Short Communications

Amniogenesis in Schreiber's long-fingered bat *Miniopterus schreibersii natalensis*

M. van der Merwe
Department of Zoology & Entomology, University of Pretoria, Pretoria, 0002, Republic of South Africa

Received 15 June 1995; accepted 30 January 1996

Schreiber's long-fingered bat, *Miniopterus schreibersii natalensis* is seasonally monoestrous, carrying a single foetus in the right uterine horn. Implantation is superficial, the amnion being a pleuramnion. Lateral folds, originating from the ends of the caudal and cephalic folds, are the main contributors in the formation of the definitive amnion. The formation of the amnion by folding is preceded by the development of a primitive amniotic cavity within the inner cell mass which eventually disappears.

Gopalakrishna & Karim (1979, 1980) identified three main types of amniogenesis in the Chiroptera. The first type involves the formation of the definitive amnion, preceded by the formation of a primitive amniotic cavity within the inner cell mass. The roof of this cavity does not contribute to the formation of the definitive amnion and eventually disappears. The definitive amnion develops later out of folds originating from the margins of the embryonic disc which grow dorsally and arch over until they fuse to form the ectodermal component of the true amnion. Extra-embryonic mesoderm envelops this layer to establish the definitive amnion. According to Gopalakrishna & Karim (1979) this mode of amnion formation is common in all pipistrellids studied in India. A similar mode of amnion formation has been described for *Myotis lucifugus lucifugus* (Wimsatt 1944) and the European *Miniopterus schreibersii* (Da Costa 1919, 1920; present study).

The second type is a schisamnion. Again a cavity develops within the inner cell mass by means of cavitation. In this case, however, the cavity is a true amniotic cavity from the beginning, because although the roof of the cavity becomes stretched during embryogenesis, it does not rupture and persists as the ectodermal component of the true amnion. Extra-embryonic mesoderm eventually covers the ectodermal component to establish the true amnion. This method of amnion formation has been reported in only a few bat species, e.g. the phyllostomatids and desmodontids. Both these groups have been considered exceptional among bats in exhibiting completely interstitial implantation (Hamlett 1935; Wimsatt 1954). In members of both these groups amniogenesis by means of cavitation has been described e.g *Glossophaga soricina* (Hamlett 1935; Rasweiler 1974) and the vampire bat *Desmodus rotundus* (Wimsatt 1954). Recently a similar method of amnion formation has been described for the superficially implanting South African vespertilionid, *Pipistrellus rusticus* (Van der Merwe 1994).

The third type of amniogenesis involves the formation of an amnion by folding as described for the free-tailed bat *Tadarida brasiliensis cynocephala* (Stephens 1962).

Schreiber's long-fingered bat, *Miniopterus schreibersii natalensis*, is seasonally monoestrous producing a single offspring in November. It has a bicornuate uterus with asymmetrically sized horns. The right horn is twice the length of the left one. All implantations occur in the right uterine horn, with 90% of the conceptuses originating from the left ovary (Van der Merwe 1986). Implantation is superficial and the blastocyst expands within the uterine horn. Orientation of the point of initial trophoblastic attachment, like the orientation of the embryonic disc, is anti-mesometrial (Van der Merwe 1982). Soon after the endodermic cells have started to delaminate from the inner cell mass during the pre-implantation period, a single cavity, the primitive amniotic cavity, develops between the remainder of the inner cell mass by means of cavitation (Van der Merwe 1980). However, this cavity as well as its roof (composed of cells of the embryonic mass and overlying trophoblast) do not participate in the formation of the definitive amniotic cavity or definitive amnion respectively. At the stage when the trophoblast covering the embryonic pole of the blastocyst starts to proliferate to form the invading syncytiotrophohlast, the floor of the primitive amniotic cavity flattens off (Van der Merwe 1982). Owing to this action, the roof of the primitive amniotic cavity, as well as the overlying cytotrophoblast, becomes stretched and progressively thinner until it eventually ruptures and disappears. The embryonic disc at this stage is roofed only by the syncytiotrophoblast (Van der Merwe 1982).

