Zoosyst. Evol. 93 (1) 2017, 105–133 | DOI 10.3897/zse.93.10489

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A review of the reproductive biology of the only known matrotrophic viviparous anuran, the West African Nimba toad, *Nimbaphrynoides occidentalis*

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http://zoobank.org/1A298760-DD05-466F-AD01-23BF7619426C

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Received 13 September 2016
Accepted 14 January 2017
Published 3 February 2017

Academic editor:
Peter Bartsch

Key Words

Amphibia
development
evolution
ovary
oviduct
pueriparity
seasonality
testes
uterus

Abstract

Amphibians, and anurans in particular, show the highest diversity of reproductive modes among tetrapods. Nevertheless, viviparity is scarce in anurans and its occurrence is even more often assumed rather than confirmed. Probably the best studied viviparous amphibian is the Nimba toad, *Nimbaphrynoides occidentalis*. During more than 40 years of research, the Nimba toad's reproductive morphology, endocrine activity of the ovary as well as the pituitary gland, and to some extent the ecological impact (seasonality, humidity, food availability) on reproduction was examined. Due to the Nimba toad's unique reproductive mode, summaries are usually included in reviews discussing amphibian reproduction and articles on reproductive biology often discuss the exceptional reproductive system of Nimba toads. However, to our knowledge a detailed synthesis, summarising all the different original studies on the toad's reproduction, is so far missing. In this paper we review and summarise all available initial publications, which often have been published in French and/or are difficult to access. A short overview is given of the climatic and environmental conditions experienced by Nimba toads and the key findings supporting a "true" viviparous reproduction with matrotrophy (maternal provision of nutrition during the gestation) and pueriparity (birth of juveniles). Then foetal development (morphological, gonad and pituitary development), and the female (ovary, oviduct, pituitary and their endocrine interactions) and the male reproductive system (testes and pituitary) are reviewed. Finally, the reproductive cycle and its link to the Nimba mountains' seasonality and ecological/ conservation implications are discussed.

Introduction

Viviparity, in the sense of retaining developing eggs or foetuses within the female genital tract, evolved numerous times in vertebrates and within tetrapods especially in squamates (Blackburn 1999, 2015, Pyron and Burbrink 2014, van Dyke et al. 2014). Internal fertilisation is necessary to allow oviductal egg retention and viviparity (Wake 2015b). As most caudates (90%, Wake 2015a) and all caecilians are internally fertilising (Gomes et al. 2012, Wake 2015a) they show the first requirement for viviparity (Wake 2015a, 2015b). Nineteen (2.7%, Buckley 2012) of the ca 700 caudate species (695 AmphibiaWeb 2017, 703 Frost 2017, both accesses 11.01.2017) and about one fourth of the ca 200 caecilian species (205 AmphibiaWeb 2017, 205 Frost 2017, both accessed 11.01.2017) are known or assumed to be viviparous (Buckley 2012, San Mauro et al. 2014, Wake 2015a, 2015b). Anurans (frogs and toads), on the other hand, show a very high diversity in reproductive modes (at least 42 modes, Haddad and Prado 2005, Iskandar et al. 2014), but internal fertilisation and viviparity are very rare. Of the ca 6,700 recognised anuran species (6,714 AmphibiaWeb 2017, 6,678 Frost 2017, both accessed 11.01.2017), viviparity is only known or assumed in 17 species (0.3%) of five genera: *Craugastor* (1 species), *Eleutherodactylus* (1), *Limnonectes* (1), *Necto-*

phrynoides (13) and *Nimbaphrynoides* (1). Hence, within the Amphibia, viviparity is common in caecilians, rare in caudates and exceptional in anurans.

Viviparous reproductive modes may differ in two fundamental traits: i) at which developmental stage offspring are born and ii) in the way foetuses receive the nutritional energy for their development. During amphibian development the adult anuran body plan is only achieved after metamorphosis is completed (Duellman and Trueb 1986, Wells 2010). In larviparous species, offspring are born at any larval stage before metamorphosis (Greven 2003) and newborn larvae are dependent on open water. In pueriparous species offspring are born after metamorphosis (Greven 2003) and water independent juveniles are born. Within caudates 14 (64%) of the viviparous species and some subspecies of *Salamandra salamandra* (Buckley et al. 2007, Wake 2015a, 2015b) and *Salamandra algira* (Beukema et al. 2010, Escoriza and Ben Hasssine 2014) are pueriparous. In anurans only one of the known 17 viviparous species is larviparous (*Limnonectes larvaepartus*; Iskandar et al. 2014, Kusrini et al. 2015), all others (94%) are pueriparous. Within caecilians no larviparous species are known, nevertheless oviparous species may lay eggs in the neurula stage (Sarasin and Sarasin 1887, Kupfer et al. 2006, Gomes et al. 2012). Hence, pueriparity is the only known viviparous mode in caecilians and more common in anurans than in caudates.

Foetal development and growth needs energy. This energy can be provided by the mother via yolk rich (lecithotrophic) eggs, or by unfertilised eggs and smaller siblings (oophagy and embryophagy, sometimes called adelphophagy), or the mother continuously nourishes the foetus during gestation (matrotrophy). A good example for lecithotrophy is direct development: large, yolk rich eggs are deposited at humid locations, outside of water, and the entire development takes place within the egg, exclusively powered by yolk (Guibé and Lamotte 1958, Hanken 2003). The best-known example for matrotrophy are mammals, in which yolk poor eggs are fertilised and very early within the development the nutritional needs of the foetus are provided for by the mother via a placenta. Nutrition through unfertilised eggs and/ or intra-oviductal cannibalism of the offspring, is known for example from *Salamandra salamandra* subspecies (Buckley et al. 2007, Buckley 2012). Matrotrophy in amphibians may be achieved through the development of a particular intra-oviductal epithelium (e.g. "zona trophica" in *Salamandra atra*) with increased mitoses, cell growth and apoptosis (Vilter 1986, Wake 2015a, 2015b). This epithelium is consumed by the foetuses, for which they developed special dentition in salamanders and caecilians (Wake 1993, 2015a, Gomes et al. 2012). In some oviparous caecilians with maternal care, special epidermal cells are produced by the mother and eaten by the offspring (skin feeding, Kupfer et al. 2006, Wake 2015a). The distinction between lecitotrophy, oophagy and matrotrophy is not as sharp as between pueriparous and larviparous species and the different types are not mutually exclusive (Wake 2015a, 2015b). All eggs contain some amount of yolk which is consumed before other nutrition is used (Wake 2015a). The best studied viviparous salamander, *Salamandra atra*, for example combines lecitotrophy, with oophagy and matrotrophy (Vilter 1986, Wake 2015a). Within this species, only two eggs, one per oviduct, receive all egg layers necessary for fertilisation. In each oviduct one foetus develops, first dependent on its own yolk, after hatching by feeding on the unfertilised eggs within the oviduct and last by matrotrophy where foetuses feed of the epithelium cells in a "zona trophica" (Vilter 1986, Wake 2015a). Within caecilians and caudates matrotrophy is known from several species (San Mauro et al. 2014, Wake 2015b). In anurans, only a single species is known in which most energy for foetal development derives from matrotrophy (Xavier 1971, Wake 1993, 2015a, 2015b). This species is *Nimbaphrynoides occidentalis* (Xavier 1977, 1986, Wake 2015b).

This unique matrotrophic (and pueriparous) anuran is a small (snout vent length (SVL) 15–27 mm, Lamotte 1959, Xavier 1971) West African bufonid, endemic to a few square kilometres (4 km², Lamotte 1959) of the high altitude grasslands of the Nimba mountains (Lamotte 1959, Lamotte and Sanchez-Lamotte 1999, Hillers et al. 2008, Sandberger-Loua et al. 2016a), a small mountain chain in the tri-border area of Guinea, Ivory Coast and Liberia (Figure 1). Shortly after its description as *Nectophrynoides occidentalis* Angel, 1943, its unique viviparous reproductive mode was recognised (Angel and Lamotte 1944a, 1944b) and led to numerous, partly very detailed studies. After the internal fertilisation of very tiny (~500 µm) yolk-poor eggs (Angel and Lamotte 1944b, Lamotte and Rey 1957, Vilter and Lugand 1959a, Xavier 1971), the eggs are retained within the lower part of the oviduct (Angel and Lamotte 1944a, Vilter and Lamotte 1956, Xavier 1971), the oviductal epithelium secrets liquid mucoproteins to nourish the foetuses (Vilter and Lugand 1959b, Xavier 1971, 1977, 1986), which are born after nine months as fully developed juveniles (e.g. Angel and Lamotte 1947, Lamotte et al. 1956, Lamotte 1959, Xavier 1971). Françoise Xavier summarised her own large body of work in two book chapters (Xavier 1977, 1986) and summaries are included e.g. in reviews by M. Wake (e.g.: Wake 1993, 2015a, 2015b) and in K.D. Wells' book (2010). Within this review we synthesise all the extensive work by F. Xavier, but as well the studies of M. Zuber-Vogeli, M. Lamotte, J. Gavaud, V. Vilter and others.

A short history of the taxonomy of *Nimbaphrynoides occidentalis*

The Nimba toad was described as *Nectophrynoides occidentalis* Angel, 1943, due to its similarity to the then two known East African *Nectophrynoides* Noble, 1926 species, *N. viviparous* (Tornier, 1905) and *N. tornieri* (Roux, 1906). For the same reason the new species was assumed to be pueriparous and lecitotrophic (Angel 1943). Later it was even hypothesised to be parthenogenetic, as during the

first field survey M. Lamotte had not recorded any males (Angel and Lamotte 1944a, 1944b). During a second field period males and matings were observed and subsequently matrotrophy assumed (Angel and Lamotte 1944b). Dubois (1986) revised the genus *Nectophrynoides*, which at that time comprised oviparous, lecitotrophic viviparous and matrotrophic viviparous species and placed them, according to their reproductive mode, into four different genera: *Spinophrynoides* (for the oviparous Ethiopian *S. osgoodi*, now placed in *Altiphrynoides* and feared to be extinct; Gower et al. 2013), *Altiphrynoides* (for the direct developing *A. malcolmi* from Ethiopia), *Nectophrynoides* (now 13 lecitotrophic pueriparous species all from Tanzania, one extinct in the wild), and *Nimbaphrynoides* (now one species from the Nimba mountains in Guinea, Ivory Coast and Liberia). The *Nimbaphrynoides* population from Liberia was described as a separate species (Xavier 1978), but because of high genetic and acoustic similarity is now considered to be a sub-species of *N. occidentalis* (Sandberger et al. 2010). Based on morphological data it was assumed that *N. occidentalis* is more closely related to *Altiphrynoides* than to *Nectophrynoides* (Wake 1980b, Grandison 1981), and most closely related to *Didynamipus sjostedti* (Grandison 1981, Graybeal and Cannatella 1995), a small, probably direct developing forest toad from Cameroon (Gonwouo et al. 2013). A recent, comprehensive phylogenetic study by Liedtke et al. (2016) confirmed *Didynamipus* as sistergroup to Nimba toads. Interestingly, while *Altiphrynoides*, *Nectophrynoides* and *Nimbaphrynoides* are part of the same clade, none of these form a sistergroup relationship with each other. This suggests that direct development and viviparity seen in these taxa possibly evolved independently of each other (Liedtke et al. 2016).

The habitat of the Nimba toad

Nimba toads are endemic to the Nimba mountains, which are a south-west, north-east oriented mountain chain in Liberia, Ivory Coast and Guinea (see Figure 1). This steep-sided mountain chain is about 40 km long and in parts up to 12 km wide (Schnell 1952). The surrounding lowlands are at about 500 m asl, whereas the highest peak, Richard-Molard, reaches 1,762 m asl (Schnell 1952, Leclerc et al. 1955, Lamotte 1959, Lamotte et al. 2003). In its southern, Liberian part the mountain ridge is lower (Lamotte et al. 2003), and after mining activities now only rarely exceeds 1,200 m asl. In the northern, Guinean part the main ridge undulates between 1,200 m asl and 1,762 m asl (Leclerc et al. 1955). It consists of banded iron formations (Billa et al. 1999), which are of high global economic importance (Berge 1974, Lamotte 1983, Schnell 1987). The presence of Nimba toads and chimpanzees as part of a rich and endemic fauna and flora led to the declaration of the Nimba mountains as a World Heritage Site in Guinea in 1981 and Ivory Coast in 1982 (UNESCO July 22, 2015/2015). Due to increasing mining exploration activities, the WHS has been listed as in danger since 1992 (Poilecot and Loua 2009, UNESCO July 22, 2015/2015). In 2014 its outlook was considered to be "critical" (IUCN January 11, 2017/2014).

