

The Fetal Membranes of the Otter Shrews and a Synapomorphy for Afrotheria

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The otter shrews of mainland Africa are the closest relatives of the Madagascar tenrecs. We sought for similarities in placentation between the two groups and, in a wider context, with other mammals of the Afrotheria clade. Specimens of the Nimba otter shrew (*Micropotamogale lamottei*) were obtained from the Ivory Coast and examples of the giant otter shrew (*Potamogale velox*) from the Hill Collection. The Nimba otter shrew has a central haemophagous organ similar to that in tenrecs. The labyrinth of the Nimba otter shrew, however, is endotheliochorial with syncytial trophoblast enclosing the maternal vessels. On the other hand tenrecs have cellular haemomonochorial placentae and an associated spongy zone, which is not present in the Nimba otter shrew. The placenta of the giant otter shrew is also endotheliochorial. The central region of its placenta is particularly interesting, since the juxtafetal portion is clearly a haemophagous region whereas the labyrinth feeding this region is endotheliochorial. Thus there is considerable variation in placental morphology within Tenrecidae. Importantly, however, both otter shrews have a large allantoic sac divided into four intercommunicating lobes by two pairs of septal folds. A similar arrangement has been described for representatives of each of the remaining five orders within Afrotheria. This is significant because previous anatomical studies have failed to establish a single synapomorphy in support of Afrotheria.

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INTRODUCTION

Otter shrews are found in West and Central Africa. They are semi-aquatic mammals resembling small otters with a large, flat muzzle, a slender body and a strong tail. The giant otter shrew, *Potamogale velox*, is fairly widely distributed, but the two smaller species, *Micropotamogale lamottei* and *Micropotamogale rumenzorii*, have restricted geographical ranges [1]. All three species are threatened by habitat loss and are listed as endangered by The World Conservation Union [2]. There are a number of studies of their anatomy, behaviour and ecology (reviewed by Vogel [3]), but little is known about their reproductive biology. Only a brief note exists on the placentation of the giant otter shrew [4].

The otter shrews are related to the Madagascar tenrecs, from which they diverged about 53 mya [5], and constitute the subfamily Potamogalinae within the family Tenrecidae. There is currently much controversy about the phylogenetic position of tenrecs. Analysis of nucleotide sequences in nuclear and mitochondrial genes provides strong support for a superordinal

clade, the Afrotheria, subsuming six orders of mammals, one of which includes tenrecs and golden moles [6,7]. So far, however, studies of morphological characters have failed to reveal a single shared, derived character (synapomorphy) in support of Afrotheria [8,9]. As a result, analyses based on morphological characters alone favour inclusion of tenrecs with other insectivores in Order Lipotyphla (e.g. Ref. [10]).

We here describe the fetal membranes of two otter shrews. In addition to providing information about placentation in this little known group and comparing it to that of the Madagascar hedgehog tenrecs [11,12], we asked whether there are any fetal membrane characters that are shared by tenrecs and the other orders of Afrotheria [13].

MATERIALS AND METHODS

We examined the placenta and fetal membranes of the Nimba otter shrew (*M. lamottei*) and compared them with those of the giant otter shrew (*P. velox*). Specimens of the Nimba otter shrew from the Ivory Coast had been trapped and drowned in bow nets and conserved in formalin by the fishermen. Eight pregnant uteri were at our disposal of which seven were well enough preserved for study. The four specimens of the giant

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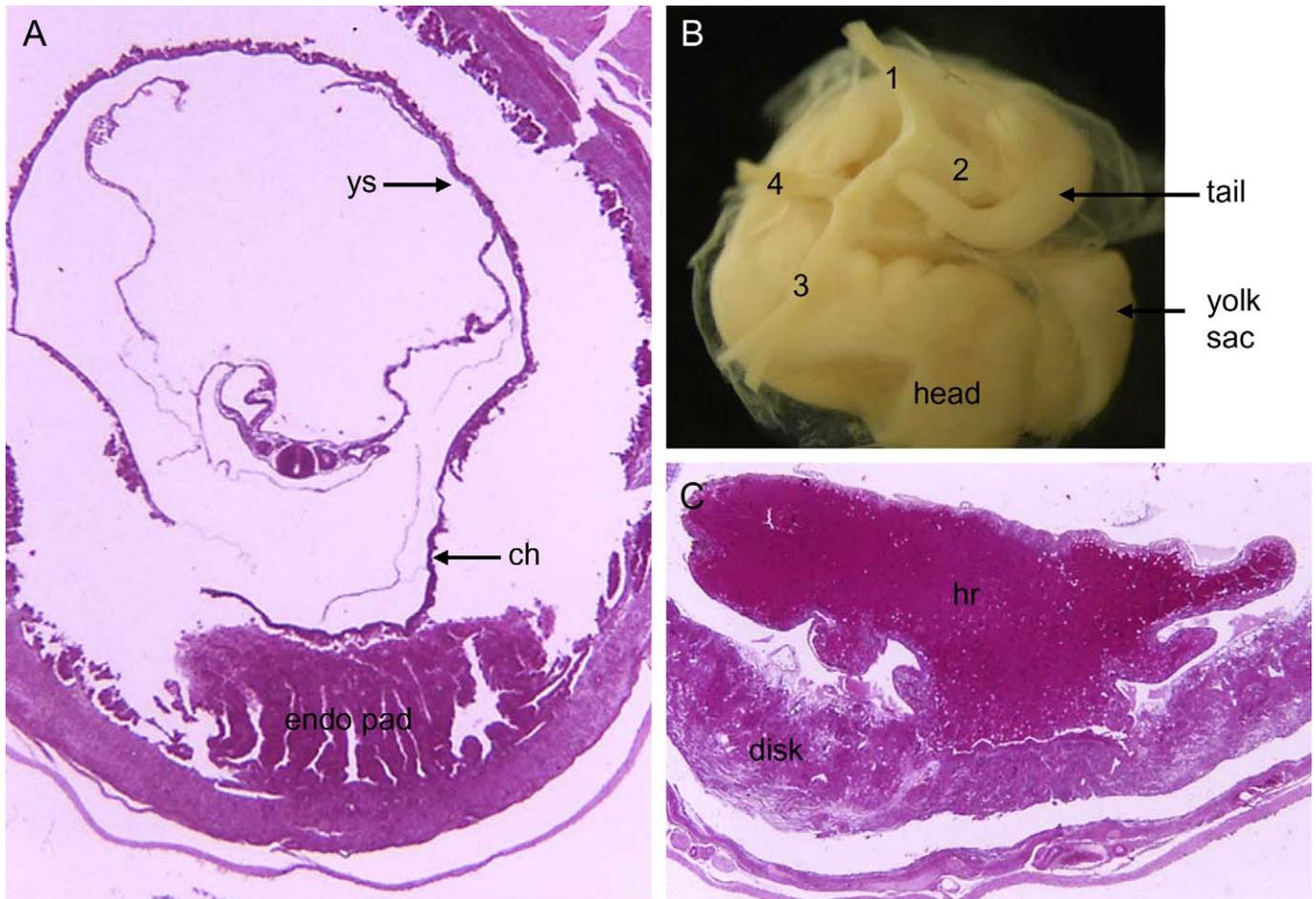


Figure 1. Placenta and fetal membranes of the Nimba otter shrew. (A) Fetal membranes in relatively early gestation (CRL 2 mm). The yolk sac (ys) has detached from the endometrium. The chorion (ch) is in contact with a thick pad of endometrium (endo pad). 30 \times . (B) Embryo with pontine flexure (CRL 5 mm). The short cord is hidden by the tail. It gives rise to four branches (1–4) that subtend folds of allantoic membrane. The yolk sac is clearly seen. 10 \times . (C) Early gestation placenta (embryo CRL 6.25 mm). The central haemophagous region (hr) mushrooms over the adjacent placental disk. 20 \times .

otter shrew from The Cameroons had been examined by J.P. Hill but are known only from a brief abstract [4]. This material, including Hill's notes and photographs, was made available to us by the Hubrecht Laboratory, which then housed the Hill Collection (present location Museum für Naturkunde, Humboldt University, Berlin).