The purpose of the present study was to determine how the pleuramnion in the superficially implanting Schreibers' long-fingered bat is formed. The main aim was to determine whether the cephalic/head, lateral and caudal folds develop simultaneously and contribute equally to its formation. There has been some controversy as far as this aspect is concerned because, although authors refer to these folds, some do not elaborate on the contribution made by each of these folds during amniogenesis. They normally only mention that the free edges of the folds meet dorsally of the embryo. In the pig these circumferential amniotic folds are in reality all directly continuous with each other (Patten 1948). This, indeed, is expected to be the case in the majority of mammals where a pleuramnion exists. In Schreibers' long-fingered bat these folds eventually become continuous, but this occurs only at a later stage of amniogenesis (see below).

Twenty Schreiber's long-fingered bats carrying embryos were collected during July and August at Sandspruit Cave No. 1 (24°37'S, 27°40'E) in the northern Transvaal bushveld.

Following paraffin-wax embedding, all the genital tracts were serially sectioned at 5 µm and the mounted sections stained with Ehrlich's haematoxylin and counter-stained with eosin.

The sections revealed that upgrowing folds from the perimeter of the embryonic disc in Schreiber's long-fingered bat, brought with them ectoderm and extra-embryonic mesoderm (Figures 1a, b). Because this is a double fold of somatopleura, the outer part comes into close contact with the syncytiotrophoblast. As growth of the folds progresses, the outer layer of the folds eventually covers more of the syncytiotrophoblast overlying the embryonic pole. Finally the folds unite dorsally,