The climate of the area is characterised by first rains in March/ April, a rainy season from May to October and a dry season from November to February/ March. Mean yearly temperature is 25°C in the lowlands (550 m asl) and 19°C at high altitudes (Leclerc et al. 1955, Lamotte 1958, Lamotte and Roy 1962, Lamotte et al. 2003). Mean annual precipitation is 2,093 mm, but is estimated to range from 1,500 mm in the lowlands to 3,000 mm on the ridges (Lamotte 1959, Lamotte et al. 2003). The rainy season at high altitude is characterised by nearly constant fog and rather continuous, but not very heavy, rain (Lamotte 1959). The dry season is characterised by larger temperature fluctuations, low humidity, little rain and dry season fires (see Figure 2, Schnell 1952, Lamotte 1958, 1959). The change from one season to the other is characterised by tornadoes and thunderstorms (Schnell 1952, Leclerc et al. 1955, Lamotte 1959). The first rains after the dry season may arrive between the end of February and April, whereas the very humid rainy season does not

Figure 1. The Nimba mountains. Left: elevation map of the Nimba mountains, with an inset map showing the position of the Nimba mountains within West Africa. Right: a large part of the Nimba mountains showing the steep slopes, the high altitude grasslands, the forests in the lowlands and the ravines.

Figure 2. Rainy and dry season at Nimba. The rainy season (top) is characterised by persistent fog and rain, whereas the dry season (middle) is characterised by little rain, high temperature fluctuations and dry season fires. After dry season fires the grasses sprout very fast (bottom, © Nèma Soua Loua).

start before June (Schnell 1952, Leclerc et al. 1955, Lamotte 1959). The transition between the rainy and the dry season is much faster and happens during a few weeks between October and November (Lamotte 1959). High elevations receive more rain than the lowlands during the rainy season, but less rain during the intermittent and dry seasons (Lamotte 1959, Lamotte and Roy 1962).

The areas outside the World Heritage Site are almost exclusively anthropogenically altered landscapes (Schnell 1952, 1987, Fournier 1987, Lamotte et al. 2003). Within the reserve the lower flanks and lowlands of the Nimba mountains are mainly covered in evergreen forests. In the South forests naturally grow as well on the mountain ridges, whereas in the North they are restricted to ravines (Lamotte et al. 1962, Fournier 1987). The lowland forests are a mosaic of primary and secondary forests (Lamotte 1947b, Schnell 1952, 1987, Fournier 1987). At higher altitudes the lowland forests transition into a montane forest characterised by lower tree diversity, the presence of *Parinari excelsa,* many epiphytes and less lianas (Schnell 1952). Savannas are mostly found in the lowlands on the eastern sides of the mountain chain, growing on thinner top-soils than forests (Schnell 1952, 1987, Fournier 1987, Lamotte et al. 2003). Within Guinea the largest parts of the high altitudes are covered in montane grasslands (Lamotte 1947a, 1947b, 1958, 1959, Schnell 1952, 1987, Lamotte and Roy 1961a, 1962, Lamotte et al. 1962, 2003a, Fournier 1987). These grasslands are characterised by the high abundance of the grass *Loudetia kagerensis* (Schnell 1952, 1987, Fournier 1987), fast regrowth after fires and despite their thin layer of top soil a large herbaceous biomass (Fournier 1987). Depending on the soil characteristics and anthropogenic impacts (e.g.: mining roads) slightly differing plant assemblages can be recognised (Fournier 1987, Schnell 1987). Standing open water is present only at two locations on the mountain top and only for some months during the rainy season (June/ July-September). These ponds are very shallow (< 5 cm). Due to the rarity of open water, the harsh climatic conditions and the isolation of the mountain a high percentage of the species in the high altitude grasslands is endemic (Lamotte and Roy 1961a), such as the Nimba toad, which occurs exclusively in the high altitude grasslands above 1,200 m asl (Lamotte 1959, Hillers et al. 2008, Sandberger-Loua et al. 2016a).

Annual seasonality and activity patterns

The very pronounced differences between the rainy and the dry season has strong effects on the toad's activity patterns (Lamotte 1959, Sandberger-Loua et al. 2016a) and their reproductive cycle (Xavier 1971, Gavaud 1977). Nimba

Figure 3. Nimba toad females. Left: a young female towards the end of the rainy season. Right: a large gestating female in June, shortly before parturition.

toads are only active during the rainy season (Lamotte 1959, Sandberger-Loua et al. 2016a). With the first rains gestating females emerge from the aestivation sites, later joined by virgin females and males (Lamotte 1959, Xavier 1971, Lamotte and Sanchez-Lamotte 1999). When rains become more permanent and humidity rarely drops below saturation, juveniles are born (compare Figure 3, Angel and Lamotte 1947, Lamotte 1959). Nimba toads mate at the end of the rainy season, in September/ October, rarely November (Angel and Lamotte 1947, Lamotte 1959, Xavier 1971, Lamotte and Xavier 1976b, Lamotte and Sanchez-Lamotte 1999). Mated females go underground, whereas unmated females and males may stay as long above ground as the rains continue (Lamotte 1959, Xavier 1971). During the dry season toads are dormant underground (Angel and Lamotte 1947, Vilter 1955, Lamotte and Xavier 1976a), hiding under rocks and within crevices (Vilter 1955), where humidity is higher and temperature less variable (Lamotte 1959). Nimba toads have a gestation period of nine months (October-June, e.g. Angel and Lamotte 1947, Lamotte et al. 1956, Lamotte 1959, Xavier 1971), of which they spend about the first six months (may vary between 4–7 months, October/ November to February/ March/ April) dormant underground and only the last 3 months (2–5 months, February/ March/ April to June) active above ground (e.g.: Angel and Lamotte 1947, Lamotte 1959).

Nimba toad females have a life expectancy of three to four, rarely five years; males reach even only up to three years (Castanet et al. 2000). On average, females give birth to nine young per gestation (range 4–20, Angel 1943, Angel and Lamotte 1944a, 1944b, Vilter 1956a, Lamotte and Xavier 1976a). However, the number of offspring born per gestation increases with age and hence, with size (Lamotte 1959, Xavier 1971). The average number of offspring per female in their first year is four, in the second 6.5, in their third 9.4 and in their fourth year 12 young (Xavier 1971). Assuming a life expectancy of three years, each female may give birth to about 20 offspring during her lifetime (32 if females survive four years). For a toad this is a very small number (Liedtke et al. 2014). This even assumes that females are mature three months after their birth and mate at the end of the rainy season of the same year. Depending on the length of the rainy season, between 30% and 70% of the respective year's newborn females become mature, nevertheless, the range indicates that an equal number of females become mature only the following year (Angel and Lamotte 1947, Lamotte and Rey 1957, Lamotte 1959). Average female lifetime reproductive output may thus even be much smaller.

In summary: Nimba toads are endemic to the high altitude grasslands above 1,200 m asl of the Nimba mountains in West Africa. Their sexual cycle and their activity are strongly linked to the local seasonality. They spend the dry season underground (4–7, on average 6 months) and they are active only during the rainy season. The active time covers the last three months (range 2–5 months) of the gestation and about three months between gestations. All Nimba toad males and 30–70% of females become mature within three months, the remaining females within 15 months. The female life-time reproductive output is very low with a maximum of 20–32 offspring.

Mating

Generally females are larger than males (females: SVL: 15–27 mm, Xavier 1971, 1986, 2009, Lamotte and Xavier 1976b, males: SVL: 15–23 mm, Lamotte and Xavier 1976b, Xavier 1986, 2009). Males have a darker back (Le Quang Trong 1967) and during most of their active above ground life recognisable nuptial pads (Angel and Lamotte 1947, 1948). Nuptial pad development is linked to spermatogenesis (Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966), and can be observed in males with an SVL of

Figure 4. Female and male cloaca of Nimba toads and a pair in amplexus. The female cloaca (top left) is close to the urostyle, whereas the male cloaca (top right) is ventrally oriented. During mating it swells and encloses the female cloaca. During the amplexus lumbalis the female is constantly horizontally swaying (bottom).

only 13 to 14 mm (Angel and Lamotte 1948). The most important secondary sex difference, however, is the position of the cloaca (Angel and Lamotte 1947, Xavier 1971, 1986). The female cloaca is very close to the urostyle, whereas the male cloaca is more ventrally positioned and the distance to the urostyle is larger (Figure 4; Angel and Lamotte 1947, 1948, Xavier 1971).

Mating occurs without a copulatory organ (Angel and Lamotte 1947, Xavier 1971, 1986). Sperm transfer is ascertained by cloacal apposition (Xavier 1971), in which the male cloaca swells and encloses the female cloaca (Angel and Lamotte 1948, Xavier 1971). In contrast to all other bufonids amplexus is lumbal and is accompanied by a particular behavioural repertoire, described by Xavier (1971): males crouch on their front legs and as soon as the female moves, follow her and grab her tightly in the groin. This is supported by the spines of the nuptial pads on the males' thumbs and first fingers. Females are often injured by these nuptial pads during mating. As soon as the female is grabbed she starts swaying from one side to the other without cease. Mating occurs mainly during the day and may take several hours to more than one day (Xavier 1971). If mating occurs during the night it is interrupted by torch light (Angel and Lamotte 1948).

Due to the long duration of mating, it was assumed that Nimba toads may ovulate during this time (Lamotte et al. 1956). However, it was later shown that females, if kept without males, also ovulate (Xavier 1974). The unfertilised eggs are kept within the enlarged distal end of the oviduct and a pseudo-gestation develops (Xavier 1969, 1971, 1974). In some other amphibians with internal fertilisation, a receptaculum seminis (spermatheca) can be found (Wake 2015a). In Nimba toads fertilisation quite likely occurs within the upper part of the oviduct, but no receptaculum seminis nor any other accumulation of sperm in any part of the oviducts was found (Xavier 1971). Nevertheless, in 25% of Nimba toad litters more than one sire is needed to explain the genotypes found (Sandberger-Loua et al. 2016b).

In summary: mating occurs without specialised copulatory organs and sperm transfer is achieved through direct contact of the morphologically differently positioned male and female cloacae. Mating position is an amplexus lumbalis, being exceptional within Bufonidae. No receptaculum was found, nor any other accumulation of sperm, nor indication of ovulation induced by mating. Mating takes several hours and polyandry occurs.

Matrotrophy

Nimba toad eggs are very yolk poor and with a diameter of 500–600 µm (on average 540 µm, Vilter and Lamotte 1956, Vilter and Lugand 1959b, Lamotte et al. 1964, Xavier 1971, 1986) and a weight of 220 µg (Xavier 1971), particularly small. Newborn toadlets on the other hand, have an average SVL of 7.5 mm (Lamotte and Xavier 1976a, Xavier 1977, range: 6–10 mm, Angel and Lamotte 1944b, Lamotte and Rey 1957, Xavier 1971), this is one third of the mother's SVL (Lamotte et al. 1956, Xavier 1971), and an average weight of 45 mg (range: 30–60 mg, Xavier 1971, 1977, Lamotte and Xavier 1976a, 1976b). In a family-wide analysis of African bufonid egg sizes, Nimba toad eggs were described as being exceptionally small and well below the average egg size for other species of comparable adult size (Liedtke et al. 2014). Two anurans of similar adult size are, for example, the direct developing *Arthroleptis crusculum* (SVL < 20 mm, Guibé and Lamotte 1958) and the lecitotrophic *Nectophrynoides tornieri* (SVL: 25–27 mm, Angel and Lamotte 1948). In *Arthroleptis crusculum,* eggs are 3.5 mm in diameter (Guibé and Lamotte 1958, 7× the size of a Nimba toad egg) and newly hatched juveniles have a SVL of 5 mm (0.67× the size of Nimba toad toadlets). In *Nectophrynoides tornieri,* eggs measure 2 mm in diameter (4× the size of a Nimba toad egg) and newborn juveniles 5.4 mm (Lamotte and Xavier 1972a, 0.72 × of Nimba toad toadlets). Hence, metamorphs in the direct developing species are 1.4 times larger than the egg, in the viviparous, lecitotrophic toad 2.7 times larger and in the matrotrophic Nimba toad 15 times larger than the egg. This huge increase in size from egg to juvenile and particularly in weight (Nimba toad juveniles are > 200 times heavier than the eggs, Vilter and Lugand 1959b, Xavier 1971), requires energy, which has to be provided by the mother.

Matrotrophy is characterized by the transport of (nutritional) energy from the mother to the foetus. It is challenging to prove this process, nevertheless matrotrophy was very early (Vilter and Lugand 1959b) assumed in Nimba toads. No placentation occurs (Xavier 1971, Lamotte and Xavier 1976b), but matrotrophy was first linked to the observation that within the oviductal liquid microscopic whitish droplets were observed ("uterine milk", Vilter and Lugand 1959b). These were likewise found in the vacuoles within oviductal epithelium cells (Vilter and

Figure 5. Foetal labial papillae. Labial papillae are forming during stage Ia (left) and are well developed at stage Ib (right). See foetal development for more information on developmental stages. Redrawn after Lamotte and Xavier (1972b).

Lugand 1959b). Additionally it was observed that very early during the foetal development labial papillae appear (Figure 5) and foetuses have well developed intestines and livers very similar to adults early on (Angel and Lamotte 1944b). The hypothesis thus was that foetuses feed on the liquid "uterine milk" via the labial papillae (Vilter and Lugand 1959b, Xavier 1971, Lamotte and Xavier 1976b). To prove the nutritional transfer from mother to foetus, Xavier (1971) injected radioactively marked amino acids into gestating females. Six percent of these radioactively marked amino acids could be detected within 30 hours in the foetuses. They first were detectable in the digestive system, then in the very large liver and after 48 hours, in foetal muscle and brain tissue (Xavier 1971, 1977, 1986). This indicates that amino acids from the mother are transferred to the foetus and that they enter the foetus through the digestive system. This supports the hypothesis that mucoproteins are synthesised and released by the distal oviduct epithelium cells that form nutrients that are being fed upon by the foetuses with their labial papillae. The exact function of the papillae is unknown. Nevertheless, temporally they are linked to the presence of white droplets and it can be speculated that they are used to mechanically induce the mucoprotein secretion. It was also shown that well-nourished females gave birth to larger juveniles than under-nourished females (Xavier 1971), which provides additional supporting evidence for the importance of maternal nutrient transfer to the developing foetuses.

