrophoblast, but the inner trophoblast and fetal stroma and capillaries do not penetrate this layer.

Masses of syncytial trophoblast occur in the maternal vessels beneath the placenta (Fig. 8C). They were originally described as endothelial in origin (van der Horst, 1948; Gabie, 1959, 1960) but we have examined some of van der Horst's slides and believe this interpretation to be erroneous. Intramural trophoblast is also observed (Fig. 8D).

Placentation in tenrecs and otter shrews

The tenrecs reached Madagascar an estimated 40 million years ago (Douady *et al.*, 2002) and underwent an adaptive
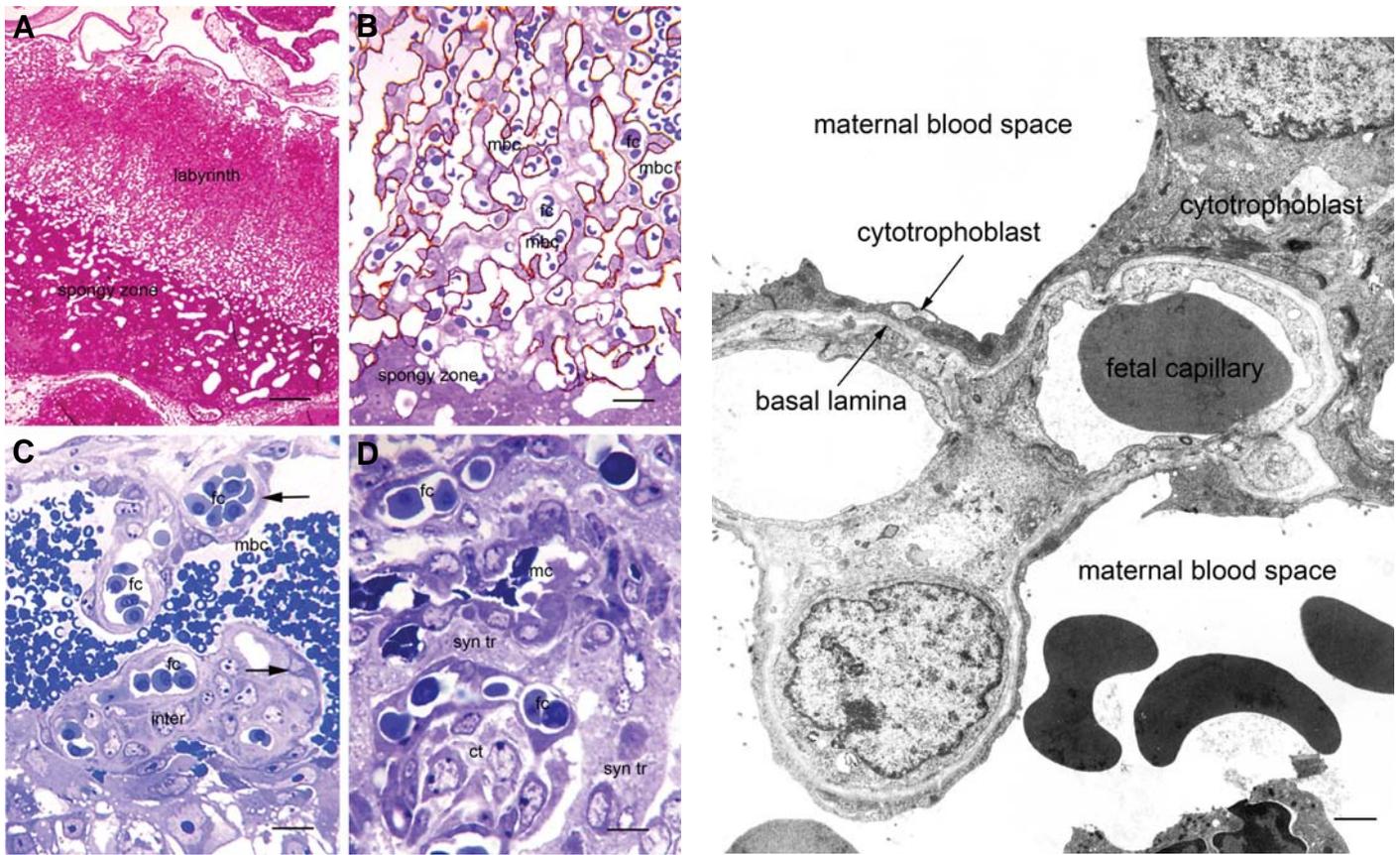
Fig. 9 (Left). Placentation in tenrecs. (A) *In the lesser hedgehog tenrec* (Echinops telfairi) *there is a prominent spongy zone beneath the labyrinth.* **(B)** *The placenta of* E. telfairi *is hemochorial and the maternal blood channels (mbc) are here outlined by staining the trophoblast surface with DBA lectin. The fetal capillaries (fc) do not stain. Courtesy of Dr. Carolyn Jones.* **(C)** *Early in gestation the placenta of the shrew tenrec* (Microgale cowani) *has an extensive villous region, although this later this is incorporated into the labyrinth. Fetal capillaries (fc) contain nucleated erythrocytes and are separated from the maternal blood space (mbc) by a thin layer of trophoblast (arrows). The villi also contain numbers of interstitial cells (inter).* **(D)** *The placenta of the Nimba otter shrew (*Micropotamogale lamottei*) is endotheliochorial. Syncytiotrophoblast (syn tr) is interposed between the fetal capillaries (fc) and the maternal ones (mc). Some fetal connective tissue is present. Reprinted from Carter* et al. *(2006) copyright © 2006 with permission from Elsevier. Scale bars 216 (A), 22 (B), 28 (C) and 9.1 (D) μm.*