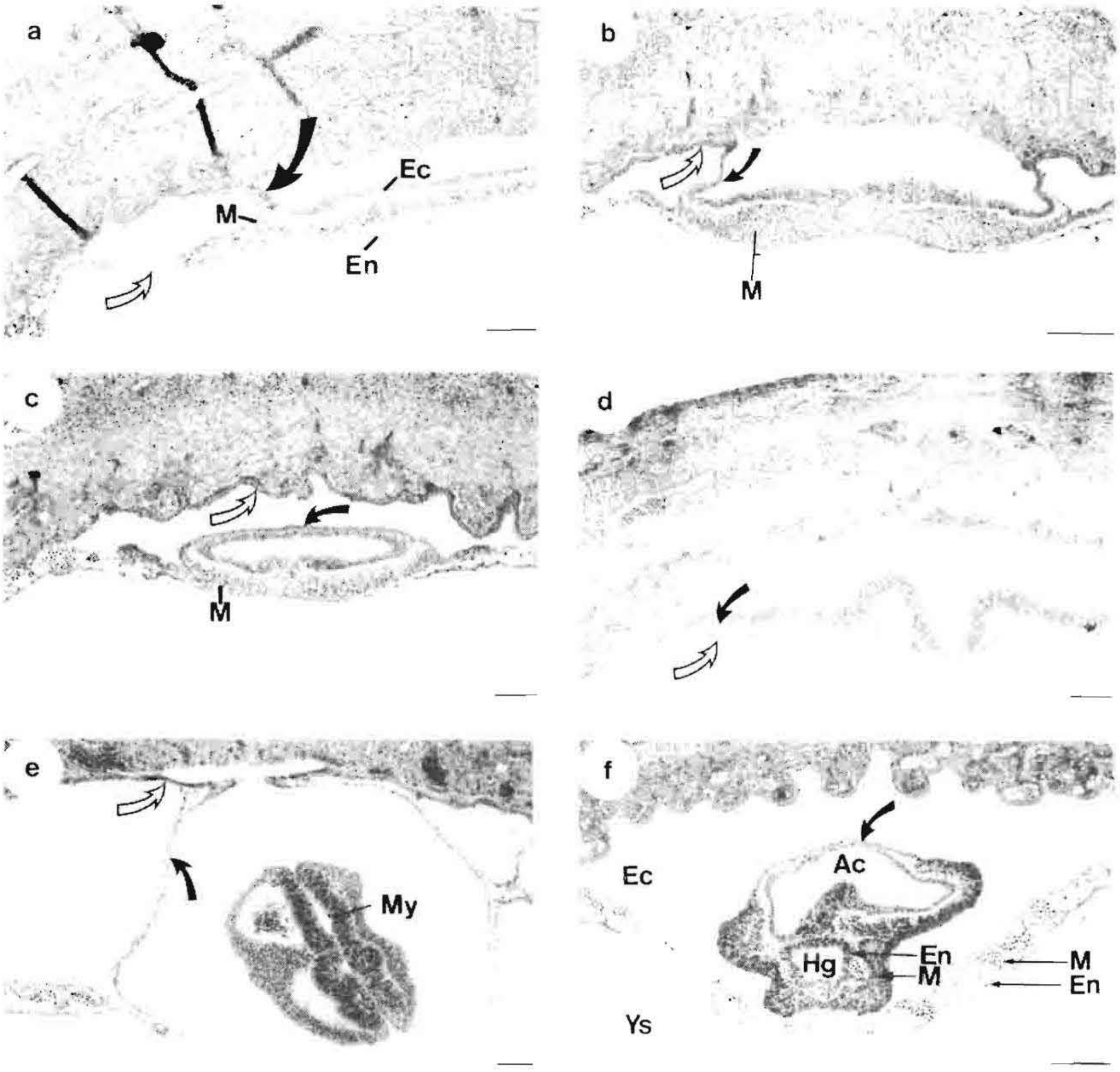

Figure 1a–f. Figure 1a Mesoderm (M) splitting on one side of the embryonic disc, some 90 µm from the posterior tip, to form a somatopleura [mesoderm (M) and ectoderm (Ec); solid arrow] and splanchnopleura [mesoderm (M) and endoderm (En); open arrow]. Bar = 100 µm. **Figure 1b** Embryo showing double fold of somatopleura with the lateral amniotic fold indicated with a solid arrow, and the rudiment of the chorion with an open arrow. The mesoderm (M) is conspicuous between the overlying ectoderm and underlying endoderm. Bar = 100 µm. **Figure 1c** Posterior part of embryo covered with a hood, i.e. after the double lateral amniotic folds have met mid-dorsally, they have split to give rise to the overlying chorion (open arrow) and the amnion (solid arrow). The ectoderm layer of the amnion is surrounded on the outside by mesoderm (M). Bar = 100 µm. **Figure 1d** Anterior side of embryo where the mesoderm has started to split into a somatic layer (solid arrow) and a splanchnic layer (open arrow). This section is roughly 200 µm from the anterior tip of the embryonic disc. Bar = 100 µm. **Figure 1e** Area of the cervical flexure, where the down-bent myelencephalon (My) can be seen. In this section the double lateral folds of somatopleura, which originated in the posterior part of the embryo, are uniting mid-dorsally. The double fold of somatopleura on either side consists of the outer future chorion (open arrow) and the future inner amnion (solid arrow). At this stage the lateral amniotic folds are prominent and continuous all the way around the embryo. Roughly 50 µm anterior of this section, the lateral folds that orientated from the ends of the pro-amnion are uniting mid-dorsally, thus leaving only a small area (50 µm by 130 µm) in the vicinity of the cervical flexure uncovered by amnion. Bar = 100 µm. **Figure 1f** Section through the posterior part of an embryo which has been undercut by the caudal and lateral body folds, thus pinching it off from the underlying yolk sac (Ys). With the forward growth of the subcaudal fold and its concomitant undercutting of the embryo, an endodermally lined pocket, or hind gut (Hg) is established in the caudal region. Because the subcaudal fold is a double layer of splanchnopleura [mesoderm (M) and endoderm (En)] its inner half forms the wall of the hind gut, while its outer half is continuous with the rest of the yolk sac. In this part of the embryo, the amnion (solid arrow) and the amniotic cavity (Ac) are already well established. The extra-embryonic coelom (Ec) is conspicuous. Bar = 100 µm.

and the inner and outer layers of the double folds separate from each other. The inner half then forms the definitive amnion, while the outer half closely underlying the syncytiotrophoblast becomes the chorion.