Another possibility to transfer nutrients from the mother to the foetuses is the ovulation of unfertilised eggs, or intra-oviductal cannibalism of other foetuses (Wake 2015a). Nimba toad follicles develop into corpora lutea after ovulation and persist for the time of gestation (Lamotte and Rey 1954, Lamotte et al. 1956, 1964, Xavier 1970b) and hence, the number of ovulated eggs can be compared with the number of foetuses in the oviduct (Lamotte et al. 1964). Usually all of the ovulated eggs develop into foetuses, but up to three more corpora lutea than foetuses were observed (Lamotte et al. 1964). While cannibalism or feeding on unfertilised eggs and other foetuses cannot be completely ruled out, it is unlikely to constitute a major source of nutrients to the developing foetuses.

In summary: newborn Nimba toads are 15 times larger and > 200 times heavier than the egg. It was shown that marked amino acids injected into mothers are detectable within 30 hours in the foetal digestive system and liver. This supports the hypothesis that foetuses take up their nutrition through the very early developed digestive system and nutrition is provided by oviductal epithelial mucous cells. Additionally, matrotrophy is supported as foetal size at birth is linked to environmental conditions during the last third of the gestation period, during which females are active and most of the foetal growth occurs.

Parturition

Parturition may take several hours to over two days, depending on the number of offspring (Xavier 1971, Lamotte 1982). Juveniles are either born legs or head first, on their venter or on their back, depending on the position they had within the distal end of the oviduct (Xavier 1971, 1986). At the end of gestation nearly all of the body cavity of the female is occupied by the oviducts (compare Figures 3 and 6a), severely restricting all other organs (Angel and Lamotte 1944a, Vilter 1956a, Xavier 1971). At this stage, the distal end of the oviduct becomes very stretched and the wall, especially the muscle layer, is very thin and supposedly too thin and weak to induce labour (Angel and Lamotte 1944a, Vilter 1956a, Xavier 1971). In any case, the injection of labour inducing pharmaceuticals had no effect on gravid *N. occidentalis* (Vilter 1956a). V. Vilter (1956a) was the first to observe females giving birth and developed a scenario on how birthing is achieved. Females press their thighs to their flanks, the feet are further away from the body, the two legs building a double W and are in that way supporting the pressure on the flanks (Figure 6b, c). V. Vilter (1956a) hypothesised that with their lungs (possibly too small and simple, Angel and Lamotte 1944b, compare Figure 7) and with some muscle support they are at the same time increasing the intraperitoneal pressure on the distal end of the oviduct from the cranial direction. The whole body is pressed to the ground, barring oviductal extension towards the ground. Vilter's (1956a) hypothesis is that with this posture the female is restricting the space within the oviduct and forces the juveniles to take the "only possible way out", through the cloaca. As support for his idea he claims that females just before or after giving birth are more sensitive to light (smaller pupils when exposed to light than females several weeks before giving birth). This would make them seek out shelters offering suitable support for the "birthing posture" and protecting them during birthing (Vilter 1956b).

There is indication that first all juveniles from one and only thereafter from the other oviduct are born (Xavier 1971). The passage through the cloaca is very fast (a few seconds), but if parturition is interrupted at this stage the juvenile dies, if juveniles already die within the oviduct they will stay there until the mother dies of sepsis (Xavier 1971). These latter observations indicate an active part of the juveniles during parturition.

In summary: Nimba toad females have not enough muscle power in their oviducts to induce labour. Hence, they induce birth through a unique "birthing posture". If foetuses die within the oviduct or during parturition, they may not be evacuated, hinting at necessary juvenile activity during parturition.

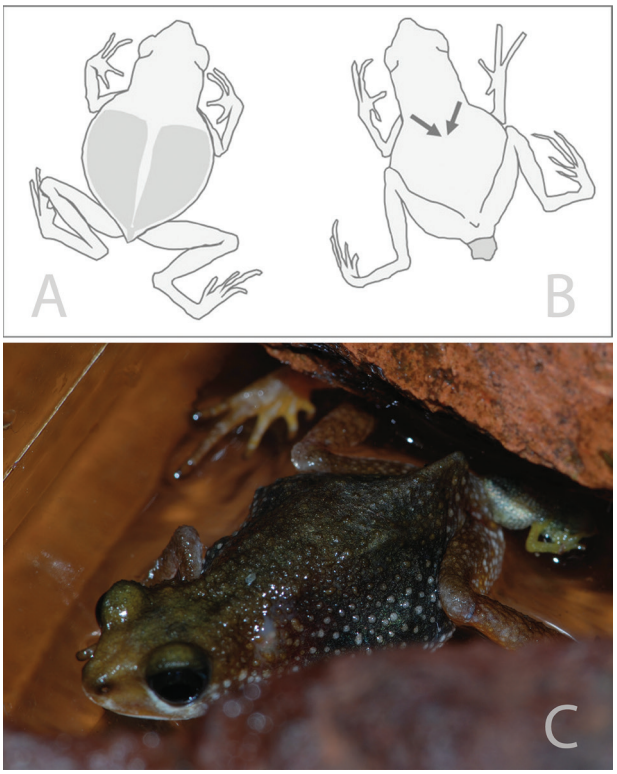

Figure 6. "Birthing posture" in Nimba toads. **A**: gestating Nimba toad; the grey shading indicates the size and position of the enlarged distal parts of the oviduct (uterus), **B**: shows the "birthing posture", in which females build a double W with their legs and increase pressure on their uteri. Compare the position of legs on the photograph on the right (**C**) of a female giving birth, showing likewise the double W, of the legs. **A** and **B** are redrawn after Vilter (1956a).

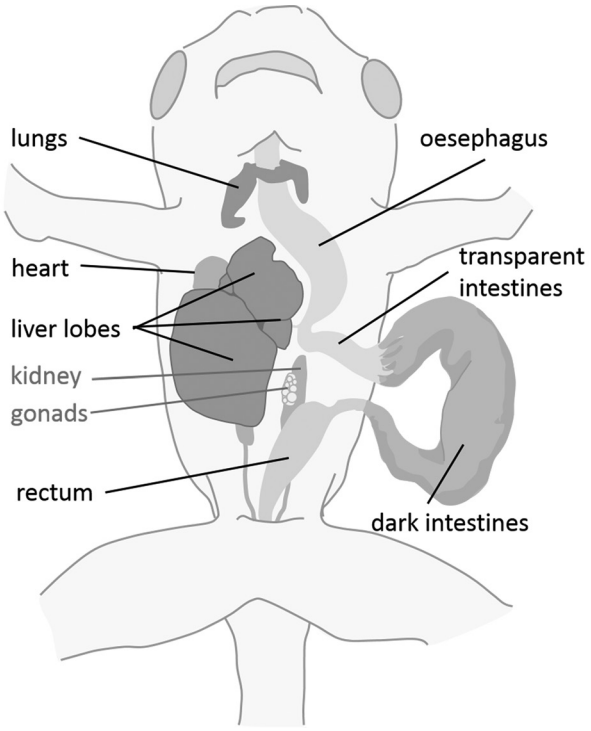

Figure 7. Foetal digestive system. Exceptional for an anuran foetus is the straight and differentiated gut, with an oesophagus, the transparent and the dark intestines (stomach) and a rectum, as well as the large liver lobes. Additionally, shown are small lungs (as well small in adults), the heart, kidney and gonads. Redrawn after Angel and Lamotte (1944a).

Foetal development

Aquatic tadpoles of other anurans generally have a tail, with more or less pronounced fins, first external and later internal gills, a coiled gut, a spiracle, and mouth parts (i.e. horny beaks, labial teeth (keratodonts) and labial papillae, Altig and McDiarmid 1999, Channing et al. 2012). Nimba toad foetuses have a large square head and large eyes (Angel and Lamotte 1944a). They have neither internal nor external gills, nor spiracle, nor coiled gut and neither keratodonts nor horny beaks at any time during their development (Angel and Lamotte 1944a, 1944b, Xavier 1971, 1986, Lamotte and Xavier 1976a, 1976b). Their tails have very narrow fins and for most of the time they possess labial papillae (Angel and Lamotte 1944a, 1944b, Xavier 1971, 1977, 1986). The gut and the large and well developed liver are already adult-like relatively early during development (Figure 7, Angel and Lamotte 1944b). These peculiarities of the foetal morphology preclude the application of Gosner stages (Gosner 1960) usually used to describe early anuran development (Xavier 1971). Therefore, we use herein the eight stages for the foetal development defined and used by Zuber-Vogeli and Bihouès-Louis (1971), Lamotte and Xavier (1972b) and Lamotte et al. (1973). For the different stages see Figure 8.

Stage 0

Stage 0 is the earliest described stage; it has a duration of less than two months. At stage 0 foetuses have a tail, some yolk is still visible, no eyes, no pigmentation and no cloacal opening. During this stage the intestines are forming. Foetus size varies between 1.0–2.7 mm (body: 0.6–1.4 mm, tail: 0.4–1.3 mm, Lamotte and Prum 1957, Lamotte and Xavier 1972b). Gonocytes (foetal germ cells) are already identifiable and large (range 25–55 µm, most 30–40 µm), and they assemble in a line between the aorta and the Wolffian ducts (Lamotte et al. 1973).

Stage Ia

Stage Ia has a duration of less than two months. Some yolk is still visible in early stage Ia foetuses but completely resorbed in older specimens. During this stage the head develops fast, the eyes appear and become pigmented, the mouth, first visible as a depression, develops into a slit and connects to the oesophagus, and papillae appear on the lower and upper lip (Lamotte and Xavier 1972b). The lobes of the liver become visible and the first pigmentation appears, hind limb buds are still absent (Lamotte and Xavier 1972b). Body size is on average 1.6 mm, tail length 1.9 mm. All gonocytes migrate into the periphery of the primordial gonads. Gonads are longer than wide and built stalks. A renal stalk protrudes into the gonad. At the end of stage Ia sex differentiation occurs, and all intracellular vitelline flakes

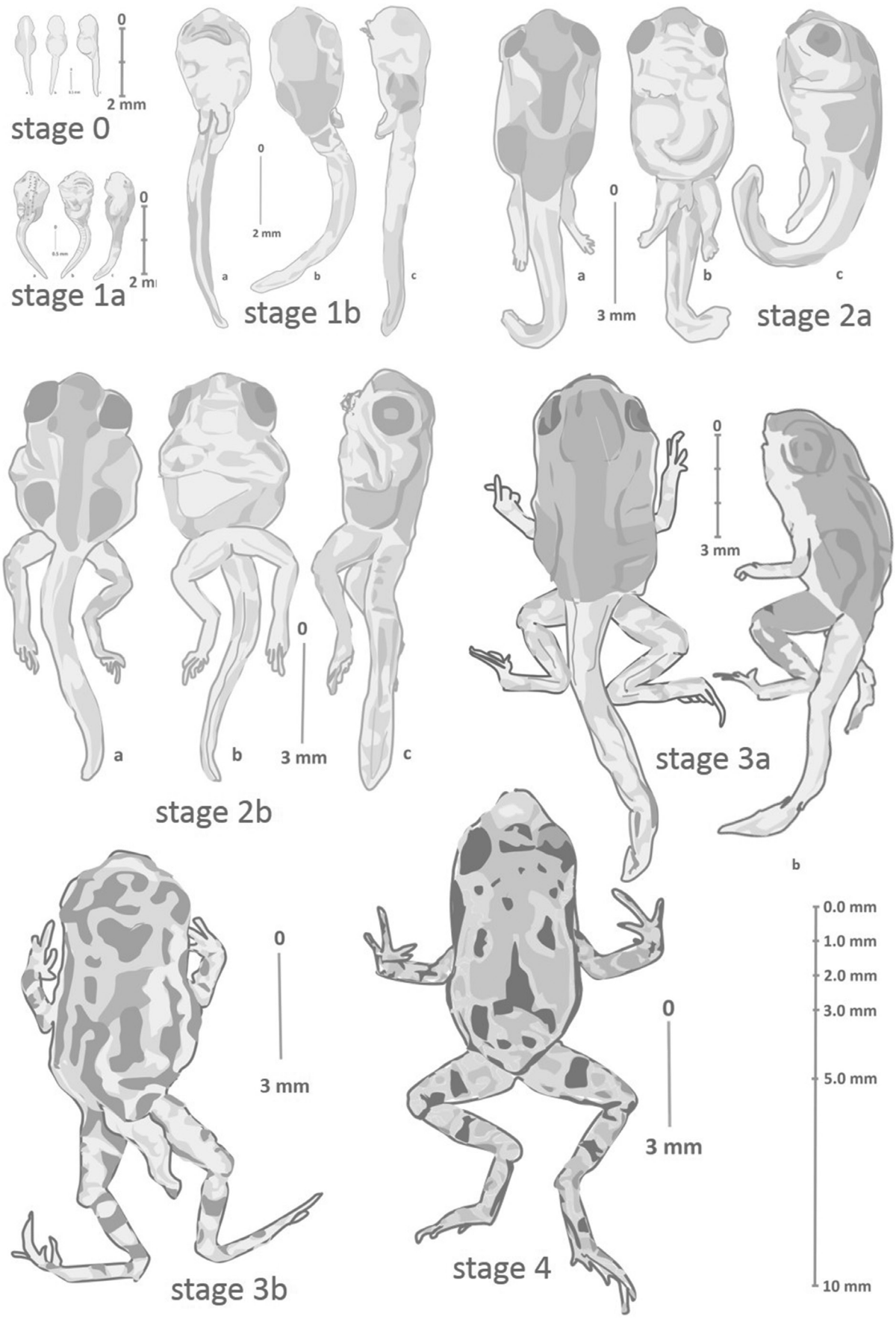

Figure 8. Embryonic development. Shown are the eight stages as found in the literature. Redrawn after Lamotte and Xavier (1972b).

are resorbed (Lamotte et al. 1973). Within the pituitary gland the pars distalis, pars intermedia, pars tuberalis and the nervous lobe are differentiated (Zuber-Vogeli and Bihouès-Louis 1971).