We made a careful examination of the gravid uteri of the Nimba otter shrew. They had been opened earlier to expose the embryos, but we were able to reconstruct the course of the umbilical vessels. Placental tissues were then embedded in plastic for thin sectioning or in paraffin for serial sectioning. Sections of the paraffin-embedded material were stained with haematoxylin and eosin; sections of the plastic-embedded material were stained with Azure B. Other paraffin sections were stained by the periodic acid Schiff's reaction (PAS). In addition, some of the sections were examined for collagen IV using a rabbit polyclonal antibody (1:2000; Chemicon, Temecula, CA) and immunostaining by the avidin–biotin–peroxidase technique with diaminobenzidine as the chromogen. Negative controls were with omission of the primary antibody.

Tissues for transmission electron microscopy (TEM) were obtained from three of the better preserved placentae of the Nimba otter shrew and postfixed in 2% osmium tetroxide. These tissues were dehydrated and embedded in Araldite epoxy resin; 1 μ m sections were stained with Azure B and examined by light microscopy, after which selected regions were thin-sectioned and examined by TEM.

RESULTS

Both species of otter shrew have a bicornuate uterus with a large corpus within which the fetuses and their membranes reside. Most uteri of the Nimba otter shrew contained two fetuses, but there were two litters of four, as in the specimen reported by Kuhn [14]. The uteri of the giant otter shrew had 1–2 fetuses.

Placenta of the Nimba otter shrew

Two specimens of *M. lamottei* were in a late implantation stage. The embryos had about nine somites, an open

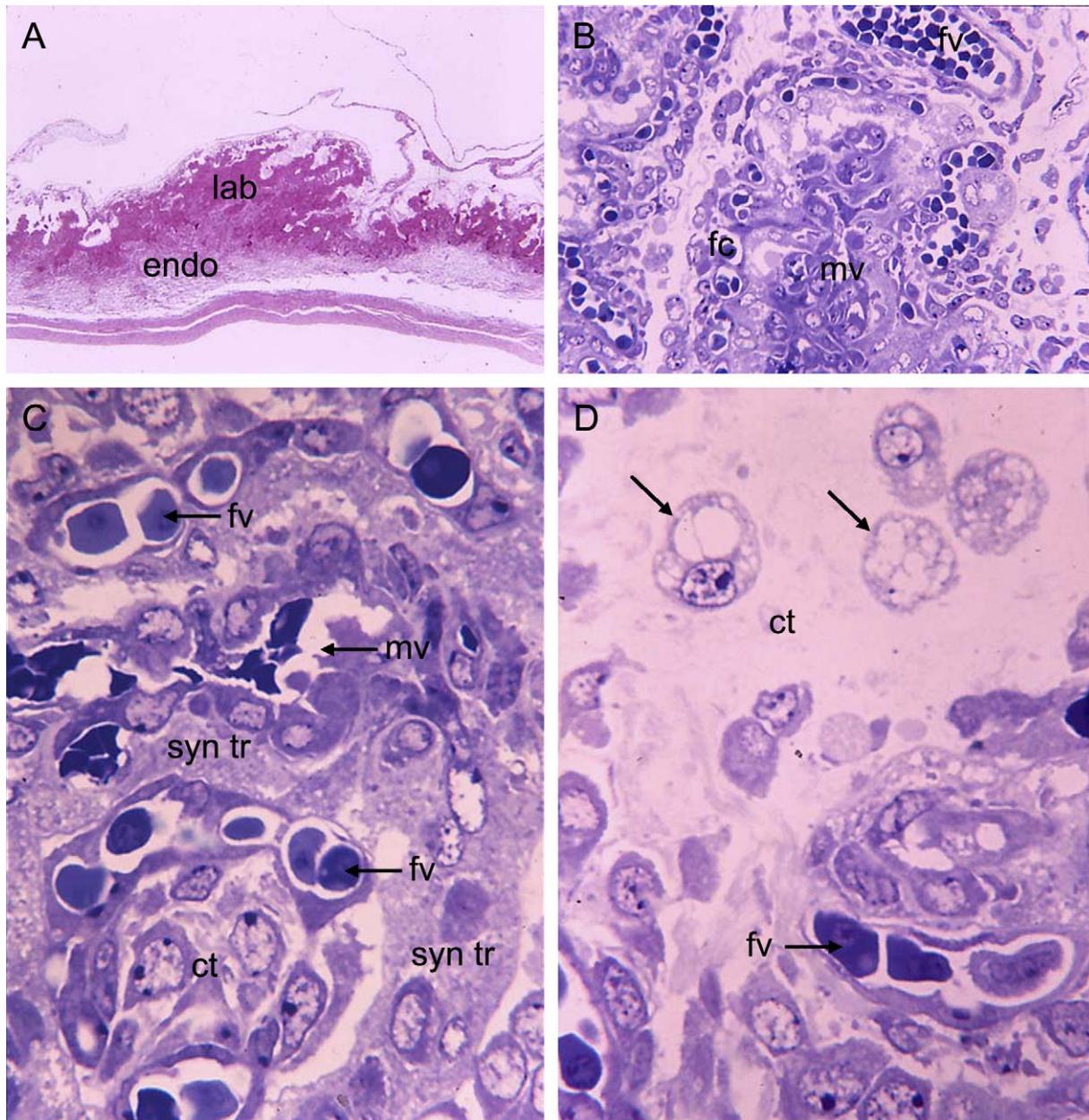


Figure 2. Placenta of the Nimba otter shrew early in gestation. (A) Overview of the placental labyrinth (lab) and underlying endometrium (endo). 30 \times . (B) Maternal vessels (mv) have basophilic endothelium that aids in distinguishing them from fetal vessels (fv) and capillaries (fc). 320 \times . (C) Fetal vessels can also be distinguished by their content of nucleated erythrocytes. They are separated from maternal vessels by syncytiotrophoblast (syn tr). Some fetal connective tissue (ct) is also present. 1100 \times . (D) The fetal connective tissue includes some vacuolated cells (arrows). 1100 \times .

anterior neuropore, and anterior but not posterior limb buds. The body wall was not completely formed and the internal and external coeloma communicated over the large yolk sac. The yolk sac had clearly been in contact with folds of the endometrium, although they were pulled away in these specimens. Blood cell formation was taking place within the yolk sac. The chorion, which had not yet been vascularized by allantoic vessels, was in contact with a thick pad of endometrium that showed dilated capillaries immediately adjacent to the trophoblast of the chorion (Figure 1A). This pad was on the antimesometrial side of the uterus. Although much of the trophoblast appeared to

be cellular, patches of syncytial trophoblast were seen both at the periphery of the chorion and in the trophoblast of the choriovitelline placenta.

Two specimens were in relatively early gestation, with embryos ranging from 5 to 6.25 mm crown-rump length (CRL). The embryos were in a visceral arch stage with prominent pontine flexure. The umbilical vessels comprised two arteries and one vein. They divided first into two, then four main branches (Figure 1B). These branches and all the subsequent branches were situated within membranes of the allantois, which formed four major compartments as the vessels approached the placenta.