Fig. 10 (Right). Interhemal region of the placenta in a lesser hedgehog tenrec *(E. telfairi)*. *Fetal capillaries are situated between two maternal blood spaces. The cytotrophoblast has a robust basal lamina.*

radiation that resulted in three subfamilies. Placentation has been studied in the Tenrecinae and Oryzorictinae but not yet in Geogalinae. The otter shrews of mainland Africa are the closest relatives of the Malagasy tenrecs and constitute a fourth subfamily, Potamogalinae.

Interhemal barrier and spongy zone

There are excellent studies of placentation in *Hemicentetes spinosus* (Goetz, 1937b, 1938a, b), *Setifer setosus* (Strauss, 1943) and *Tenrec ecaudatus* (Goetz 1937a) but we shall confine our remarks on Tenrecinae to *Echinops telfairi* (Carter *et al.*, 2004, 2005). The greater part of the placental disk consists of a labyrinth underlain by a spongy zone (Fig. 9A). The interhemal region (Figs. 9B, 10) is unusual in that the trophoblastic component is a single layer of cytotrophoblast. These trophoblast cells have thick areas especially near the nuclei and extensive thin flanges (Fig. 10). The luminal surface has isolated patches of microvilli, and pinocytotic vesicles are numerous both apically and basally. The

labyrinthine zone ends abruptly at the margins of the placental disk. However, the endoderm and connective tissue of the allantois and a layer of cytotrophoblast extend beyond the placental disk as a paraplacental region.

Placentation in *Microgale* and two other genera of the subfamily Oryzorictinae is different from that in Tenrecinae (Enders *et al.*, 2007). Developmentally a more villous portion of the placental disk forms before the formation of a compact labyrinth (Fig. 9C). Although the definitive placenta is cellular hemomonochorial, it lacks the spongy zone found in the Tenrecinae.

The Nimba otter shrew *Micropotamogale lamottei* has an endotheliochorial labyrinth with syncytial trophoblast enclosing the maternal vessels (Fig. 9D). There is no spongy zone (Carter *et al.*, 2006). The placenta of the giant otter shrew *Potamogale velox* is also endotheliochorial (Carter *et al.*, 2006).

Histotrophic nutrition

Areolae have been observed in the paraplacental region of

Tenrec ecaudatus (Goetz, 1937a), but the hedgehog tenrecs are better known for their prominent hemophagous regions. In *Echinops telfairi* this develops early from polarized trophoblast (Fig 11A) overlying the glands at the centre of the implantation site (Carter *et al.*, 2005). Later the trophoblast becomes highly folded to form a prominent organ at the centre of the placental disc. The primary folds have allantoic endoderm at one surface and columnar cytotrophoblast at the other. These trophoblast cells have numerous lipid droplets and vesicles, and often contain large yellow pigment crystalloids (Fig 11B). There is scant evidence of phagocytosis in later stages of pregnancy, but the crystals of hematoidin impart a yellow hue to the entire structure (Carter *et al.*, 2004). In Oryzorictinae there is an early, simple and lateral rather than central hemophagous region. In the otter shrews, however, there is again a prominent hemophagous region (Enders *et al.*, 2007). In *Potamogale velox*, the central region (Fig. 11C) at first sight appears to be a placental disk (Hill, 1938), yet the juxtafetal portion is clearly a hemophagous region whereas the labyrinth leading to this region is endotheliochorial (Carter *et al.*, 2006).