Stage Ib

Stage Ib has the longest duration (about two months). It is characterised by the appearance of the hind limb buds and their separation into thigh and lower leg. Front limbs develop under the transparent opercular skin fold. Pigment cells start to appear on the flanks. The digestive system and large liver-lobes are well developed (Lamotte and Xavier 1972b). Total length of foetuses varies between 3.8–6.2 mm (body: 1.8–2.4 mm, tail: 2–4 mm, Lamotte and Xavier 1972b). The small ovaries (200–300 µm) are pear-shaped and stalked, sometimes with a central cavity. They contain oocytes and oogonia in pre-meiosis, which are arranged in the periphery of the ovary. During this stage the first follicle cells appear and some oogonia start growing. The testes have a compact appearance, are small (300 × 70 µm) and become more elongated. The cells within the testes show no apparent order, but large spermatogonia can be observed (Lamotte et al. 1973). Within the pituitary gland the first glycoprotein type 1, 2 and 3 cells, and protein type 1 cells appear (Zuber-Vogeli and Bihouès-Louis 1971, Zuber-Vogeli and Doerr-Schott 1984). The latter are distributed in all areas of the pars distalis (Zuber-Vogeli and Doerr-Schott 1984). For cell type descriptions see the section on the (female) pituitary gland below.

Stage IIa

This stage has a duration of about one month and its beginning coincides with the emergence of females from their underground aestivation sites. It is characterised by the further differentiation of the hind limbs into thigh, lower leg and foot. The limbs are still short, and at the end of this stage the toes appear but are not separated. Foetuses of this stage have a large liver and the cloacal tail piece (sensu Gosner 1960) is still present. A clear boundary between body and tail becomes apparent; the eyes protrude and increase in size. Pigment cells increase in number; the pigmentation starts intensifying at the head and the darker colour arrives in the middle of the back at the end of this stage, the dark circles at the flanks persist. The hind limbs begin to be dorsally pigmented (Lamotte and Xavier 1972b). Foetus size varies between 6.4 and 12.5 mm (body 2.4–5.3 mm, tail 4–7.5 mm, hind limbs: 0.9–3.4 mm, Lamotte and Xavier 1972b). The ovary continues increasing in size, oocytes increase in number and their cytoplasm increases in volume (Lamotte et al. 1973). During this stage glycoprotein type 2 cells (corticotropic cells) and melanotropic cells are present in the pars distalis and pars intermedia. Protein type 2 cells (somatotropic cells) were rare before but appear in this stage in the dorso-caudal region of the pars distalis (Zuber-Vogeli and Bihouès-Louis 1971).

Stage IIb

This stage has a duration of less than one month. It is characterised by the elongation of the limb and to a lesser extent by their differentiation. The metatarsal tubercle appears, toes become separated and the front limbs continue developing beneath the opercular skin fold and start distending the thin membrane. The eyes grow quickly and the pupil appears. The mouth is still surrounded by papillae (Lamotte and Xavier 1972b). At this stage the pituitary gland changes, becomes spherical, the sinus of the pars distalis becomes extended; the position of the pars intermedia is modified as the median protuberance is thickening. Mitoses of the glycoprotein type 1 cells are observed (Zuber-Vogeli and Bihouès-Louis 1971). About 1/10 of the surface of the thyroid gland becomes vascularised (Lamotte and Prum 1957).

Stage IIIa

This stage has a duration of a few weeks. It is characterised by the start of the tail resorption, the rupture of the opercular skin fold and the breaking through of the front limbs. Front and hind limbs are coloured dorsally with the species-specific stripes. The head elongates, nares are visible, the pupils are further developed and the labial papillae start to decrease in size (Lamotte and Xavier 1972b). Some of the oogonia continue growing until the first ovulation. Within testes spermatocytes start maturing, conjunctive tissue appears which builds the basis of the future seminiferous lobules. The intra-testicular oocytes, present in low numbers until that stage, start decreasing in size (Lamotte et al. 1973). Protein type 1 cells and glycoprotein type 1 cells are still visible and distributed in all areas of the pars distalis, but for the latter secretory activity is reduced (Zuber-Vogeli and Doerr-Schott 1984).

Stage IIIb

This stage has a duration of a few weeks. It is characterised by the resorption of the tail and the labial papillae. The head becomes more elongated; the pupils are fully developed. Foetuses already show the characteristic juvenile colouration (Lamotte and Xavier 1972b). The ovaries are large (300–600 µm, Lamotte et al. 1973). The activity of the glycoprotein type 1 cells is reduced, glycoprotein type 3 cells are very active and protein type 1 cells, melanotropic and protein type 2 cells still present (Lamotte et al. 1973, Zuber-Vogeli and Doerr-Schott 1984). The thyroid is highly vascularised (1/3 of the surface) and the colloid amount reduced drastically (Lamotte and Prum 1957).

Stage IV

This stage has a duration of just a few days. At this stage foetal development is completed, the juvenile toads are a bit stockier and have slightly longer extremities and proportionately larger eyes compared to adults. Labial papillae are absent, the nares moved lateral. SVL rang-

es between 6–10 mm, hind legs ca. 9 mm, front legs, 5.6 mm and on the feet all tubercles are visible (Lamotte and Prum 1957, Lamotte and Xavier 1972b). Large oocytes grow particularly fast before birth. Some lobes are present in testes; the first vascularised interstitial tissue appears; Sertoli cells appear at the beginning of the second cycle of meiosis. Most often spermatocyte maturation begins with birth, nevertheless spermatogenesis was observed even before birth (Lamotte and Prum 1957, Lamotte et al. 1973). Thyroid activity increases again within this stage (Lamotte and Prum 1957). The pituitary gland of newborn toads does not differ from the pituitary gland of adults (Zuber-Vogeli and Bihouès-Louis 1971).

In summary: Nimba toad foetuses are characterised by the absence of a coiled gut, internal and external gills, spiracle, horny bill and labial teeth, and the presence of a gut similar to those of adults, large livers, a large head with large eyes, a mouth with papillae and the early development of the reproductive, locomotor, digestive, and respiratory systems. The foetal development is linked to the different seasons experienced by the adults. During the first 5–6 months, the time mothers spend underground during the dry season, development is slow (stages 0-IIa, Xavier 1971, 1986, Lamotte et al. 1973, Lamotte and Xavier 1976b). Foetal development and growth is accelerated from the moment of emergence and the start of the active life of females (Xavier 1971, 1986, Lamotte et al. 1973, Lamotte and Xavier 1976a).

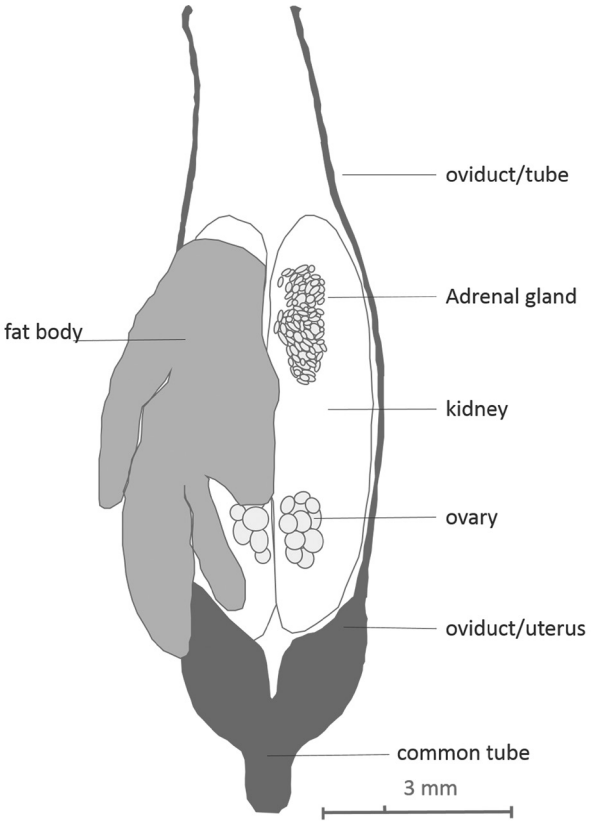

Figure 9. The female reproductive system. Redrawn after Lamotte et al. (1964).

Female reproductive system

Within this section the morphology and temporary changes of the oviduct, ovary and the pituitary gland are described and their possible interactions are discussed. The most complete description of the female reproductive system is given in the doctoral thesis of Xavier (1971).

Oviduct

The oviduct is rather simple, it consists of two parallel strings (Figure 9, Lamotte and Tuchmann-Dubplessis 1948, Xavier 1971, 1986), which can be separated into three parts: each string in the tube or oviduct sensu stricto, the uterus, which is the lower enlarged part, in which the foetuses develop (non-homologous to the mammalian uterus) and the common tube, a short tube which unites the lower-most part of both uteri (Xavier 1971, 1976).

Tube or oviduct sensu stricto. The tube consists of an inner epithelium, a connective tissue, a thin muscle layer and is surrounded by a thin envelop (Xavier 1971). The epithelium is ragged and two cell types can be distinguished, ciliated cells and mucous cells. Based on histology and the epithelial secretions produced, the tube can be separated into four parts (from anterior to posterior): i) a ciliated part, ii) a part in which mainly acidic secretions occur, iii) an acidic and neutral part, in which acidic and neutral secretions are observed and iv) a neutral part with exclusively neutral secretions (Xavier 1971, 1973).

Within a reproductive cycle the tube changes little. It is hypertrophic and active only before and during ovulation (diameter between 350–450 µm, Vilter 1957) at the end of the rainy season (Vilter 1957, Xavier 1971, 1973, 1974, 1976, 1986, Lamotte and Xavier 1976a). After ovulation activity stops and the tube diameter decreases in size until it reaches its thinnest diameter during dormancy (Vilter 1957, Xavier 1971). Some weeks after emergence, activity is slowly taken up again, but only speeds up some weeks after parturition (diameter at parturition: 100–110 µm) for the preparation of the new ovulation (Vilter 1957, Xavier 1971, 1974). During gestation the tube does not change much (Lamotte and Tuchmann-Dubplessis 1948, Xavier 1971).

Uterus. As a modification of the tubular oviduct sensu stricto, the uterus also consists of an epithelium, connective tissue, a thin muscle layer and an envelope (Lamotte and Tuchmann-Dubplessis 1948, Xavier 1971). Depending on the season only mucous cells or mucous and ciliated cells can be recognised (Angel and Lamotte 1948, Lamotte and Tuchmann-Dubplessis 1948, Xavier 1971, 1973, 1986). Some weeks after birthing the uterus is 2–3 mm long, 0.5–1 mm wide and 0.5 mm high (Xavier 1971), just before ovulation it already increased in size to 3–4 mm length, 1–2 mm width and 0.5–2 mm height (Angel and Lamotte 1948, Lamotte and Tuchmann-Dubplessis 1948, Xavier 1971). During the last months of gestation the uterus is very large, filling most of the abdomen and squeezing all other organs (Lamotte and Tuchmann-Dub-

plessis 1948, Xavier 1971). Within one reproductive cycle three phases can be distinguished: a proliferation phase, starting 12 days after parturition and lasting until ovulation, a secretion phase, starting with ovulation and ending with parturition and an apoptosis phase during the first 12 days after parturition (Xavier 1971, 1973, 1977, 1986).

The proliferation phase starts in July/ August when the new epithelium is built from the connective tissue (Xavier et al. 1970, Xavier 1971), at first ciliated and mucous cells are present and within mucous cells mitoses can be observed (Xavier 1971, 1973, 1986). At the same time blood vessels appear during the first gestation and increase in number in the following gestations (Xavier 1971, 1986). In September, shortly before ovulation, the ciliated cells disappear, the epithelium is folded into longitudinal ridges, blood vessels form within the ridges of the epithelium, between the connective tissue and epithelium (Xavier 1971).