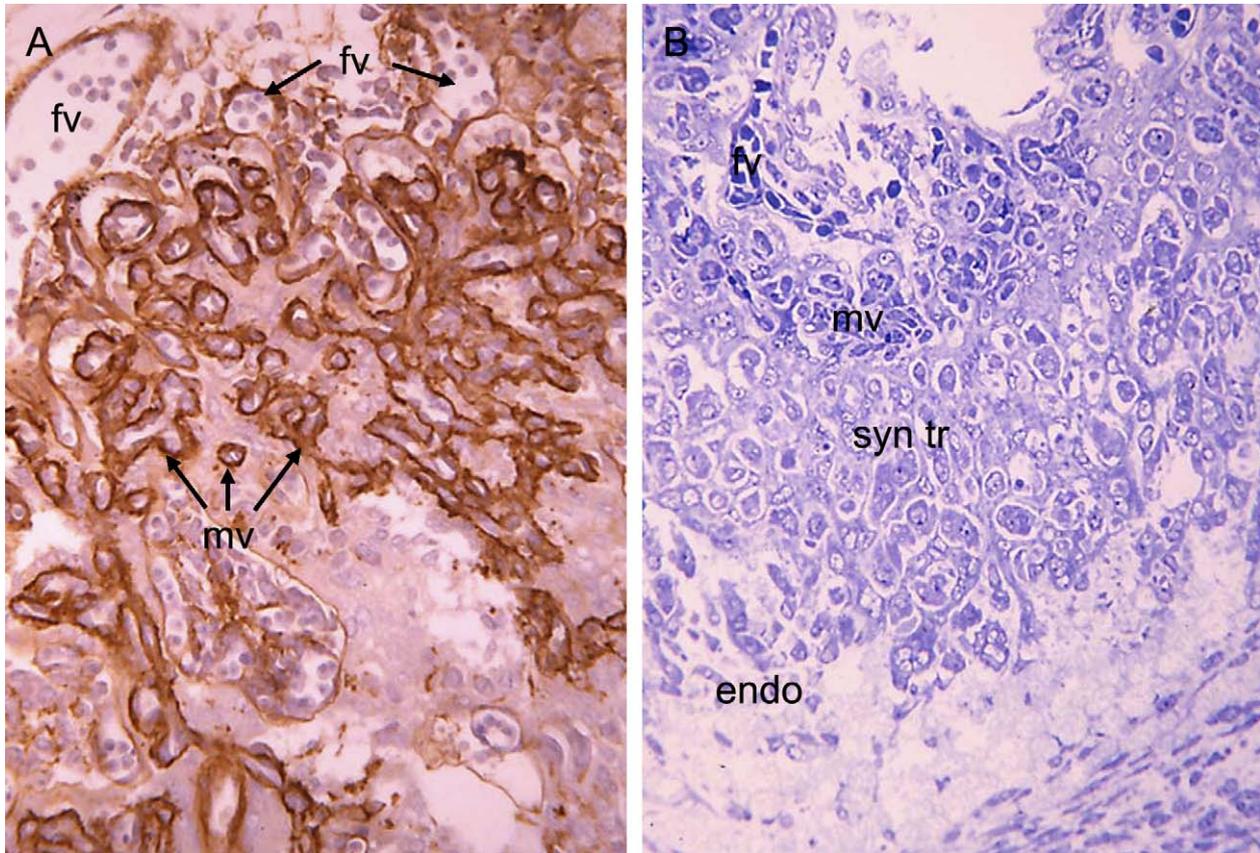


Figure 3. Placenta of the Nimba otter shrew early in gestation. (A) Labyrinth immunostained for type IV collagen. The thick interstitial membrane between the endothelium of maternal vessels (mv) and syncytiotrophoblast is well seen. Fetal vessels (fv) have a thin basal lamina. 270 \times . (B) At the maternal–fetal interface, a thick band of syncytiotrophoblast (syn tr) extends from the dark maternal vessel (mv) above to the pale endometrium below. The syncytiotrophoblast surrounds numerous individual cells. There is a fetal vessel with erythrocytes just above the maternal vessel. 270 \times .

The irregular placental disk consisted of an extensive labyrinth with a central haemophagous region that mushroomed over the adjacent portion of the disk (Figure 1C). The labyrinth consisted of highly convoluted maternal vessels surrounded largely by syncytial trophoblast (Figure 2A). The endothelium of the maternal vessels was basophilic and an irregular but thick interstitial lamina was seen between the endothelium and the surrounding syncytial trophoblast (Figure 2B). Fetal vessels containing nucleated erythrocytes roughly followed the syncytial trophoblast in an undulating pattern but were otherwise not closely associated with the syncytium overlying the loops of maternal vessels (Figure 2C). Some of the cells within the fetal connective tissue were vacuolated; others nearer to the syncytial surface were rounded and slightly basophilic (Figure 2D). The thick interstitial membrane between the maternal vessels and the syncytial trophoblast was immunologically reactive for type IV collagen, as was the much thinner basement membrane of the fetal vessels (Figure 3A). At the maternal–fetal interface a thick mass of syncytium extended into the endometrium and in places surrounded some partially decidualized endometrial stromal cells (Figure 3B).

Peripheral to the labyrinth, the chorioallantois formed a relatively simple paraplacenta consisting of a single layer of

cuboidal trophoblast cells, poorly vascularized connective tissue, and a squamous allantoic endodermal lining. The uterine luminal epithelium in this area was relatively unfolded.

The haemophagous region was saccular with only a few folds of trophoblast (Figure 4A). The trophoblast cells were columnar but at this stage only a few contained engulfed erythrocytes, and little pigment was present within the cells (Figure 4B). However, there were numerous fetal vessels underlying the cellular trophoblast.

The yolk sac, although large, had been completely displaced from any association with the trophoblast and was free in the exocoelom.

Definitive placenta

A single midgestational stage and two later gestational stages provided material for the definitive placenta of *M. lamottei*. Progressive changes were seen in both the labyrinth and the haemophagous region.

In the area of the labyrinth, the junctional zone between the trophoblast and the endometrium was much reduced in thickness, even in the youngest of these three specimens (Figure 5A). The maternal connective tissue cells underlying this area were extensively decidualized both at the junction and

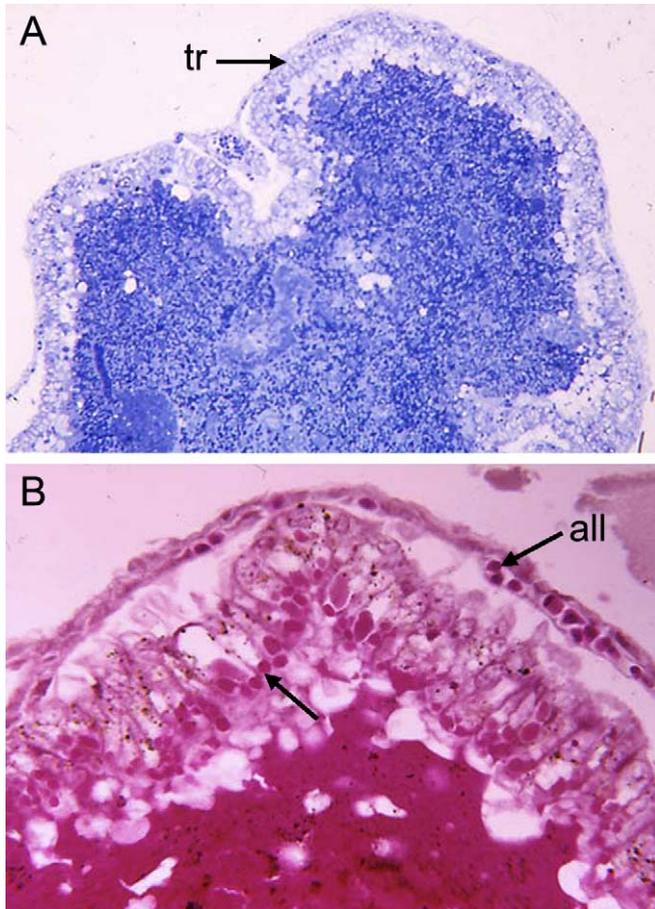


Figure 4. Placenta of the Nimba otter shrew early in gestation. (A) The haemophagous region is saccular with only a few folds of trophoblast (tr). 80 \times . (B) The columnar trophoblast cells contain some ingested erythrocytes (arrow). They are overlain by allantoic endoderm and mesoderm (all) with fetal blood vessels. 320 \times .

around the maternal vessels within the endometrium. Projections of the endometrium extended into the labyrinth as endometrial folds (Figure 5B). Within the fetal connective tissue the fibroblasts had undergone epithelioid differentiation, being basophilic in the earlier specimen and almost completely packing the fetal connective tissue adjacent to the trophoblast in the older specimens (Figure 5C). There was an increase in thickness of the labyrinth, and the maternal vessels within the labyrinth were extensively convoluted. There was a progressive decrease in distance between fetal and maternal vessels with the fetal vessels indenting the syncytial trophoblast (Figure 5D).