Implications for systematics

The history of insectivore systematics is admirably reviewed by Symonds (2005). The most recent developments have been removal of tenrecs and golden moles to a separate order Afrosoricida and partition of the remainder between Erinaceomorpha (hedgehogs and gymnures) and Soricomorpha (shrews, moles and solenodons).

There is some support for the Erinaceomorpha-Soricomorpha split from differences in early development and placentation (Table 1). In hedgehogs implantation is interstitial, amniogenesis occurs by cavitation, the interhemal barrier is hemochorial and there is a prominent spongy zone. In shrews and moles implantation is superficial, amniogenesis is by folding, the interhemal barrier is endotheliochorial and a spongy zone is absent. As in analyses of other morphological characters (Asher, 2001), *Solenodon* with its hemochorial placenta does not quite fit.

It is difficult to form a coherent picture of placentation in Afrosoricida (Table 2). There are striking differences even between the subfamilies of tenrecs with the interhemal barrier being endotheliochorial in otter shrews and hemochorial in Tenrecinae and Oryzorictinae. One feature of Afrosoricida that has been highlighted is division of the allantoic sac into four lobes in *Potamogale* and *Chrysochloris*. This is widely accepted as a synapomorphy for Afrotheria (Mess and Carter, 2006, Seiffert, 2007; Asher and Lehmann, 2008). It could equally be the result of homoplasy as has occurred in the evo-

lution of other characters in mammals (Luo, 2007).

Debate on the systematics of insectivoran grade mammals is far from over. In a recent paper, Wible *et al.* (2007) challenged the monophyly of superorders Laurasiatheria and Afrotheria since in their analysis *Potamogale* nested within laurasiatherian insectivores. As Symonds (2005) remarked, with reference to Simpson's (1945) famous pronouncement on Lipotyphla, "The wastebasket may have been emptied, but its contents are currently strewn over the floor." A major challenge in mammalian systematics remains the integration of molecular and morphological data for living and fossil forms (Springer *et al.*, 2004).

What needs to be done?

The wealth of data offered by comparative genomics cannot be exploited without data on the phenotype. Functional studies should focus on the three insectivoran grade mammals selected for whole genome sequencing. As described above, our knowledge of placentation in the tenrec *Echinops telfairi* is fairly complete although it would be nice to have details of events up to and including blastocyst implantation. *Sorex araneus* was the shrew studied by Hubrecht (1894) and there is more recent data on *S. fumeus* (Wimsatt and Wislocki, 1947; Wislocki and Wimsatt, 1947). Our first priority therefore must be to apply modern techniques to the hedgehog *Erinaceus europaeus* as there have been no substantial studies of the genus since Hubrecht (1889). Because *Erinaceus* would need to be trapped in the wild, it might be more feasible to study the African genus *Atelerix* which is available from breeders.