The secretion phase lasts the entire gestation period and is characterised by the secretion of mucoproteins by all mucous cells. Nevertheless, the importance of various factors changes over the duration of the gestation. During the first part of the gestation, mainly within the first part of dormancy, the muscle layer increases, the vascularisation of the connective tissue increases and mitoses can be observed (Angel and Lamotte 1948, Xavier 1971, 1977, 1986, Lamotte and Xavier 1976a). At the end of the gestation, when foetuses are at stages III and IV (the time the labial papillae start being resorbed, Xavier 1971, Lamotte and Xavier 1972b) the secretion of mucoproteins decreases and glycoproteins are secreted and the blood vessels open into the uterine cavity (Xavier 1971, 1973, 1986).

The apoptosis phase is the shortest and lasts only for about 12 days (Xavier 1971, 1973, 1986), nevertheless it has the largest impact on the uterus. After parturition the uterus collapses, the muscle layer and the connective tissue are arranged in lateral folds, but the epithelium shows no structure after collapse (Vilter and Lamotte 1956). The reason for the different reactions is that the epithelium detaches from the connective tissue and is finally phagocytised within the uterine cavity (Xavier 1971, 1973, 1976, 1977, 1986). The secretion of glycoproteins, starting already at the end of the secretion phase, is intensified and this leads to the disconnection of the epithelium (Xavier 1971, 1973, 1977, 1986). At the same time, the blood vessels are surrounded and phagocytised by many mast cells, and from the connective tissue a new epithelium is building (Xavier 1971, 1973, 1977, 1986). After 12 days the uterus has the same appearance as that of virgin females, the epithelium is ragged, contains mucous and ciliated cells, and some mitoses can be observed.

Common tube. The common tube consists of an epithelium, connective tissue, a muscle layer and an envelope. The muscle layer is thicker than in the oviduct sensu stricto. It follows the cyclic changes of the uterus, with the only exception that the apoptosis phase and the glycoprotein secretion are missing. The transition from the secretion to the proliferation phase is achieved as ciliated cells appear (Xavier 1971).

In summary: the oviduct is separated into the tube, the uterus (distal end of the oviduct) and the common tube. The tube is only active before ovulation and hence, changes the least of the three parts. The uterus supports the foetuses during gestation, undergoes the largest size changes, and rebuilds its epithelium after every gestation. The common tube follows a similar development as the uterus, but is missing the apoptosis phase, in which the epithelium is completely exchanged.

Ovary

The ovaries are situated in the posterior third of the body (Xavier 1971). They are small, of irregular shape (1–2.5 mm wide and 0.5–1 mm large, Xavier 1971, 1974) and have a weight of 0.15–0.75 mg (Xavier and Ozon 1971). They contain oogonia and follicles of different sizes, the total number rarely exceeding 60 (Angel and Lamotte 1944b, Lamotte and Rey 1954, 1957, Lamotte et al. 1964, Xavier et al. 1970, Xavier 1974). Not all eggs are ovulated at the same time; 4–20 eggs, most often 8–9 eggs, per ovulation were observed (Angel and Lamotte 1944b, Lamotte et al. 1964, Xavier 1971, 1977, 1986). The number of ovulated eggs depends on the female's size (age), with larger females ovulating more eggs. First-gestating females may ovulate 1–8 eggs, whereas older females may ovulate between 14–18, and the largest females up to 20 eggs (Lamotte et al. 1964, Xavier 1971, 1986).

Follicles consist of a theca layer and granulosa cells (Xavier 1971, 1977, 1986). Most follicles in an ovary have a size between 150–200 µm diameter (Angel and Lamotte 1944a, 1944b); mature follicles on the other hand are larger with a diameter of 500–650 µm (Lamotte and Rey 1954, Lamotte et al. 1956, Xavier 1971). At birth oocytes all have the same size (< 220 µm) but within fast-developing females during mating season two types of follicles can be observed, the majority is < 300 µm and a few are mature with a size between 500–620 µm (Lamotte and Rey 1957).

After ovulation, follicles decrease slightly in size (280–320 µm diameter, Lamotte and Rey 1954) and develop into corpora lutea (Lamotte and Rey 1954, Lamotte et al. 1956, Vilter and Lugand 1959a, Xavier et al. 1970, Xavier 1971, 1974, Lamotte and Xavier 1976a). A corpus luteum develops when granulosa cells invade the follicle cavity and the theca thickens (Lamotte and Rey 1954, Xavier 1971, 1974). The corpora lutea persist during the entire gestation (Lamotte and Rey 1954, Lamotte et al. 1956, Vilter and Lugand 1959a, Xavier et al. 1970, Xavier 1971), nevertheless they decrease in size after emergence (Lamotte and Rey 1954, Lamotte et al. 1956, Xavier 1971, 1977, 1986). Large follicles that did not reach maturity in time, and did not ovulate their ova, undergo atresia (Xavier et al. 1970, Xavier 1971).

Hence, within the ovary two phases can be observed, a follicular phase, which is characterised by follicle growth, and a luteal phase, during which the corpora lutea are present (Xavier and Ozon 1971, Xavier 1971, 1986, Lamotte and Xavier 1976a). The follicular phase starts slowly with

emergence, vitellogenesis takes place after parturition, and the phase ends with ovulation (Lamotte et al. 1964, Xavier 1971, 1977, 1986). This phase is characterised by the growth of the follicles involved in the next ovulation and vitellogenesis (Xavier and Ozon 1971, Xavier 1971, 1977, 1986). The presence and activity of 17α-hydroxylase and 3β-hydroxy-steroid dehydrogenase (3β-HSD) was observed within the follicles, particularly before ovulation (Ozon and Xavier 1968, Xavier et al. 1970, Xavier 1971). Only before ovulation the theca cells of the follicles show enzymatic activity (Xavier et al. 1970), during which oestrogens are produced until ovulation (Xavier 1971, 1986). During the same time granulosa cells of the follicles produce progesterone (Xavier and Ozon 1971, Xavier 1971, 1986). Pre-ovulation oestrogen, progesterone, as well as 4-adrostenedione and testosterone are produced within the granulosa or theca cells of mature follicles (Xavier 1976).

The luteal phase starts with the gestation. Corpora lutea decrease in size during the active life of females after emergence, and are rapidly disappearing after parturition (Vilter and Lugand 1959a, Lamotte et al. 1964, Xavier 1970a, 1970b, 1971, 1976, 1977, 1986, Xavier et al. 1970, Xavier and Ozon 1971). During the luteal phase progesterone, but no oestrogen is produced (Ozon and Xavier 1968, Xavier et al. 1970, Xavier and Ozon 1971, Xavier 1971, 1986). Progesterone levels are linked to number and size of corpora lutea and hence, decrease after emergence (Xavier et al. 1970, Xavier and Ozon 1971, Xavier 1971, Lamotte and Xavier 1976a). Larger females with more corpora lutea have higher progesterone levels (Xavier and Ozon 1971, Xavier 1971). During the dry season corpora lutea are larger and progesterone levels are high and this time coincides with the time of slow foetal growth and the absence of follicle growth. The two ovarian phases are non-exclusive as both are overlapping from emergence until parturition. Nevertheless, during this overlapping time corpora lutea decrease in size, and follicle growth is slow (Ozon and Xavier 1968, Xavier 1970a, 1970b, 1971, 1986, Xavier et al. 1970, Xavier and Ozon 1971).

In summary: Nimba toads have small ovaries, which contain only few follicles (< 60), of which a small proportion (4–20) reaches maturity every year. After ovulation follicles develop into corpora lutea, which are present during the entire gestation period. Within follicles androgens (oestrogen and testosterone) are produced within the theca cells, whereas the granulosa cells produce progesterone. During the luteal phase, exclusively progesterone is produced by the granulosa cells. Progesterone levels are highest during the dry season, when foetal and follicle development is slow or absent.

Pituitary gland

The pituitary gland is comparatively small (Vilter et al. 1959), and does not differ between virgin females and males after emergence (Zuber-Vogeli 1968). After parturition the volume of the pituitary decreases dramatically, but increases again after emergence and is maximal just before birthing (Vilter et al. 1959). Three types of glycoprotein cells (in some publications called basophilic, amphophilic or cyanophilic cells) and two types of protein cells (sometimes termed acidic cells) were determined (Zuber-Vogeli 1968). Different names were used for the same pituitary cell types, between different as well as within the same publications. To avoid confusion and standardise usage, we use the following names: glycoprotein type 1 cells (other names: gonadotropic cells, basophilic PAS purple cells, gonadotropic type I and FSH-cells), glycoprotein type 2 cells (corticotropic cells, basophilic PAS red cells, gamma cells, gonadotropic cells II), glycoprotein type 3 cells (thyrotropic cells), protein type 1 cells (prolactin-like cells, somatotropic cells, orangeophilic cells, alpha cells), protein type 2 cells (prolactin-like cells, somatotropic cells, erythrosonophilic cells). Within the two protein cell types, the challenge is that it was first assumed that the protein type 2 cells are the prolactin-like cells, but it was later demonstrated that they produce somatotropin (STH, Zuber-Vogeli et al. 1975).

The glycoprotein type 1 cells are widely distributed within the pituitary gland (Zuber-Vogeli 1968, Xavier 1971), these cells and their mitochondria are large (Zuber-Vogeli and Doerr-Schott 1976). During dormancy glycoprotein type 1 cells are small or even absent (Zuber-Vogeli 1968, Xavier 1971, Zuber-Vogeli and Xavier 1973, Zuber-Vogeli et al. 1975). They increase in size and activity after emergence and are most active between parturition and ovulation (Zuber-Vogeli and Herlant 1964, Zuber-Vogeli 1968, Zuber-Vogeli and Xavier 1973, Zuber-Vogeli et al. 1975). In females they are active during the follicular phase (Xavier 1971) and in males during spermatogenesis (Zuber-Vogeli et al. 1975). Hence, it was assumed that they are equivalent to the gonadotropic cells. The glycoprotein type 2 cells are scarcely found within the rostro-ventral part of the pituitary (Zuber-Vogeli and Herlant 1964, Zuber-Vogeli and Doerr-Schott 1976), have a well-developed Golgi apparatus and endoplasmic reticulum (Zuber-Vogeli and Doerr-Schott 1976). These cells are not visible during dormancy, are few and small at emergence, and most prominent in July/ August (Zuber-Vogeli 1966, 1968). In females it was assumed either that they may have no function (Zuber-Vogeli and Xavier 1973), or that they are corticotropic cells (Zuber-Vogeli et al. 1975, Zuber-Vogeli and Doerr-Schott 1976). The least is known about the glycoprotein type 3 cells. They are scarce and had to be excluded from many studies due to this fact (e.g. Zuber-Vogeli and Doerr-Schott 1976), the only thing known is that they are distributed throughout the gland (Zuber-Vogeli and Herlant 1964).

Protein type 1 cells are large, widely distributed cells within the pituitary gland (Zuber-Vogeli and Herlant 1964, Zuber-Vogeli et al. 1975) and are often ciliated (Zuber-Vogeli et al. 1975, Zuber-Vogeli 1978), their cytoplasm contains many granules (Zuber-Vogeli 1978) and their Golgi apparatus shows activity (Zuber-Vogeli and Doerr-Schott 1976). In the presence of artificially injected bromocriptine, the cells are smaller, have smaller nuclei and the present granules increase homogeneously within the cytoplasm, and M. Zuber-Vogeli (1978)

assumed that bromocriptine is hindering the exocytosis of prolactin. Within non-gestating females protein type 1 cells increase in number after emergence (Zuber-Vogeli 1968). In gestating females protein type 1 cells are abundant during dormancy and abundant at emergence (Zuber-Vogeli 1968). After birth they increase in number and have a large nucleus, large Golgi apparatus, many mitochondria, a well-developed endoplasmic reticulum and exocytose was observed 16 days after parturition (Zuber-Vogeli 1978). One month after parturition they are abundant and well developed (Zuber-Vogeli 1978). Protein type 2 cells are more localised at the dorsal and caudal poles of the pituitary gland (Zuber-Vogeli and Herlant 1964, Zuber-Vogeli 1968, Zuber-Vogeli and Doerr-Schott 1976). At the beginning of gestation the Golgi apparatus is well visible (Zuber-Vogeli and Doerr-Schott 1976). Protein type 2 cells are abundant during dormancy, decrease in number with emergence and disappear with parturition (Zuber-Vogeli and Xavier 1973, Zuber-Vogeli 1978). Hence, it was assumed that their presence is linked to the presence of corpora lutea and it was wrongly assumed that they are prolactin-like cells (Zuber-Vogeli 1968, Xavier 1971, Zuber-Vogeli and Xavier 1973). Later it was found that they are somatotropic cells (Zuber-Vogeli et al. 1975, Zuber-Vogeli and Doerr-Schott 1976).

Within one sexual cycle this means that at the beginning of the gestation glycoprotein and protein type 1 cells are present and protein type 2 cells are appearing. Glycoprotein type 2 cells are less abundant but easily and brightly stainable (Zuber-Vogeli and Xavier 1973). At emergence of the females from their dormancy sites protein type 1 cells are present, protein type 2 cells disappear, glycoprotein type 1 cells restart activity, glycoprotein type 2 cells are present, and glycoprotein type 3 cells are scarce (Zuber-Vogeli and Xavier 1973). Twenty days after parturition protein type 1 cells increase in number and glycoprotein type 1 cells are secreting (Zuber-Vogeli and Xavier 1973). One month after parturition protein type 1 cells are abundant and the glycoprotein type 1 cells are large and well granulated (Zuber-Vogeli and Xavier 1973). It was concluded that the pituitary is controlling the follicle growth, ovulation and corpora lutea development and maintenance (Vilter and Lugand 1959a, Xavier 1986).