The formation ot the definitive amnion is evident only after the primitive streak stage, and the sequence of its formation appears to be the opposite of that described for reptiles and birds by Balinsky (1975). In Schreiber's long-fingered bat the rudiment of the amnion and chorion first appears as a transverse caudal fold (somatopleura) at the posterior end of the embryo. Although individual variation is expected, it appears from the present material that splitting of the mesoderm, to give rise to the somatic and splanchnic mesoderm layers (which eventually participate in the formation of the caudal amniotic and body fold respectively), is not necessarily restricted to the most posterior tip of the embryonic disc, but could in fact start at any point in the vicinity of the tip, either on one or both sides. In Figure 1a it can be seen that splitting of the mesoderm (to form an outer somatopleura and an inner splanchnopleura) is initiated on the one side of the embryonic disc only. In this specimen, the mesoderm has already split from the posterior tip over a distance of 280 μm along the one side of the embryonic disc, being most prominent 90 μm from the tip. It is thus obvious that when splitting of the mesoderm occurs, on one or both sides, it will expand both in an anterior and posterior direction. However, with the posterior tip of the embryonic disc nearby, the mesoderm of that area will soon be involved, thus establishing the somatopleura (which will give rise to the caudal amniotic fold) and the splanchnopleura (which will give rise to the caudal body fold). A caudal amniotic fold, however, does not become prominent because shortly after its formation the lateral ends of this fold are prolonged forward along both sides of the embryo (Figure 1b). These folds approximate each other and are fused from the back towards the front of the embryo.

The development of the transverse cephalic/head fold (pro-amnion) was only initiated at a later stage, because there was no sign of this fold, even when the tail-end of the embryo was already covered with a hood (Figure 1c). In this specimen 65 μm of the posterior end of the embryonic disc was covered with an amnion. However, on the anterior side, splitting of the mesoderm had only recently begun, covering an area of 420 μm from the anterior tip along the sides of the embryonic disc (Figure 1d). On both sides, splitting of the mesoderm is most conspicuous about 200 μm from the anterior tip of the disc, giving the impression that splitting of the mesoderm started there and then spread in an anterior (around the tip of the disc) and posterior direction. At that stage, however, there was still no sign of an anterior amniotic fold.

It is expected that the cephalic amniotic fold becomes prominent only at the time when flexion of the cephalic region occurs. Lateral folds originating from the ends of the cephalic amniotic fold then extend backwards, and unite with those that have originated from the ends of the caudal fold in the region of the cervical flexure (Figure 1e).

Formation of the body folds which undercut the embryo cephalically and caudally, together with an increase in the length of the embryonic body, cause the embryo to overhang the extra-embryonic layers (Patten 1948). When exactly these folds, which undercut the embryo, and thus separate it from the extra-embryonic structures, form in Schreiber's long-fingered bat, is not certain. Whether the folds of splanchnopleura which undercut the head and tail (subcephalic and subcaudal folds) develop simultaneously, or whether development of the cephalic fold is precocious to that of the caudal fold, is also not certain. However, it is clear that the posterior amniotic fold forms well in advance of both the subcaudal fold as well as the cephalic and subcephalic fold. In one embryo where the amnion already formed a hood over the posterior end of the embryo, covering about 8% (65 μm) of the 840 μm long embryonic disc (Figure 1c), there was still no sign of a caudal body fold or a cephalic and subcephalic fold. At this stage the mesoderm was only beginning to split over the anterior side of the embryonic disc (see above).

As far as the body folds are concerned, it is expected that development of the subcephalic fold will be precocious to that of the subcaudal fold, owing to earlier flexion of the cephalic region. Simultaneously with the formation of the subcephalic fold, formation of the cephalic amniotic fold is anticipated, because during flexion the head region actually dips into the underlying extra-embryonic region. In a more advanced embryo, undercutting and flexion of the cephalic region was more developed. In the same embryo, however, flexion was not conspicuous in the caudal region although the caudal and lateral body folds had already pinched off the hind gut (Figure 1f).