Despite the poor conditions of preservation, it was possible by TEM to demonstrate clearly both the syncytial trophoblast and the endotheliochorial nature of the placenta (Figure 6). Maternal vessels within the labyrinth had endothelial cells that had numerous ribosomes, many of which were associated with irregular strands of endoplasmic reticulum. They were surrounded by a thick interstitial membrane. Blunt unbranched projections from the endothelial cells passed through the interstitial membrane at irregular intervals and contacted

the syncytiotrophoblast. The syncytial surface towards the maternal vessels varied from smooth to scalloped but appeared to lack microvillous regions. The polyribosomes in the syncytiotrophoblast were predominantly arranged on stacks of four or more parallel strands of endoplasmic reticulum. The fetal capillaries had a thin basal lamina, in striking contrast to the interstitial membrane between the syncytium and maternal vessels.

The margins of the labyrinth occasionally overlapped the paraplacenta. In the paraplacenta, a single layer of cellular trophoblast separated maternal and fetal connective tissue (Figure 7A). Again the paraplacenta was poorly vascularized. The maternal connective tissue associated with this region was poorly decidualized. As at earlier stages the umbilical vessel branches supplying the placenta and paraplacenta were situated within membranes of the allantois (Figure 7B).

The haemophagous region showed progressive folding, including trophoblast villi with a connective tissue core but without allantoic endoderm (Figure 7C). In the youngest of the three specimens, many of the trophoblast cells contained erythrocytes and some showed yellow pigment. In the older specimens very little space was left between the folds of the haemophagous region and yellow crystalloid pigments were present both in the trophoblast and in the lumen (Figure 7D). Few erythrocytes remained between the folds but some debris, which may have included sloughed trophoblast cells and numerous crystalloids, was seen.

Placenta of the giant otter shrew

There were one singleton and three twin pregnancies of *P. velox*. Fetal size (greatest length) varied from 9 to 37 mm. In twin pregnancies an interembryonic septum separated the two embryos. It comprised portions of the allantoic membranes of both conceptuses with a fused conjoint trophoblast layer. The allantoic sac was large and divided into four intercommunicating lobes by two pairs of septal folds that extended from one side of the uterus to the other (Figure 8A). The free edges of these septa carried the main branches of the umbilical vessels. These arose by division of the cord first into two primary branches and then into two secondary branches (Figure 8B). Hill [4] reported that a yolk sac was present as a choriovitelline placenta, but only at the early stages.

Sections of the two earliest specimens in the Hill collection were at stages prior to definitive placenta formation. These sections showed the initial penetration of the uterine epithelium by trophoblast (Figure 9).

The definitive placenta of the giant otter shrew consists of an extensive endotheliochorial placental labyrinth (called the placental field by Hill [4]) and a central protruding zone that includes a haemophagous region (called the 'relatively small placental disk' by Hill [4]). The labyrinth receives its fetal vasculature directly from branches of the allantoic vessels, whereas recurrent branches go to the central zone (Figure 8B). The labyrinth is extensively folded with the typical looping structure associated with convoluted maternal vessels

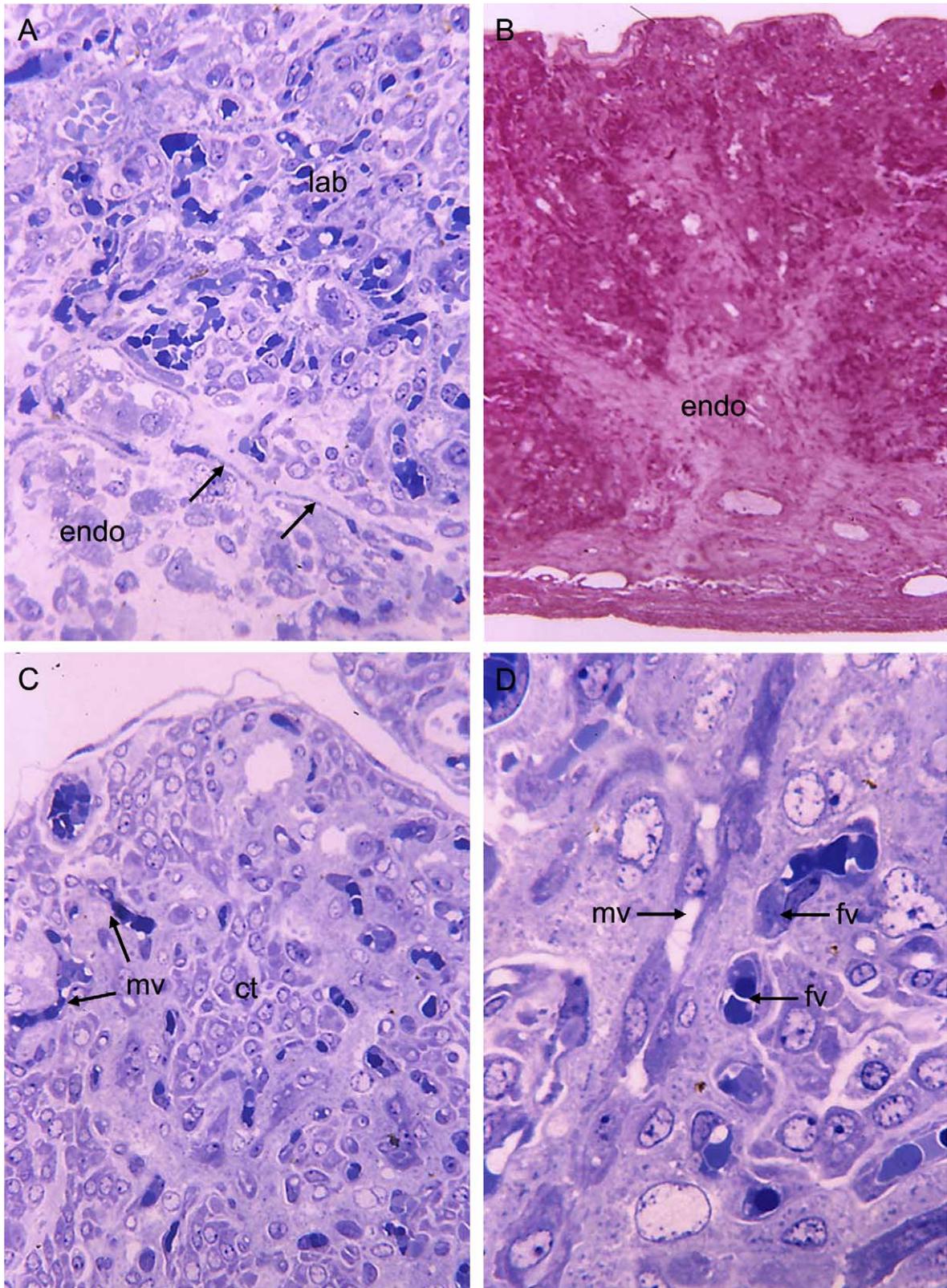


Figure 5. Definitive placenta of the Nimba otter shrew. (A) The labyrinth (lab) has expanded and the junctional zone (arrows) with the endometrium (endo) is reduced in thickness compared to earlier stages. 430 \times . (B) The endometrium projects into the labyrinth as endometrial folds. 100 \times . (C) Maternal vessels (mv) in the labyrinth are convoluted. Connective tissue fibroblasts (ct) have undergone epithelioid differentiation. 430 \times . (D) Fetal vessels (fv) indent the syncytiotrophoblast. Note the pale syncytiotrophoblast between the labelled maternal and fetal vessels and the large empty-appearing nuclei associated with the syncytiotrophoblast. 1100 \times .