In summary: five different cell types can be distinguished within the pituitary gland, three glycoprotein cell types and two protein cell types. The glycoprotein type 1 cells are visible and active during the toad's active life, follicle development and vitellogenesis. This indicates a connection between the glycoprotein type 1 cells and the ovarian follicular phase, which makes them gonadotropic cells. The protein type 1 cells are well visible and active during most of the time, with a slight decrease in activity for some time after emergence. They had been shown to secret prolactin.

Interaction between different organs

Within the female reproductive cycle three time periods lead to changes within the reproductive system: i) before toads become dormant ovulation and mating occur and gestation starts. Within the oviduct this leads to the cease of activity within the tube, the start of the secretion phase within the uterus and within the ovary to the start of the luteal phase, in the pituitary glycoprotein type 1 cells become scarcer and protein type 2 cells appear; ii) With emergence at the beginning of the rainy season, the uterus starts to increase considerably in size as foetal growth and development takes up speed, within the ovaries the corpora lutea start decreasing in size, the follicles slowly start development and within the pituitary the glycoprotein type 1 cells start to appear, protein type 1 and 2 cells become less abundant; iii) With the birth of juveniles in June the tube increases developmental speed, the uterus collapses and rebuilds a new epithelium, within the ovary the corpora lutea disappear and follicle growth intensifies, within the pituitary the glycoprotein type 1 cells show very high activity, the protein type 1 cells increase their activity as well and protein type 2 cells are absent. Hence, the question arises, whether these changes in the different organs are coincidences or one change is triggering the change in another organ. To answer this question several experiments were carried out. If not stated otherwise studies are described in Xavier (1971).

Ovary and foetus development. During the dry season corpora lutea are present, large and active, and it was shown that they produce progesterone. Foetal development is slow during dormancy (Xavier 1976). Ovariectomy (the surgical removal of ovaries) of females early during gestation leads in 50% of females to parturition of normally developed foetuses three months earlier than in non-ovariectomised females (Xavier et al. 1970, Xavier and Ozon 1971, Xavier 1971). In the other 50%, which are assumed to be first-gestating females, ovariectomy leads to abortion (see below). Implantation of progesterone into gestating females after emergence leads to normally developed foetuses, but parturition occurs 2–3 months later than in non-progesterone treated females. This indicates that progesterone produced by the corpora lutea slows down developmental speed of foetuses naturally during dormancy and experimentally during the active life of gestating females (Xavier 1970a, 1970b, 1971, 1976, 1986).

If females are separated from males during the mating season, ovulation occurs and a "pseudo-gestation" develops (Xavier 1969, 1974). Within the first four months the uteri are long bags (5 × 3 mm) containing separated eggs and within the ovary corpora lutea develop normally (stage 1, summarised from: Vilter and Lugand 1959a, Xavier 1969, 1971, 1974). Within the next 1 to $1^{1}/_{2}$ months the eggs are still separated but the corpora lutea decrease and follicles increase in size (stage 2). After emergence uteri are thinner (epithelium, connective tissue and muscle layer), less transparent and the mucous cells reduce production and later ciliated cells appear, and the unfertilised eggs start accumulating (stage 3). The accumulated eggs are then lysed, the mucous cells stop secreting and the uterus changes into the same stage as

for virgin females (stage 4). In general, the mucous layer is less thick and less ragged than in gestating females and the uterus does not substantially change. The pseudo-gestation is shorter than a gestation and leads to ovulation 3–4 months earlier (June) than in gestating females (September/ October). If no fertilisation occurs a new pseudo-gestation is established (Xavier 1974). Hence, the absence of fertilisation and with that of foetuses leads at first to normal developments within the ovary and the uterus, but particularly after emergence the pseudo-gestation cycle is faster and shorter. This may indicate that the presence of foetuses is needed for normal uterus and ovary cycles during that period.

Ovary and oviduct. The tube's endocrine cycle is synchronised with the follicular phase of the ovary. Follicles and tube start slowly developing after emergence and are most active before and during ovulation, and are not active during dormancy. In females ovariectomised after parturition the tube does not show any activity towards the end of the rainy season, usually the time of highest activity. In females ovariectomised at about the time of ovulation, the tube maintains high activity for the next 6 months and activity is terminated only after two years. This indicates that the ovary is controlling tube activity (Xavier 1971). At the time of ovulation within the follicles oestrogen is secreted by the theca cells and progesterone by the granulosa cells. One year after ovariectomy Xavier (1971) injected oestrogen, testosterone and progesterone at the time of ovulation. She made the following observations: oestrogen (oestradiol) and testosterone initiated hypertrophy and development of the epithelial cells within the tube, the injection of progesterone initiated the thickening of the muscle layer. Only if oestrogen and progesterone were injected simultaneously the tube did show normal development during ovulation. If first oestrogen and three weeks later progesterone was injected this triggered the secretion of the mucous cells within the tube. If first oestrogen and after one-month progesterone was injected this triggered the decrease in tube activity. Hence, oestrogen and progesterone together are needed for normal tube development during ovulation and progesterone is needed to stop tube activity (Xavier 1971, 1986).

Another connection between ovary and tube became apparent by an observation of Lamotte et al. (1964). The number of ovulated eggs in one ovary corresponds very closely to the number of foetuses developing within the uterus of the same side. In unilaterally ovariectomised females, most eggs developed into foetuses in the uterus of that side on which the ovary was still present (Figure 10A, Xavier 1971). If one tube was closed (bound) of the same side as the ovary was still present, most eggs developed into foetuses in the uterus of the non-manipulated side, and very few ended up in the bound oviduct or within the body cavity (Figure 10B). If one uterus was removed and females were unilaterally ovariectomised (Figure 10C) or kept both ovaries (Figure 10D), most eggs were found in the remaining uterus (Xavier 1971). This indicates a preference of eggs to be present in the uterus of the same side than the ovary they came from. Additionally, one uterus may host all eggs ovulated from two ovaries, and that foetuses are not able to pass from one uterus into the other passing the common tube, as in that case eggs should have been present in both uteri in the experiment shown in 10B (Xavier 1971, 1977, 1986).

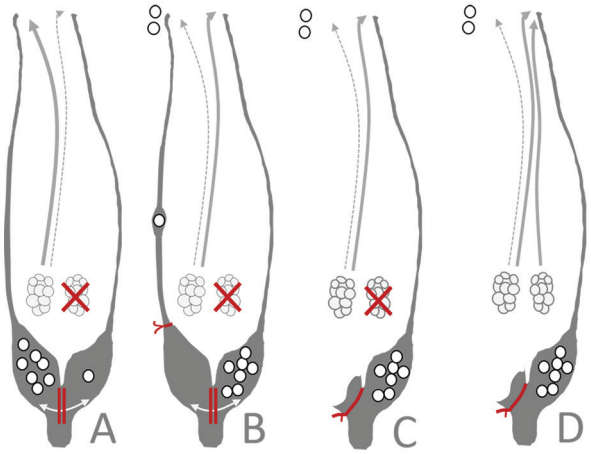

Figure 10. Experiments on female ovary, oviduct and uterus removal. Shown are the positions of eggs in the uteri after unilateral ovariectomy (**A**), after unilateral ovariectomy and the connection between oviduct and uteri bound at the side with the still present ovary (**B**), unilateral ovariectomy and the oviduct and uterus at the side of the still present ovary removed (**C**) and the unilateral removal of the oviduct and the uterus without ovariectomy (**D**). The red cross indicates the ovary removed, the red lines indicate positions where either oviduct (**B**) or the uterus (**C** and **D**) were bound. The two-headed arrow with the two red lines indicates that eggs apparently do not wander from one uterus to the other passing the common tube. Redrawn after Xavier (1971).

The uterus does not follow the same cycle as the follicles and the tube. The largest change of the uterus is during gestation. Nevertheless, during the follicular phase after parturition in ovariectomised females the injection of oestrogen initiated the development of the mucous layer and some blood vessels, the injection of testosterone the development of the connective tissue and some blood vessels, and the injection of progesterone the development of the muscle layer and an epithelium without ciliated cells. Only if oestrogen and three weeks later progesterone were injected together, did the uterus develop as in non-ovariectomised females (Xavier 1971, 1986). This indicates that the hormones produced by the ovary are controlling uterus development between gestations.

The removal of ovaries at the beginning of gestation leads to abortion 3–4 months after the operation in 50% of gestating females. Whether ovariectomy leads to abortion depends on the female's age. In all females 3–4 months old (hence, born the same year) and within 50% of females 15–16 months old, ovariectomy resulted in abortion. In all older females the gestation continued. As females may either get mature within 3–4 months, or

one year later, females 15–16 months of age may be gestating for the first or the second time. Françoise Xavier (1971) concludes that ovariectomy leads to abortion only in females gestating for the first time. She links this to the progesterone levels produced by the ovaries, before ovariectomy. Older females show 4× higher progesterone levels than females gestating for the first time. Françoise Xavier assumes that a progesterone threshold needs to be reached to trigger uterus development. Additionally, within the uterus structures needed for gestation are in part still present (e.g.: blood vessels) in older females, but need to build in first-gestating females. Françoise Xavier assumed that foetuses develop faster as progesterone is missing and on the other hand the uterus development is hindered. This leads to asynchrony between foetuses and uterus in females gestating for the first time and finally to abortion (Xavier 1970a, 1971, 1977, 1986).

After emergence, ovariectomy of gestating females never results in abortion, irrespective of the female's age. This indicates that at this stage of the gestation the ovary has no longer an important effect on gestation (Xavier 1971, 1986, Zuber-Vogeli and Xavier 1973).

Hysterectomy (the removal of uteri) at the beginning of the gestation leads to atresia of small follicles and the corpora lutea disappear faster. After ovulation in non-hysterectomised females only nearly mature follicles are destroyed by atresia. Hence, ovary and uterus are influencing each other. To examine whether this connection is linked to the uterus, or to the developing foetuses, foetuses were removed by caesarean section (Xavier 1971). During early gestation the same modifications of the ovary are observed, but caesarean section after emergence leads to a collapse and apoptosis of the uterus, similar to that after birth, but does not change the ovaries (Xavier 1971). Considering that hysterectomy and caesarean section lead to atresia of small follicles, but pseudo-gestation (see above) does not, this raises the question whether atresia of small follicles is triggered by the traumata of the operations, or if mating (which is missing in pseudo-gestating females) triggers some other development which is similarly changed.

Ovary and pituitary gland. After ovariectomy at the beginning of gestation the glycoprotein type 1 cells are contracted and de-granulated. The protein type 2 cells, which are normally appearing with the corpora lutea, are absent, but protein type 1 cells are very abundant. Ovariectomy after emergence does not lead to large changes within the pituitary gland. Ovariectomy one month after parturition leads to the degranulation of the glycoprotein type 1 cells and they develop into "castration cells". Hence, the absence of the ovary has the largest effect on the pituitary at the beginning of the gestation and after parturition during vitellogenesis (Zuber-Vogeli and Xavier 1973).

Examining the effect of the pituitary on the ovary is much more problematic, as females die within the next 15–25 days after the flattening (destruction) of the pituitary, indicating that the pituitary has other vital functions in Nimba toads (Xavier 1971). Destruction of the pituitary at the beginning of the gestation, leads to atresia of small follicles > 180 μm and smaller corpora lutea develop, but does not lead to abortion within 15–25 days. Destruction of the pituitary gland after emergence leads to abortion within the next six days. As the destruction of the pituitary obviously has lethal effects on the female, F. Xavier hypothesised that abortion might be linked to the large energy demands of foetuses during that period, which cannot be supplied by the injured female. Destruction of the pituitary just before parturition has no negative impacts on parturition or foetuses (Xavier 1971).

Summary of interactions. The ovary seems to have the largest effect on the other reproductive organs from June to emergence at the beginning of the rainy season. Nevertheless, the ovary has different functions between parturition and ovulation (vitellogenesis) and during the first six months of the gestation females rest underground (dormancy). During vitellogenesis follicles grow and develop quickly and they synthesise oestrogen, testosterone and progesterone. Ovariectomy at the beginning of this period hinders the development of the oviduct – tube and uterus. This non-development can be circumvented with the injection of oestrogen and progesterone, indicating, that these hormones are vital for oviduct development during vitellogenesis. As well within the pituitary gland, glycoprotein type 1 cells (gonadotropic cells) develop into "castration cells" if the ovaries are removed at the beginning of vitellogenesis. During dormancy the corpora lutea are large, numerous and active and synthesise progesterone. Progesterone slows foetal development during dormancy and if experimentally applied as well after emergence. Ovariectomy of first-gestating females leads to abortion, which was as well linked to lower progesterone levels in first-gestating, than in older females. Within the pituitary protein type 2 cells appear with the development of corpora lutea. In neutered females they are absent during the whole dry season. Hence, most of the year the ovary influences the development of the pituitary gland, the tube and the uterus and during dormancy as well foetus development.