In the context of comparative placentation the formation and nature of the interhemal barrier in moles needs to be revisited. Work is underway on a member of the Talpinae, *Talpa occidentalis* (Siniza and Zeller, 2007). However, the real bone of contention is
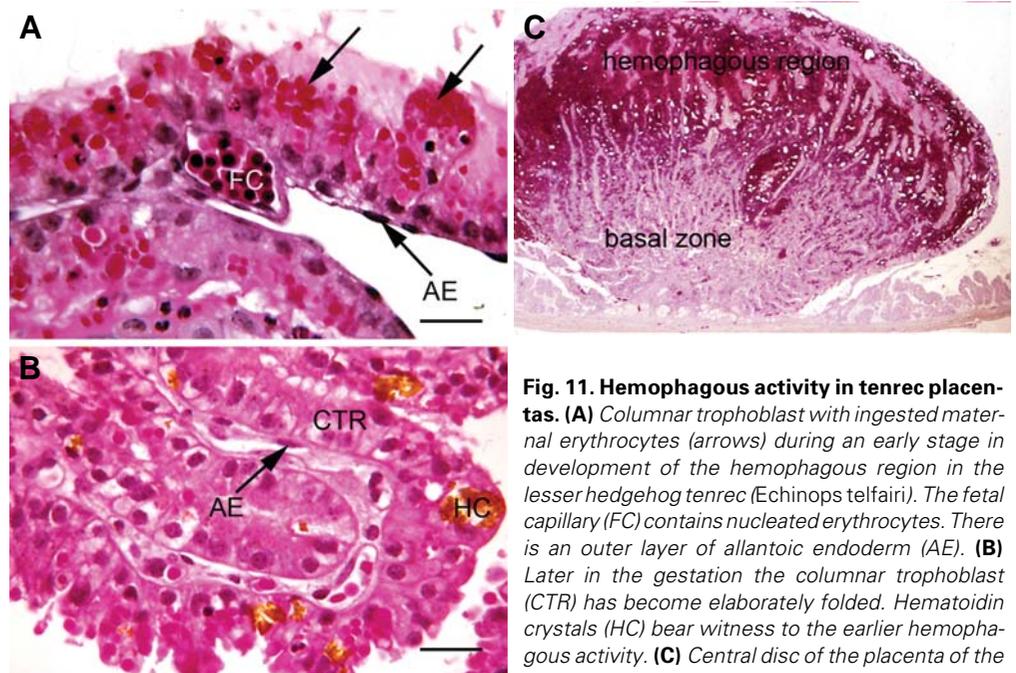
Fig. 11. Hemophagous activity in tenrec placentas. (A) *Columnar trophoblast with ingested maternal erythrocytes (arrows) during an early stage in development of the hemophagous region in the lesser hedgehog tenrec (*Echinops telfairi*). The fetal capillary (FC) contains nucleated erythrocytes. There is an outer layer of allantoic endoderm (AE).* **(B)** *Later in the gestation the columnar trophoblast (CTR) has become elaborately folded. Hematoidin crystals (HC) bear witness to the earlier hemophagous activity.* **(C)** *Central disc of the placenta of the giant otter shrew (*Potamogale velox*). The outer zone forms the hemophagous region whilst the basal zone is part of the exchange area. Reprinted from Carter* et al. *(2006) © 2006 with permission from Elsevier. Scale bars 22 (A-B), 218 (C) µm.*

whether members of Scalopinae have epitheliochorial placentation. At present the evidence for this rests on the interpretation of a single specimen by Prasad *et al.* (1979). What is needed is a developmental series of *Scalopus* or *Scapanus*. An intriguing feature of placentation in *Suncus murinus* is the presence of large numbers of invasive binucleate trophoblast cells (Sansom, 1937). The origin and fate of these cells begs renewed study. Thirdly it would be instructive to study placentation in golden moles at the ultrastructural level.

Whenever new studies are planned it is important to put tissues aside for later genomic research. Tissue reagents are available that stabilize RNA (Wildman, 2008). A bank of placental tissue could prove a resource equal in value to the embryological collections that have been conserved by earlier generations of researchers (Carter, 2008).

Finally we confess to curiosity about placentas that have never before been studied. Would *Geogale* offer yet a fourth variant on tenrec placentation? Does *Myosorex*, in many ways intermediate between soricine and crocidurine shrews, sport a trophoblastic sheath? What surprises await us in the placentas of gymnures and Asiatic shrew-moles?

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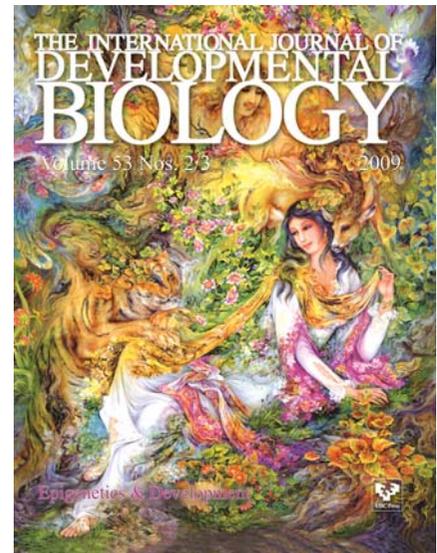M Placzek and P W Ingham
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