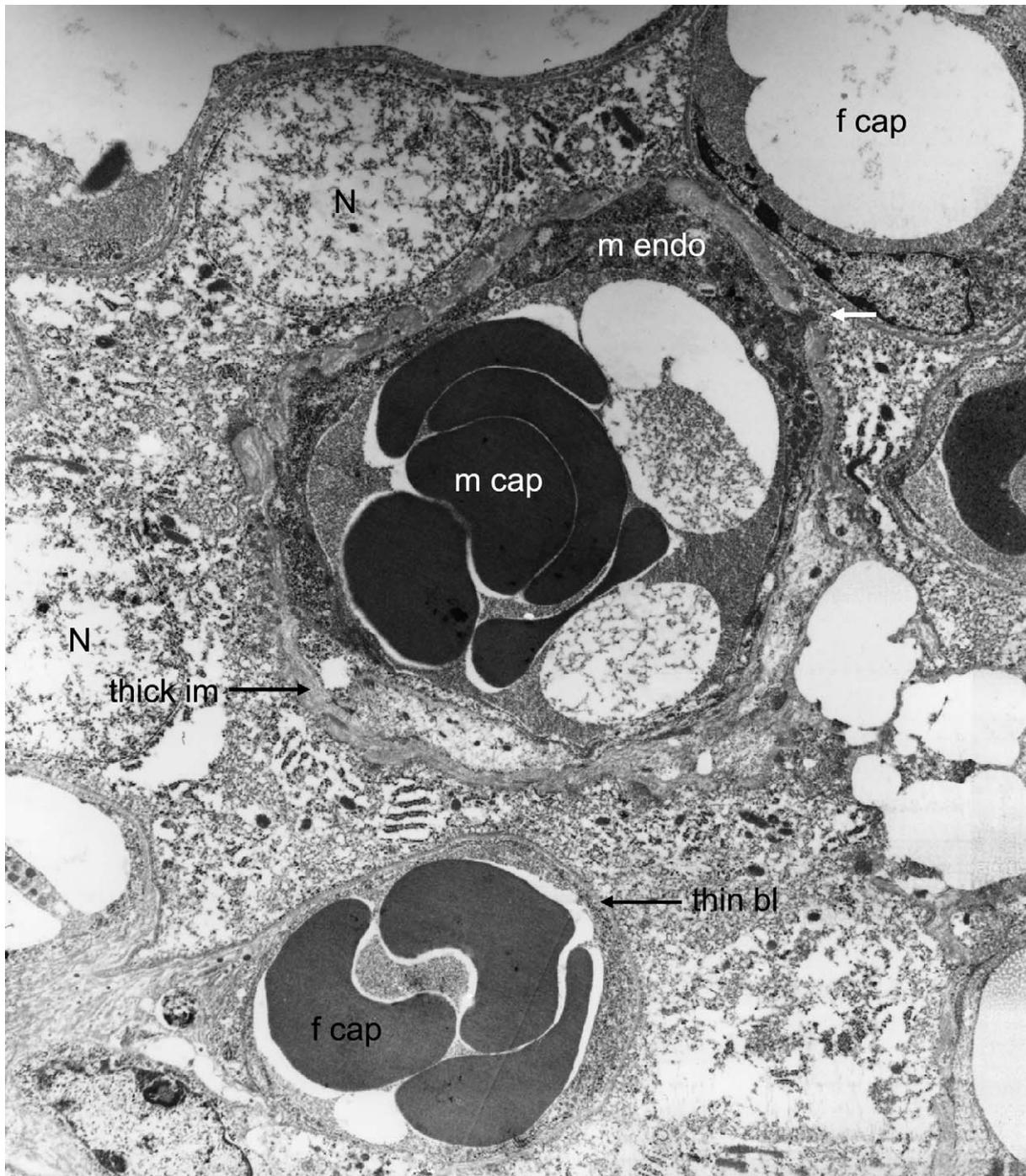


Figure 6. Definitive placenta of the Nimba otter shrew. Compare the thick interstitial membrane (im) of the maternal capillary (m cap) with the thinner basal lamina (bl) of the fetal capillary (f cap). A projection from the maternal endothelium (m endo) through the interstitial membrane is indicated by the white arrow. Despite poor preservation, the syncytial nature of the trophoblast is clearly seen; N, trophoblast nucleus. 5800 \times .

(Figures 10A, B). Fetal trophoblast and its loose connective tissue are interposed between the looped coils of maternal vessels.

The central zone is extraordinary (Figure 11A) as it includes two very different regions (Figure 11B). At the base of the projecting disk the maternal vessels are surrounded by syncytial trophoblast lined in turn by cellular trophoblast, with fetal

vessels then contained in loose connective tissue (Figure 11C). Towards the fetal surface of the projecting disk, the maternal endothelium and syncytial trophoblast disappear, and maternal blood spaces are lined by columnar cellular trophoblast (Figure 11D). The cellular trophoblast is clearly haemophagous with cells containing maternal erythrocytes and granules that Hill attributed to iron. The consequence of this arrangement is