Between emergence and parturition foetuses develop quickly, stretching the uterus and restricting the other organs within the female. This development is only stopped by the destruction of the pituitary gland, which is lethal to females. Development can be slowed down by the injection of progesterone. On the other hand, the absence of foetuses in pseudo-gestating females leads to faster development of the ovary (decrease of corpora lutea and development of follicles), but has no effect on the pituitary gland. If foetuses are removed through caesarean section after emergence, with the apoptosis phase the uterus starts a new cycle, earlier than in normally gestating females. These results are in accordance with the hypothesis that the presence and development of foetuses are important for normal development of ovaries, and through them on the oviduct and the pituitary gland. On the other hand, change of the females from the inactive, low nutrition life underground to an active high nutrition life above ground and external factors might be important too.

Male reproductive system

The reproductive system of Nimba toad males received less attention than that of females. Respective research focused more on the environmental and cyclic dependencies of the reproductive cycle than on morphology and physiology of the reproductive system. Primary publications are by M. Zuber-Vogeli (Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966) and J. Gavaud (Gavaud 1976a, 1977).

Nimba toad males lose at 13 mm SVL their juvenile colouration (Angel and Lamotte 1948) and become mature already about 3 months after they are born. They are much more active than females, mainly calling, fighting with other males and harassing females (Sandberger-Loua et al. 2016b). This more active behaviour of males could increase their predation risk and lead to higher energetic costs. Males have a positive energy budget only between emergence and July. During the mating season and particularly the dry season, males lose up to 30% of their body weight, attributed to lower food intake rates (Lamotte 1972).

In summary: males have been studies less than females; are smaller, darker and at least during the mating season more active than females. Due to their more active life-style they may suffer from higher predation pressure and possibly as well higher energy demands.

Testes

Nimba toad testes are small (< 2 mm) ovoid masses (Gavaud 1976a), situated in the dorsal body cavity and connected to the kidneys (Gavaud 1976a) by the mesentery (Angel and Lamotte 1948). Testes are attached to the fat-body and the kidneys; they contain numerous efferent canals at all times, but the space they occupy within the testes varies with the annual cycle (Gavaud 1976a). The seminiferous tubes are filled with gonad cells, and all but primary spermatogonia are grouped in pyramidal cysts (Gavaud 1976a). Cysts surround Sertoli cells and within each cyst the mitoses and meioses are synchronised (Angel and Lamotte 1948, Gavaud 1976a). Gonad cells are attached by the Sertoli cells to the cysts (Gavaud 1976a). Only a fraction of the primary spermatogonia develop into spermatozoa (Gavaud 1976a). Endocrine activity through the presence of 5α-dehydrotestosterone (5α-DHT) was observed (Gavaud 1976b) within Sertoli cells and some cells of the connective tissue (Gavaud 1976a). This tissue is pigmented during the dry season and unpigmented during the rainy season (Angel and Lamotte 1948), giving the testes a dark appearance during the dry and a whitish appearance during the rainy season (Gavaud 1976a).

Most bufonids have a Bidder's organ, a part of the testes that contains oocytes, and which is separated from the testes by a separate envelope. During Nimba toad foetal development testes usually contain 1 or 2 (rarely 5 or 6) oocytes per male (Lamotte et al. 1973). Oocytes are always positioned at the periphery and often grouped at one of the poles of the testes, but always within the testes and not separated by an envelope (Lamotte et al. 1973). Intra-testicular oocytes are small. They are not well developed at foetal stage Ia and Ib, and grow slowly until stage IIb. Stage III is the time of accelerated spermatogenesis and during this stage intra-testicular oocytes decrease rapidly in size and have disappeared by stage IV (Lamotte et al. 1973). Hence, in contrast to other bufonids, the presence of oocytes in male Nimba toads is only transitory during foetal development.

The spermatozoa show no apparent modification compared to those of other bufonids. The only differences are that the perforatorium ends slightly posterior to the acrosome vesicle, the distal centriole is penetrated throughout its length by the central singlets of the axoneme and no mitochondrial collar is present, but mitochondria are located around the anterior axoneme (Scheltinga and Jamieson 2003). It is assumed that due to the viviparous reproduction (and the absence of spermatheca in females and hence presumably low levels of sperm competition) testes are small, cysts are few and only a few spermatozoa are produced during each mating season (Angel and Lamotte 1948, Lamotte et al. 1973, Gavaud 1976a).

In summary: Nimba toad testes are ovoid masses, which are white during the rainy and dark during the dry season. During embryonic development the testes may contain a few oocytes, which disappear before birth and are never surrounded by an envelope. The seminiferous tubes are always numerous, but vary their size during the reproductive cycle. Most gonad cells are grouped around Sertoli cells and within each group development is synchronised. The spermatozoon is a typical bufonid spermatozoon with little modification.

Annual reproductive cycle

Male adaptations to the viviparous reproductive mode are less distinct than in female Nimba toads; however, the male reproductive cycle is likewise tightly linked to the climatic cycle of the environment. Nimba toad males have a discontinuous reproductive cycle, (Zuber-Vogeli and Xavier 1965, Gavaud 1976a, 1976b). In contrast to temperate anurans spermatogenesis is not triggered by temperature, but by humidity (Zuber-Vogeli and Xavier 1965, Gavaud 1976a).

The dry season dormancy is characterised by low metabolism (Gavaud 1976a), invisible colourless nuptial pads without spines (Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966, Gavaud 1976a), testes and seminiferous ducts decrease in size during the first half of dormancy and stay the same in the second half (testes weight 0.1–0.4 mg, Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966, Gavaud 1976a). Some primary spermatogonia are present (Zuber-Vogeli 1966, Gavaud 1976a, 1977) and are with their Sertoli cells attached to the walls of the seminiferous tubes (Gavaud 1976a). Primary spermatogonia increase in number during the first few months of dormancy until in mid-January when they fill 20–35%, and Sertoli cells 15–32% of the testes volume until the end of the dry season (Gavaud 1976a). As Sertoli cells increase in num-

ber they detach from the walls of the testes and migrate into the centre of the seminiferous tubes (Gavaud 1976a). During this time secondary spermatogonia were rarely observed (Gavaud 1976a).

In April, when males emerge, one month after females, testes are still black and nuptial pads are not yet visible and their fat bodies are very small (Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966). Most of the primary spermatogonia are still attached to the walls with their Sertoli cells, so that the seminiferous tubes are hollow (Zuber-Vogeli and Xavier 1965). Larger (SVL ca.19 mm) males may be advanced in their reproductive development compared to smaller males (SVL: 17–18 mm, Zuber-Vogeli and Xavier 1965). With emergence, the secondary spermatogonia appear (Zuber-Vogeli 1966, Gavaud 1976a, 1977), which continue to divide until they fill the whole testes in June (Gavaud 1977). In May testes start increasing in size (Zuber-Vogeli and Xavier 1965, Gavaud 1976a), and primary spermatocytes (45%), secondary spermatocytes (25%), and spermatids (15%) fill most of the testes volume (Gavaud 1976a). Pyknosis of some cells can be observed (Gavaud 1976a).

In June nuptial pads start to turn black, testes increase further in size (Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966) and secondary spermatogonia occupy 35–55% of the testes (Gavaud 1976a), meiosis occurs and all stages can be observed, but no secondary spermatocytes nor spermatids (Zuber-Vogeli 1966, Gavaud 1976a). July/ August is the time of spermatogenesis (Zuber-Vogeli and Xavier 1965, Gavaud 1976a, 1976b), testes continue to increase in size (Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966, Gavaud 1976a) and nuptial pads become more pronounced (darker and larger spines, Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966). At around this time recently born males reach 13–15 mm SVL and spermatogenesis is accelerated (Angel and Lamotte 1948). The quantity of secondary spermatogonia decreases dramatically from about 50% to 7% of the testes volume (Gavaud 1976a). At the same time spermatozoids mature and detach themselves from Sertoli cells (Gavaud 1976a).

During the mating season the nuptial pads are blackest and spiniest (Figure 11, Zuber-Vogeli and Xavier 1965, Zuber-Vogeli 1966). Within the testes, mobile spermatozoa are abundant (Zuber-Vogeli and Xavier 1965, Gavaud 1976a) whereas cysts of spermatids and some spermatogonia are only rarely observed (Zuber-Vogeli and Xavier 1965, Gavaud 1976a). During the mating season only some spermatogonia divide, but spermatogenesis does not continue (Gavaud 1976a). After the mating season spermatozoids degenerate, nuptial pads become transparent, testes darken and decrease in size (Zuber-Vogeli and Xavier 1965, Gavaud 1976a) and only divisions of primary spermatogonia can be observed at the beginning of the dry season (Gavaud 1976a).

Similarly to females, the male reproductive cycle is linked to three important seasonal points, the mating season and the subsequent dormancy underground (slow or no development), emergence during the next rainy season (beginning of spermatogenesis with the appearance of secondary spermatogonia), and in June/ July when rain becomes permanent spermatogenesis intensifies (appearance of spermatocytes). Despite this strong link individual males may finish their spermatogenesis at different times due to individual differences in developmental speed (Zuber-Vogeli and Xavier 1965, Gavaud 1976a). Larger males mate earlier than smaller males older than one year (Zuber-Vogeli and Xavier 1965). As spermatogenesis speed is individual, this may indicate that the reproductive cycle is influenced by several environmental variables and/ or internally determined. Experimentally, Gavaud (1977) could show that spermatogenesis and particularly its developmental speed is strongly linked to the annual environmental cycle. Jaqueline Gavaud conducted two experiments, one to determine important environmental variables for the slowed or stopped spermatogenesis during the dry season (Gavaud 1976a), and a second one determining the environmental variables important for the correct timing of spermatogenesis during the rainy season (Gavaud 1977). In these studies, she could show that spermatogenesis depends mainly on prey availability and humidity levels, of which negative effects could be intensified by reversed light intensity (1000 lux during the dry season, or 10 lux during the rainy season). She compared the reproductive development of males within the experiments to wild caught males. During the dry season, comparable testes development was achieved with nutrition once per month (50 mg), an aerial humidity of 35% and a 12h light regime at 10 lux. During the wet season comparable development to wild males was observed with nutrition every second day (25 mg), 90% humidity and a light regime of 12h with 1000 lux. Generally, little food, low humidity and less light lead to slower or no gonad development than much food, high humidity and much light (Gavaud 1977). The endocrine activity of testes is highest in July. She assumes that the beginning of spermatogenesis after emergence and the slowing down or stopping of spermatogenesis after the mating season is mainly influenced by external factors (nutrition and hu-

Figure 11. Male during the mating season, showing pronounced nuptial pads on the thumbs. © Joseph Doumbia

midity, Gavaud 1976b, 1977), whereas the onset of mitoses of primary and secondary spermatogonia towards the end of the dry season is triggered by endogenous factors (Gavaud 1976b, 1977).

In summary: the male reproductive cycle is interrupted during the dry season, when only divisions of primary spermatogonia are observed. Only after emergence, which is later in males than in females, spermatogenesis starts and is accelerated after June, when rain is more permanent, and results in the presence of spermatozoids during the mating season. During the mating season only primary spermatogonia divide. Spermatogenesis is accelerated through the availability of prey and high humidity. Nevertheless, individual males differ in the quantity and speed of the different gonad cell stages and larger males develop faster and mate earlier than smaller (but nevertheless, sufficiently old) males.

Male pituitary gland

Monique Zuber-Vogeli examined the male pituitary gland and focused on the annual reproductive cycle (Zuber-Vogeli 1966). The male pituitary gland contains the same five cell types as in females. Following the same terminology as for the female pituitary, the protein type 1 cells are the most abundant during the entire year. She assumed that they are somatotropic cells as in most vertebrates. As in females the glycoprotein type 3 cells are very rare and difficult to find. Three cell types undergo seasonal changes: the glycoprotein type 1 and 2 cells and the protein type 2 cells. The glycoprotein type 1 cells show the largest variability and are changing in accordance to the male reproductive cycle. They are less abundant or absent during the dry season, they are abundant and contain filled vacuoles in July (the time of most intensive spermatogenesis) and during the mating season (Zuber-Vogeli 1966). The glycoprotein type 2 cells are surrounding the anterior pole of the pituitary and are rare at emergence, but increase in number and activity until July/ August, when nuptial pad colouration and spermatogenesis is the strongest. Monique Zuber-Vogeli (1966) links the glycoprotein type 1 and 2 cells to the gonadotropic and the luteinising cells, respectively, described in *Rana temporaria* (van Oort 1961). The protein type 2 cells are present only in June/ July when spermatogenesis is the most active, and function is unclear. Nevertheless, it seems that within the male pituitary gland the cell types which show the largest changes within one year are linked to the reproductive cycle.

In summary: in the male pituitary, all five cell types were present, but only three of these five showed temporal modification. The glycoprotein type 1 cells are linked in males and females to gonad development and are rare during the dry season and most abundant during spermatogenesis/ vitellogenesis in July/ August and during the mating season. The protein type 1 cells are always the most abundant in males and females and show some, but little variability. The glycoprotein type 3 cells, which are assumed to be the thyrotropic cells, are always rare in both sexes. The glycoprotein type 2 cells and the protein type 2 cells differ in their annual activity between the sexes. Glycoprotein type 2 cells show no variability in females, but in males they are absent during the dry season and increase in number after emergence until they reach their maximum in July/ August and they were linked to nuptial pad development. The largest discrepancy is between female and male protein type 2 cells. In females they are abundant and active during the dry season, whereas in males they are only present in July/ August.