In *Tadarida brasiliensis cynocephala* the amnion develops exclusively by folding. Stephens (1962) mentioned that this method of amnion formation is not common in any of the bats described. Wimsatt (1944) *op cit* in Stephens (1962) mentions that in the Vespertilionidae the definitive amnion appears to be formed not by an actual folding process, but by an upgrowth of ectodermal elements from the margin of the embryonic disc. Wimsatt (1944) does not clarify this point further. Elsewhere in the same article he refers to folds when he states that the elevating folds of the definitive amnion bring with them from the periphery of the embryonic disc a layer of cytotrophoblast to underlie the syncytiotrophoblast which until then had alone formed the roof of the amniotic cavity. In a later article on the American vampire bat, *Desmodus rotundus murinus*, Wimsatt (1954) clearly states that the definitive amnion in the vampire bat is formed by cavitation and not by folding as in most other bats. It would appear that the type of amnion formation described by Stephens (1962) may be more common than was originally thought. At least in Schreiber's long-fingered bat the only significant difference appears to be the formation of the primitive amniotic cavity and its roof which does not participate in the formation of the definitive amniotic cavity or definitive amnion respectively. The roof of the amniotic cavity eventually ruptures and disappears resulting in the embryonic disc being roofed only by syncytiotrophoblast (Van der Merwe 1982). This, however, is in sharp contrast to the findings of Chari & Gopalakrishna (1984) and Gopalakrishna & Chari (1985). From their studies on *M. s. fuliginosus* the authors claim that after the roof of the primitive amniotic cavity has become torn, the embryonic plate is exposed to the potential uterine lumen with the roof formed by the superficial layer of endometrium.

Stephens (1962) mentioned that the peculiarity of the prominence of the tail fold of the amnion in amniogenesis has not been described before. He felt that it may possibly be due to the extreme delay of the migration of mesoderm into the pro-amniotic region. The absence of this mesoderm may be a factor in preventing the head fold of the amnion from migrating in the usual manner. The same phenomenon is found in Schreiber's long-fingered bat, where mesoderm activity in the tail region is advanced to that in the pro-amniotic region. In one of the younger embryos, prior to splitting of the mesoderm, the mesoderm layer in the tail region is composed of more than one layer while it is still single-layered in the pro-amniotic region. This, together with the fact that splitting of the mesoderm is initiated over the posterior side of the embryo would suggest that mesoderm migration into the tail region is more advanced compared to the situation in the pro-amniotic region.

Acknowledgements

I am grateful to the University of Pretoria for financial assistance and to Mr I.D. Wentzel for access to his property for the collection of study material.

References

BALINSKY, B.I. 1975. An introduction to embryology. W.B. Saunders Company, Philadelphia.

CHARI, G.C. & GOPALAKRISHNA, A. 1984. Morphogenesis of the foetal membranes and placentation in the bat, *Miniopterus schreibersii fuliginosus* (Hodgson). *Proc. Indian Acad. Sci. (Anim. Sci.)* 93: 463–483.

DA COSTA, A.C. 1919. Sur le processus de formation de l'amnios chez *Miniopterus schreibersii* Natterer. *Compt. rend. Soc. Biol., Paris* 82: 588–590.

DA COSTA, A.C. 1920. Sur la formation de l'amnios chez les Chéiroptères (*Miniopterus schreibersii*) et, en général, chez les mammifères. *Mem. Soc. Port. Sci. Nat., Biol. Sér.* 3: 1–51.

GOPALAKRISHNA, A. & CHARI, G. 1985. Early development, implantation of the blastocyst and amniogenesis in the bat, *Miniopterus schreibersii fuliginosus* (Hodgson). *Proc. Nat. Acad. Sci. India* 55, Section B, Part II: 1–9.

GOPALAKRISHNA, A. & KARIM, K.B. 1979. Fetal membranes and placentation in Chiroptera. *J. Reprod. Fert.* 56: 417–429.

GOPALAKRISHNA, A. & KARIM, K. B. 1980. Female genital anatomy and morphogenesis of foetal membranes of Chiroptera and their bearing on the phylogenetic relationships of the group. *National Academy of Sciences, India: Golden Jubilee Commemoration Volume* Pp 1–50.