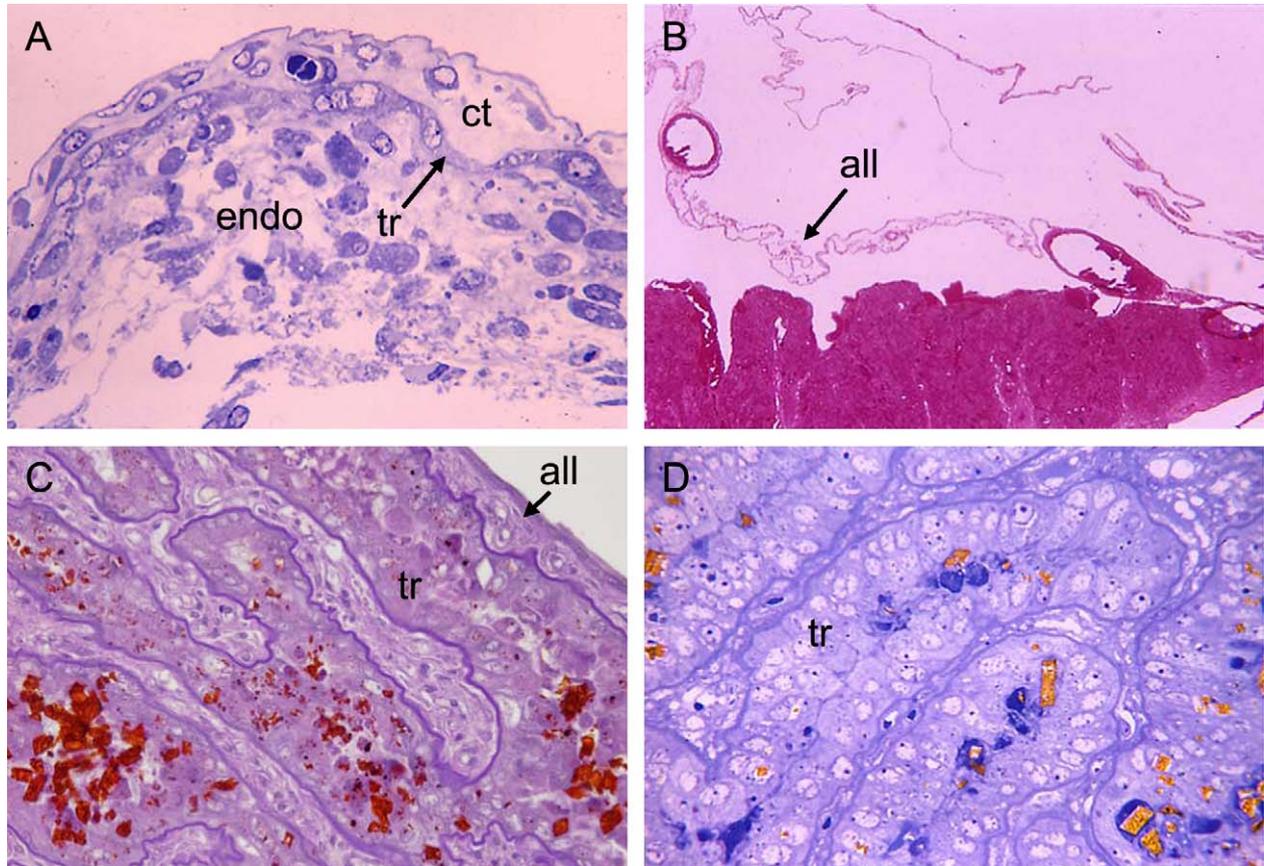


Figure 7. Definitive placenta of the Nimba otter shrew. (A) Paraplacental region. A thin layer of trophoblast (tr) separates fetal connective tissue (ct) from the endometrium (endo). 490 \times . (B) Branches of the umbilical vessels enclosed in allantoic membranes (all). 20 \times . (C) Haemophagous organ. PAS staining of the basal membranes highlights the extensive folding of the trophoblast. 280 \times . (D) Haemophagous organ. Yellow crystalloid pigments are seen in the trophoblasts and in the lumen. 320 \times .

a basal area that appears to be endotheliochorial, and more distal area that appears to be a haemophagous region. Because of this arrangement there would probably be little maternal circulation in the endotheliochorial portion of the disk, making this area relatively insignificant for oxygenation of fetal blood.

DISCUSSION

A good deal is known about placentation in the spiny tenrecs [15–17]. Recently we described the development of the central haemophagous region, which is a prominent feature of these placentae [12], and showed that the placenta of *Echinops telfairi* is haemomonochorial [11]. The present study reveals that placentation in the otter shrews differs in several important respects from that in the spiny tenrecs.

The arrangement of the major exchange regions of the chorioallantoic placenta is quite different. The placenta of the Nimba otter shrew has a labyrinth that is endotheliochorial with syncytial trophoblast enclosing the maternal vessels, whereas *Echinops* has a haemochorial labyrinth with a single layer of cellular trophoblast in the interhaemal barrier. In addition, *Echinops* has an underlying spongy zone, which is

absent in the otter shrew. The major part of the placenta of the giant otter shrew is also endotheliochorial. The central region of the placenta of this species is particularly fascinating, however, since the juxtafetal portion is clearly a haemophagous region whereas the labyrinth feeding this region is endotheliochorial. This central region constitutes a different way of forming a haemophagous region but could be considered a possible intermediate stage in transition to a haemochorial placenta. An intriguing feature of the placental barrier in the Nimba otter shrew is the sturdy interstitial membrane underlying the maternal endothelium. This is reminiscent of the situation in a New World monkey, *Callithrix jacchus*, where the thick membrane is thought to provide support for the persisting maternal blood vessels in the labyrinth which becomes hemochorial only later in gestation [18].

The golden moles are grouped with tenrecs and otter shrews in one order. This taxon is sometimes referred to as Afrosoricida [6,7], but was first described as Tenrecoidea [19] (for discussion see Ref. [20]). Their placenta resembles that of spiny tenrecs in having a discoid, haemochorial labyrinth and a spongy zone [21,22]. However, golden moles have no haemophagous regions in their placentae. Thus there

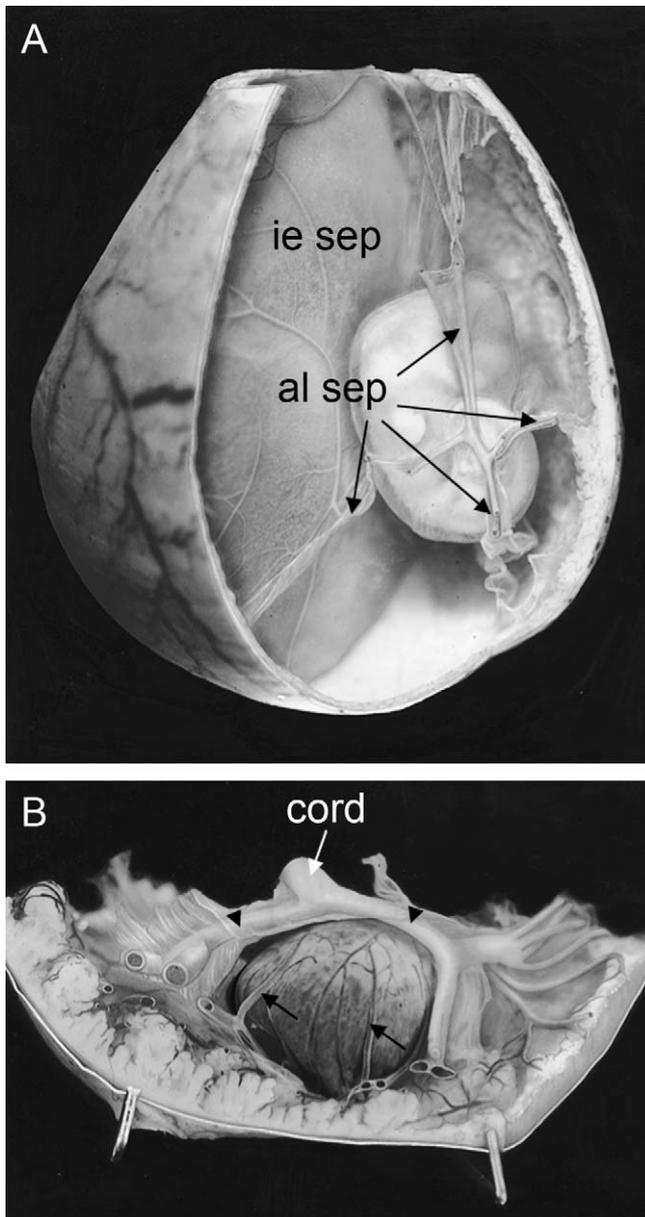


Figure 8. Placenta and fetal membranes of the giant otter shrew. (A) Twin pregnancy with one embryo (length 10.5 mm) exposed by removal of the uterine wall to which the placental disk was attached. The second embryo is hidden behind an interembryonic septum (ie sep). The allantois is divided into four lobes by allantoic septa (al sep) that carry branches of the umbilical vessels. (B) The cord is short and forms two primary branches (arrowheads) and four secondary branches that subtend the allantoic septa. The central disk is supplied by recurrent branches (arrows). Retouched photographs from the collection of J. P. Hill reproduced by courtesy of the Hubrecht Laboratory.

is significant variation in placental morphology between different members of the order. Consideration of the five

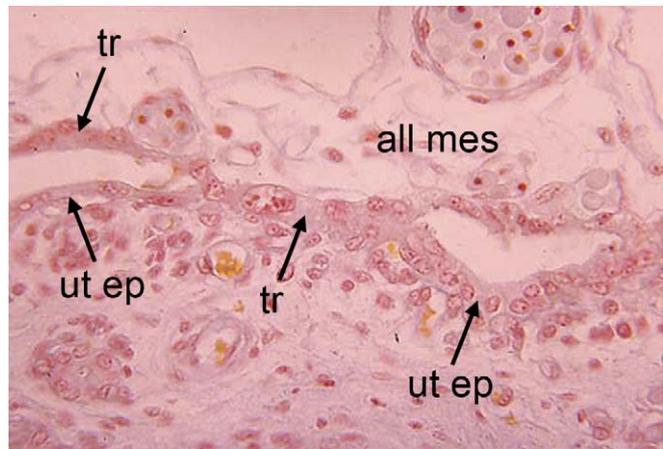


Figure 9. Fetal membranes of the giant otter shrew prior to formation of the definitive placenta. The uterine epithelium (ut ep) is intact in some areas whilst in others it is being penetrated by trophoblast (tr). Also seen are allantoic mesoderm (all mes), containing fetal vessels with nucleated erythrocytes, and allantoic endoderm. 320X. From the collection of J. P. Hill, courtesy of the Hubrecht Laboratory.

other orders assigned to Afrotheria reveals differences in gross form, ranging from zonary to discoid, in the interhaemal barrier, and in the presence or absence of a paraplacenta [23]. This diversity in structure of the chorioallantoic placenta within and between orders offers no support for their putative phylogenetic affinities [6–8].

However, all members of Afrotheria have large allantoic sacs, free uninverted yolk sacs, and branching of the allantoic vessels above the placental disk. A large allantoic sac is probably a conserved character and as such of limited value as a guide to phylogenetic relations [24]. Of greater interest is the partitioning of the allantois into four lobes by septa that convey branches of the umbilical vessels to the placenta. This is a well-known feature of placentation in the African elephant [25,26], manatee [27], rock hyrax [28] and armadillo [29,30]. A similar arrangement has been described in one of the elephant shrews [31] and likely is present in others [32]. In both otter shrews, the allantoic vessels branch well above the placental disk and the branches subtend allantoic folds. Thus, if they are taken as representative of the Tenrecoidea, this character state is present in some members of all six orders that comprise Afrotheria.

It is widely accepted that study of morphological characters has failed to reveal a single synapomorphy in support of Afrotheria [8,9]. We submit that study of embryonic development and fetal membranes, first used in a phylogeny of placental mammals by Thomas Huxley [33], may provide morphological support for the conclusions reached by molecular phylogeneticists.

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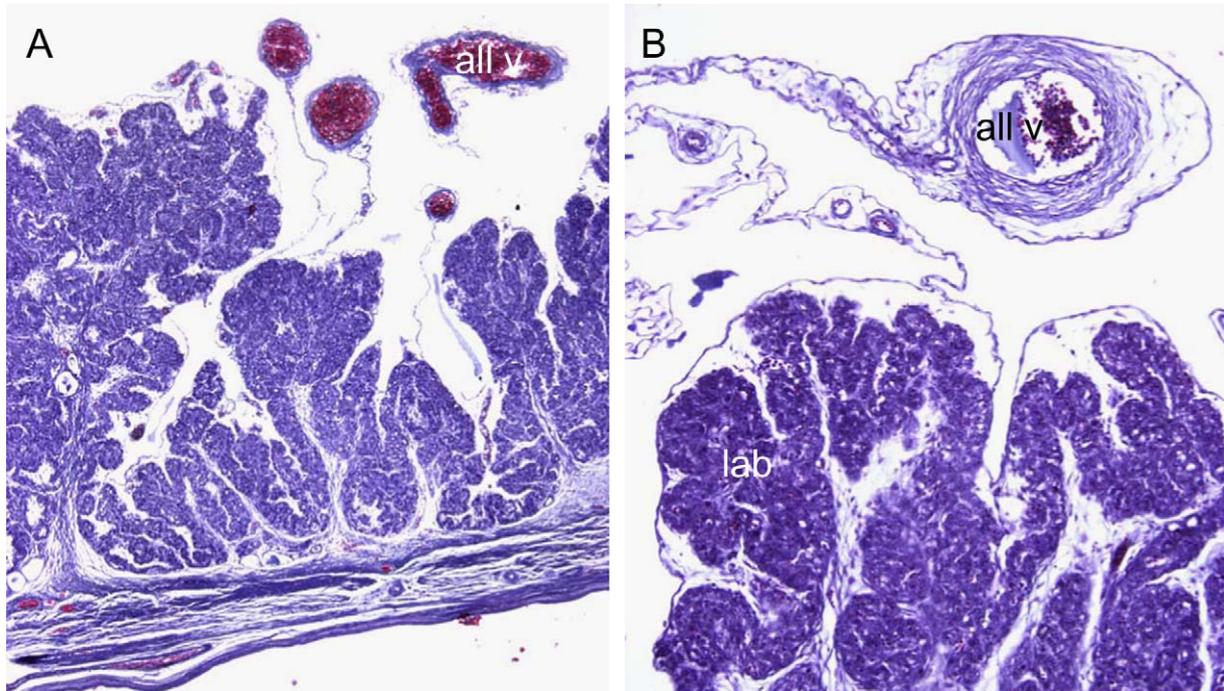


Figure 10. Placenta of the giant otter shrew from a pregnancy with 35 mm embryos. (A) The endometrium is thrown into irregularly convoluted ridges to which the trophoblast of the allantochorion is closely apposed; all v, allantoic vessel. 60 \times . (B) The labyrinth (lab) is extensively folded with the looping structure associated with convoluted maternal vessels. 120 \times . From the collection of J. P. Hill, courtesy of the Hubrecht Laboratory.

REFERENCES

- [1] Rahm U. Beobachtungen an der ersten in Gefangenschaft gehaltenen Mesopotamogale ruwenzorii (Mammalia – Insectivora). Rev Suisse Zool 1961;68:73–90.
- [2] IUCN – The World Conservation Union. IUCN Red List of threatened species. Available from: <<http://www.redlist.org>> [downloaded on 26 November 2004].
- [3] Vogel P. Contribution à l'écologie et à la zoogéographie de *Micropotamogale lamottei* (Mammalia, Tenrecidae). Rev Ecol (Terre Vie) 1983; 38:37–49.
- [4] Hill JP. The macroscopic features of the placenta of the water shrew (*Potamogale velox*). Biomorphosis 1938;1:331–2.
- [5] Douady CJ, Catzeflis F, Kao DJ, Springer MS, Stanhope MJ. Molecular evidence for the monophyly of Tenrecidae (Mammalia) and the timing of the colonization of Madagascar by Malagasy tenrecs. Mol Phylogenet Evol 2002;22:357–63.
- [6] Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, et al. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 2001;294:2348–51.
- [7] Waddell PJ, Kishino H, Ota R. A phylogenetic foundation for comparative mammalian genomics. Genome Inform Ser Workshop Genome Inform 2001;12:141–54.
- [8] Douady CJ, Chatelier PI, Madsen O, de Jong WW, Catzeflis F, Springer MS, et al. Molecular phylogenetic evidence confirming the Eulipotyphla concept and in support of hedgehogs as the sister group to shrews. Mol Phylogenet Evol 2002;25:200–9.
- [9] Yang F, Alkalaeva EZ, Perelman PL, Pardini AT, Harrison WR, O'Brien PC, et al. Reciprocal chromosome painting among human, aardvark, and elephant (superorder Afrotheria) reveals the likely eutherian ancestral karyotype. Proc Natl Acad Sci U S A 2003;100:1062–6.
- [10] Asher RJ. Cranial anatomy in tenrecid insectivorans: character evolution across competing phylogenies. Novitates 2001;3352:1–54.
- [11] Carter AM, Blankenship TN, Künzle H, Enders AC. Structure of the definitive placenta of the tenrec, *Echinops telfairi*. Placenta 2004;25: 218–32.
- [12] Carter AM, Blankenship TN, Künzle H, Enders AC. Development of the haemophagous region and labyrinth of the placenta of the tenrec, *Echinops telfairi*. Placenta 2005;26:251–61.
- [13] Carter AM. Evolution of the placenta and fetal membranes seen in the light of molecular phylogenetics. Placenta 2001;22:800–7.
- [14] Kuhn HJ. An adult female *Micropotamogale lamottei*. J Mammal 1971;52: 477–8.
- [15] Goetz RH. Studien zur Placentation der Centetiden. I. Eine Neu-Untersuchung der Centetes Placenta. Z Anat Entwickl 1937;106:315–42.
- [16] Goetz RH. Studien zur Placentation der Centetiden. III. Die Entwicklung der Fruchthüllen und der Placenta bei *Hemicentetes semispinosus* (Cuvier). Z Anat Entwickl 1938;108:161–200.
- [17] Strauss F. Die Placentation von *Ericulus setosus*. Rev Suisse Zool 1943;50: 17–87.
- [18] Merker HJ, Bremer D, Barrach HJ, Gossrau R. The basement membrane of the persisting maternal blood vessels in the placenta of *Callithrix jacchus*. Anat Embryol 1987;176:87–97.
- [19] McDowell SB. The Greater Antillean insectivores. Bull Am Mus Nat Hist 1958;115:115–213.
- [20] Asher RJ. Insectivoran-grade placental mammals: character evolution and fossil history. In: Rose KD, Archibald JD, editors. The rise of placental mammals: Origins and relationships of the major extant clades. Baltimore: Johns Hopkins University Press; 2005.
- [21] De Lange D. Contribution to knowledge of the placentation of the Cape golden mole (*Chrysochloris*). Bijdr Dierkunde Amsterdam 1919;21:161–73.
- [22] Gabie V. The placentation of *Eremitalpa granti* (Broom). J Morphol 1960; 107:61–78.
- [23] Carter AM, Enders AC, Kunzle H, Oduor-Okelo D, Vogel P. Placentation in species of phylogenetic importance: the Afrotheria. Anim Reprod Sci 2004;82–83:35–48.
- [24] Luckett WP. Cladistic relationships among primate higher categories: evidence of the fetal membranes and placenta. Folia Primatol (Basel) 1976;25:245–76.
- [25] Amoroso EC, Perry JS. The foetal membranes and placenta of the African elephant (*Loxodonta africana*). Phil Trans R Soc Lond B 1964; 248:1–34.
- [26] Allen WR, Mathias S, Wooding FB, van Aarde RJ. Placentation in the African elephant (*Loxodonta africana*): II morphological changes in the uterus and placenta throughout gestation. Placenta 2003;24:598–617.
- [27] Wislocki GB. The placentation of the manatee (*Trichechus laterostris*). Mem Museum Comp Zool, Harvard Coll 1935;54:158–78.