HAMLETT, G.W.D. 1935. Notes on the embryology of a phyllostomid bat. *Am. J. Nat.* 56: 327–349.

PATTEN, B.M. 1948. Embryology of the pig. McGraw-Hill Book Company Inc., New York.

RASWEILER, J.J. 1974. Reproduction in the long-tongued bat, *Glossophaga soricina*. II. Implantation and early embryonic development. *Am. J. Anat.* 139: 1–34.

STEPHENS, R.J. 1962. Histology and histochemistry of the placenta and foetal membranes in the bat, *Tadarida brasiliensis cynocephala*. *Am. J. Anat.* 111: 259–286.

VAN DER MERWE, M. 1980. Delayed implantation in the Natal clinging bat *Miniopterus schreibersii natalensis* (A. Smith, 1834). *Proc. Vth Int. Bat Res. Conf. Albuquerque* pp. 113–123.

VAN DER MERWE, M. 1982. Histological study of implantation in the Natal clinging bat (*Miniopterus schreibersii natalensis*). *J. Reprod. Fert.* 65: 319–323.

VAN DER MERWE, M. 1986. Reproductive strategies of *Miniopterus schreibersii natalensis*. *Cimbebasia* (A). 8: 107–111.

VAN DER MERWE, M. 1994. Schizamniogenesis in the rusty bat, *Pipistrellus rusticus*. *S. Afr. J. Zool.* 29: 220–223.

WIMSATT, W.A. 1944. An analysis of implantation in the bat, *Myotis lucifugus lucifugus*. *Am. J. Anat.* 74: 355–411.

WIMSATT, W.A. 1954. The foetal membranes and placentation of the tropical American vampire bat, *Desmodus rotundus murinus*. *Acta anat.* 21: 285–341.

First record of the sea anemone *Metridium senile* from South Africa

C.L. Griffiths, L.M. Kruger and C. Ewart Smith
Marine Biology Research Institute, University of Cape Town,
Rondebosch 7700
e-mail: clgriff@ucthpx.uct.ac.za

Received 6 March 1996; accepted 11 March 1996

A sea anemone new to the South African fauna is reported from Table Bay Harbour and is identified as *Metridium senile*. The population was probably introduced from Europe and although well established, appears to be restricted to the harbour area.

Numerous exotic species have been either deliberately or accidentally introduced into terrestrial and freshwater ecosystems throughout South Africa, often with severe ecological consequences (MacDonald, Kruger & Ferrar 1986). By contrast, relatively few marine introductions have been documented (Griffiths, Hockey, Van Erkom Schurink & Le Roux 1992) and only two such species have established significant naturalized populations. These are the Mediterranean mussel, *Mytilus galloprovincialis*, which is now the dominant intertidal invertebrate along the west and southwestern coasts of the country (Van Erkom Schurink & Griffiths 1991), and the European shore crab, *Carcinus maenas*, which is locally abundant in Table Bay, but remains restricted to a few sheltered sites elsewhere (Griffiths *et al.* 1992).

In September 1995 one of us (CES) recorded the presence in Table Bay Harbour of a colony of sea anemones with lobed oral discs fringed by abundant, feathery tentacles — features inconsistent with any previously known anemone from the region. We subsequently photographed and collected several individuals from the Alfred Basin. Fully extended anemones were salmon pink in colour, with a column approximately 100 mm in height and 30 mm in diameter. The oral disc was expanded into five elongate lobes and fringed with extremely numerous, short 'feather-duster'-like tentacles. Specimens collected alive and held under laboratory conditions contracted to about 30 × 30 mm and maintained shorter lobes on the disc (Figure 1). These features undoubtedly identify the specimens as belonging to the genus *Metridium*, within the monogeneric family Metridiidae, a group not previously recorded from southern Africa.

Sea anemones of the genus *Metridium* are common from the littoral zone to depths of several hundred metres along both east and west coasts of North America and along the Atlantic coast of Europe (Fautin *et al.* 1989). Populations have also been reported from Japan (Uchida 1938) and Argentina (Riemann-Zurneck 1975). Systematic relationships within the genus have long been a subject of confusion, some