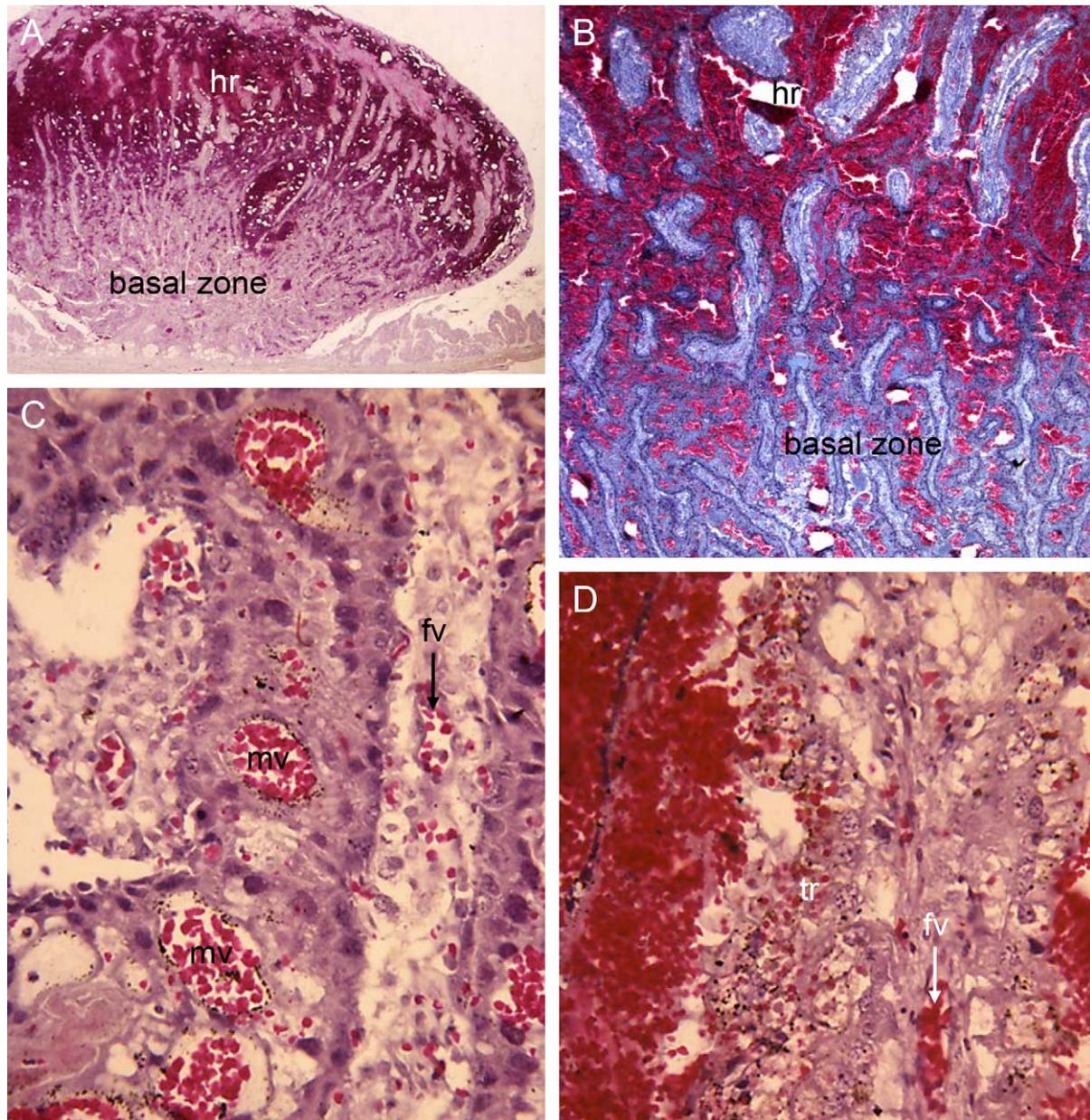


Figure 11. Central zone of the placenta of the giant otter shrew. (A) General overview to show the haemophagous region (hr) and the basal zone. 9 \times . (B) Transition between the haemophagous region and the basal zone. 40 \times . (C) Basal zone in which maternal vessels (mv) are surrounded by syncytiotrophoblast then cytotrophoblast with fetal vessels (fv) within loose connective tissue. 430 \times . (D) Haemophagous region with cellular trophoblast (tr) containing erythrocytes and pigment granules thought to be iron. 430 \times . From the collection of J. P. Hill, courtesy of the Hubrecht Laboratory.

- [28] Wislocki GB, van der Westhuysen OP. The placentation of *Procavia capensis*, with a discussion of the placental affinities of the Hyracoidea. *Contrib Embryol Carnegie Inst* 1940;28:65–88.
- [29] Mossman HW. The fetal membranes of the aardvark. *Mitteilung Naturforsch Gesellsch Bern* 1957;14:119–28.
- [30] Taverne MAM, Bakker-Slotboom MF. Observations on the delivered placenta and fetal membranes of the aardvark *Orycteropus afer* (Pallas 1766). *Bijd Dierkunde Amsterdam* 1970;40:154–62.

- [31] De Lange D. Communication on the attachment and the early development of *Macroscelides* (= *Elephantulus*) *rozeti* Duv., the North African jumping shrew. *Bijd Dierkunde* 1949;28:255–85.
- [32] Oduor-Okelo D, Katema RM, Carter AM. Placenta and fetal membranes of the four-toed elephant shrew, *Petrodromus tetradactylus*. *Placenta* 2004; 25:803–9.
- [33] Huxley TH. *Lectures on the elements of comparative anatomy*. London: Churchill; 1894.