Summary and discussion

Viviparity in Nimba toads

Within anurans, Nimba toads have a highly derived and unique reproductive mode. They retain eggs and foetuses within their oviducts and are pueriparous and matrotrophic. As in other viviparous amphibian species, they have internal fertilisation, a reduction in number of developing eggs, and a prolonged developmental period (Wake 1992, 2015a). Several morphological and physiological adaptations are present in females, foetuses and, to a lesser extent, in males of *N. occidentalis*. The reproductive systems are small. This seems to be a trend in viviparous amphibians (Wake 2015a, 2015b). Oviducts are straight; the lower end of the oviduct is enlarged. From toads of the East African genera *Altiphrynoides* and *Nectophrynoides* it is known that the oviducts can be divided into a thinner anterior part (tube) and a sometimes dilated lower part, uterus (Wake 1980, Xavier 1986). This is not surprising for the pueriparous, lecitotrophic *Nectophrynoides tornieri*, but more puzzling for the direct developing *Altiphrynoides malcolmi* (Wake 1980). In Nimba toads, the uterine mucous layer first secrets mucoproteins, later glycogen. That the oviductal mucous layer is providing nutrition is also known from *Salamandra atra* and several caecilians (Vilter 1986, Gomes et al. 2012, Wake 2015b). In *S. atra* a "zona trophica" in the (apical) pole of the oviduct is providing epithelial cells that detach from the connective tissue by apoptosis (Vilter 1986). Within studied caecilians and Nimba toads the whole oviduct wall may provide nutrition (Xavier 1971, Gomes et al. 2012). In *S. atra* and caecilians, foetuses develop a "foetal dentition" to scrape off the epithelial cells (Vilter 1986, Gomes et al. 2012, Wake 2015a, 2015b), whereas Nimba toad foetuses develop labial papillae, and feed on liquids secreted by the epithelial cells ("uterine milk"). That epithelial cells secret mucoproteins is also known for *Rhinoderma darwinii* (Goicoechea et al. 1986). In this species males keep their offspring within their vocal sacs from the moment young show muscular movement until metamorphosis (Garrido et al. 1975, Jorquera et al. 1982, Goicoechea et al. 1986). The epithelial cells of the male vocal sac secret mucoproteins (Garrido et al. 1975), which are first absorbed by the foetal skin, later presumably ingested (Goicoechea et al. 1986). Recently was shown that the pouch prooding *Gastroteca excubitor* transfers nutrients from the mother to the devoping embryos (Warne and

Catenazzi 2016). After parturition, the uterus of *N. occidentalis* collapses, the existing mucous layer disconnects from the connective tissue and is lysed within the uterine lumen, while a completely new mucous layer is built from the connective tissue. An apoptotic phase exchanging the uterine mucous layer after parturition/ spawning is absent in *N. tornieri* (Xavier 1986) and has not been described for *S. atra* (Vilter 1986) nor for any of the viviparous caecilians (Gomes et al. 2012, Wake 2015a, 2015b). Hence, at present Nimba toads are the only viviparous (in the sense of oviductal egg retention) amphibian known to provide liquid foetal nutrition and whose uterus has an apoptotic phase.

In Nimba toads the ovaries are small and contain small follicles, of which only very few mature within each reproductive cycle. For example, in *A. malcolmi* and *N. tornieri* ovaries are considerably larger and contain more follicles at very different developmental stages (Wake 1980b, Xavier 1986). In *Didynamipus sjostedti* 18 mature and ten very small ova were observed (Grandison 1981). In *Nimbaphrynoides, A. malcolmi and Nectophrynoides*, follicles develop into corpora lutea, but they are smaller, less persistent and less active in the East African species than in Nimba toads (Wake 1980b, Xavier 1986). No corpora lutea were reported for the pueriparous, lecitotrophic *Eleutherodactylus jasperi* (Wake 1978), but are present in oviparous, as well as pueriparous caecilians (Wake 1993, Gomes et al. 2012). Corpora lutea are hypothesised to be important to determine birthing stage (larviparous/ pueriparous) in subspecies of *S. salamandra* and to maintain gestation (Wake 2015a, 2015b). In Nimba toads it was shown that the ovaries and possibly the progesterone produced by the corpora lutea are important in the first weeks of first-gestating females to maintain the gestation. In older females, the observed effect of progesterone is not to maintain the gestation, but to decrease foetal developmental speed. In Nimba toads and *S. atra* ovariectomy, and hence the removal of corpora lutea in later stages of the gestation (after emergence and after the first year, respectively), has no effect on gestation duration and maintenance (Xavier 1971, Vilter 1986). At this stage the presence of foetuses in the uteri seems to be more important in Nimba toads than the presence of ovaries. Hence, at least in these two species, corpora lutea are not important for the maintenance of gestation and timing of parturition. Nevertheless, the hormones produced within the follicles (namely oestrogen and progesterone, possibly testosterone) are important for the preparative development of the oviduct and uterus. In the marsupial frog *Gastrotheca riobambae,* oestrogen, progesterone and the presence of foetuses are as well important for pouch development and the maintenance of the gestation at least for the first weeks (del Pino 1983). In summary, the Nimba toad female reproductive system is characterised by several adaptations to viviparity, including the endocrine function of the ovary. Characteristics of the ovary seem to be similar to other viviparous or back-brooding anurans.

Nimba toad foetuses have no internal, nor external gills, no spiracle, no coiled gut and neither labial teeth nor horny beaks at any time during their development, but they possess labial papillae, a gut similar in structure to that of adults, well developed livers, and their development takes nine months. It was hypothesised that within viviparous amphibians metamorphosis is prolonged (Wake 2015a). As Nimba toads lack many tadpole specific characteristics, metamorphosis is restricted to the development of the front limbs under the opercular skin fold and the rupture of the latter in stage IIIa, the resorption of the tail and the labial papillae during stage IIIb. Poorly developed mouthparts are generally found in species without a free swimming tadpole stage and within direct developers gills and spiracle are only present for a short time (Wake 1978). In *N. tornieri*, *N. viviparus* and *A. malcolmi* neither beak, nor labial teeth, nor papillae are present (Lamotte and Xavier 1972a, Wake 1980b). The free-swimming tadpoles of *Schismaderma* have jaw-sheaths, labial teeth, marginal papillae, a sinistral spiracle midway along the body and a characteristic, half-moon shaped flap on the head (Channing et al. 2012). *Nectophrynoides tornieri* are known to have a sinistral spiracle (Grandison 1978, Wake 1980b). The publications on Nimba toad foetal development state that the development of the gut and livers is very early (e.g. Lamotte et al. 1973). Hence, it seems that the most obvious morphological differences of Nimba toads, the several rows of labial papillae and early development of the gastrointestinal system in the foetus and the uterine secretion and apoptosis in females, are linked to matrotrophy.

Based on morphology Grandison (1981) and Graybeal and Cannatella (1995) postulated that the two viviparous lineages, *Nimbaphrynoides* and *Nectophrynoides*, might not be sister taxa. This was recently confirmed by Liedtke et al. (2016), who showed that while the two viviparous lineages are part of the same clade, they do not appear to be very closely related, which suggests that viviparity may have evolved independently in each of them. Here, we have identified six characteristics linked to viviparity specific of Nimba toads, which provide strong evidence that viviparity evolved indeed independently in this species. These characteristics comprise three traits which are common in other amphibians, but not usually found in other viviparous species (small and yolk poor eggs, prolonged mucoprotein secretion of the oviduct/ uterus epithelium after fertilisation, labial papillae of the foetuses), two behavioural or ecologically important traits ("behavioural birthing" and the developmental break during the dry season) and one characteristic which to our knowledge is not known of any other amphibian, but of mammals (complete apoptosis and rebuilding of the uterine epithelium after parturition). First, small yolk-poor eggs are common in oviparous amphibians with free swimming tadpoles, but are not known from other viviparous anurans. Second, in amphibians the oviduct secrets mucoproteins to produce the egg layers prior to oviposition and/ or fertilisation (Shivers and James 1970). In Nimba

toads the upper part of the oviduct has the same function, but the lower enlarged part (uterus) secrets mucoproteins to nourish the foetuses. Hence the temporal shift of the mucoprotein secretion within the uterus after fertilisation and during gestation (Xavier 1986) is exceptional. Third, foetal labial papillae are not uncommon in anurans and several functions of the papillae were hypothesised (hormonal secretion, enhancing the sucking capabilities of lotic tadpoles etc., McDiarmid and Altig 1999). In direct developers and lecitotrophic viviparous species foetuses have no or only very reduced labial appendices present for a limited duration (papillae, labial teeth etc.). Only in matrotrophic caecilians and adelphophagous salamanders, foetuses have special foetal teeth (Wake 2015a). Hence, the uniqueness is, that Nimba toad foetuses have labial appendices even so they are viviparous and no other labial feature than these papillae. The papillae are certainly linked to feeding as they appear and disappear with the mucoprotein secretion activity of the endometrium (Lamotte and Xavier 1972b), but the exact function is not known. Apart from the mucoprotein secretion of the oviduct/ uterus, which is found in all amphibians (Shivers and James 1970), these traits are more likely present in oviparous species with free-swimming tadpoles than in direct developing and ovoviviparous species. *Didynamipus sjostedti* and one *Altiphrynoides* species (*A. malcolmi*) have large yolk-rich eggs; *A. osgoodi* and *Schismaderma* have small eggs and are oviparous (Liedtke et al. 2016). This shows that within this clade egg sizes and reproductive modes vary greatly.

The other three unique traits of the Nimba toad reproduction can be regarded as adaptations to an unpredictable environment and time constraints due to the seasonality. First, the developmental break during the dry season coincides with an inactive life with presumably a low energy budget (Xavier and Ozon 1971, Lamotte 1972). The duration of this inactivity is synchronised with the duration of the dry season and its duration varies between 3–6 months depending on the onset of the monsoon (Lamotte 1959). Irrespective of the month with first rains (February – April), Nimba toad females emerge with these first rains, and the earlier they emerge, the larger are the juveniles born in June (Lamotte 1959, Xavier 1971). The larger the newborns, the higher is their survival probability. Hence, the dry season duration is unpredictable and Nimba toads are flexible in responding to this unpredictability. Second, after parturition the endometrium is exchanged and rebuilt. We speculate that this is due to the temporal constraint, that a functional endometrium needs to be present within three months after parturition to allow the new foetuses to develop. It might be more effective and faster to rebuild the endometrium, than to re-arrange the old one within a collapsed uterus, additionally this may decrease the mother offspring conflict. Whether this alone is a sufficient explanation for the evolution of a unique trait not described of other viviparous amphibians needs further consideration. Additionally, determination whether it does not occur in other viviparous amphibians is necessary. In Nimba toads the apoptosis phase lasts for 12 days only (Xavier and Ozon 1971) and probably might not have been recorded in less well studied amphibian species. Third, female Nimba toads are not able to expel juveniles at the end of the gestation and hence, induce parturition by behaviourally restricting the space for juveniles (Vilter 1956a) and young are involved in the birthing process (Xavier and Ozon 1971). Other than in mammals, no female mechanism has evolved to end a gestation and no morphological or physiological traits evolved to enhance parturition. Changes in progesterone levels (e.g. produced by the corpora lutea) are often discussed as triggers for parturition. As in Nimba toads ovariectomy has no effect on parturition and gestation duration, no indication exists that corpora lutea produced progesterone is important for parturition. Nevertheless, the glycogen secretion of the uterine endometrium could set a time frame for parturition. The inducing mechanism for the glycogen secretion is unknown. The absence of known parturition inducing mechanisms could be due to a lack of opportunity or necessity. The Nimba toad uterus has a thin muscle layer (Xavier 1971), and it should not be very challenging to increase it, giving it the power to expel juveniles from the uterus. On the other hand, the "birthing posture" allows for parturition, and might be easier to be controlled and if necessary interrupted by females. This may allow them to give birth to single or few offspring and interrupt birthing, if predators or other unpredictable threats are approaching. The behavioural induced parturition does not allow to give birth to dead juveniles, ending in the death by sepsis of the mother (Xavier 1971). This may indicate that intra-uterine death of juveniles is rare and the evolutionary advantages of behavioural parturition (temporal flexibility) might be greater than the necessity to induce labour by muscle power. The three traits mentioned here have a strong temporal link with the environmental unpredictability or with the Nimba mountains seasonality. This may indicate that the Nimba mountains environment and seasonality had a strong influence on the evolution and/ or maintenance of viviparity.

Ecology of Nimba toads

Within one year two important periods mainly determine the reproductive cycle of a female: one, at the end of the rainy season with ovulation, mating and the beginning gestation, the second in June with the birth of juveniles. These seasonal periods are important for males as well, as during mating season spermatozoids are present, but disappear afterwards, in June at the time juveniles are born, spermatogenesis is intensified. Nevertheless, one further important period in the season is the emergence at the beginning of the rainy season, which results as well in changes within the reproductive system. Hence, within one year three periods lead to important changes within the Nimba toad reproductive system, one might be triggered by reproductive and/ or environmental clues (late rainy season), one is characterised by environment – the beginning of the rainy season (emergence), and one is

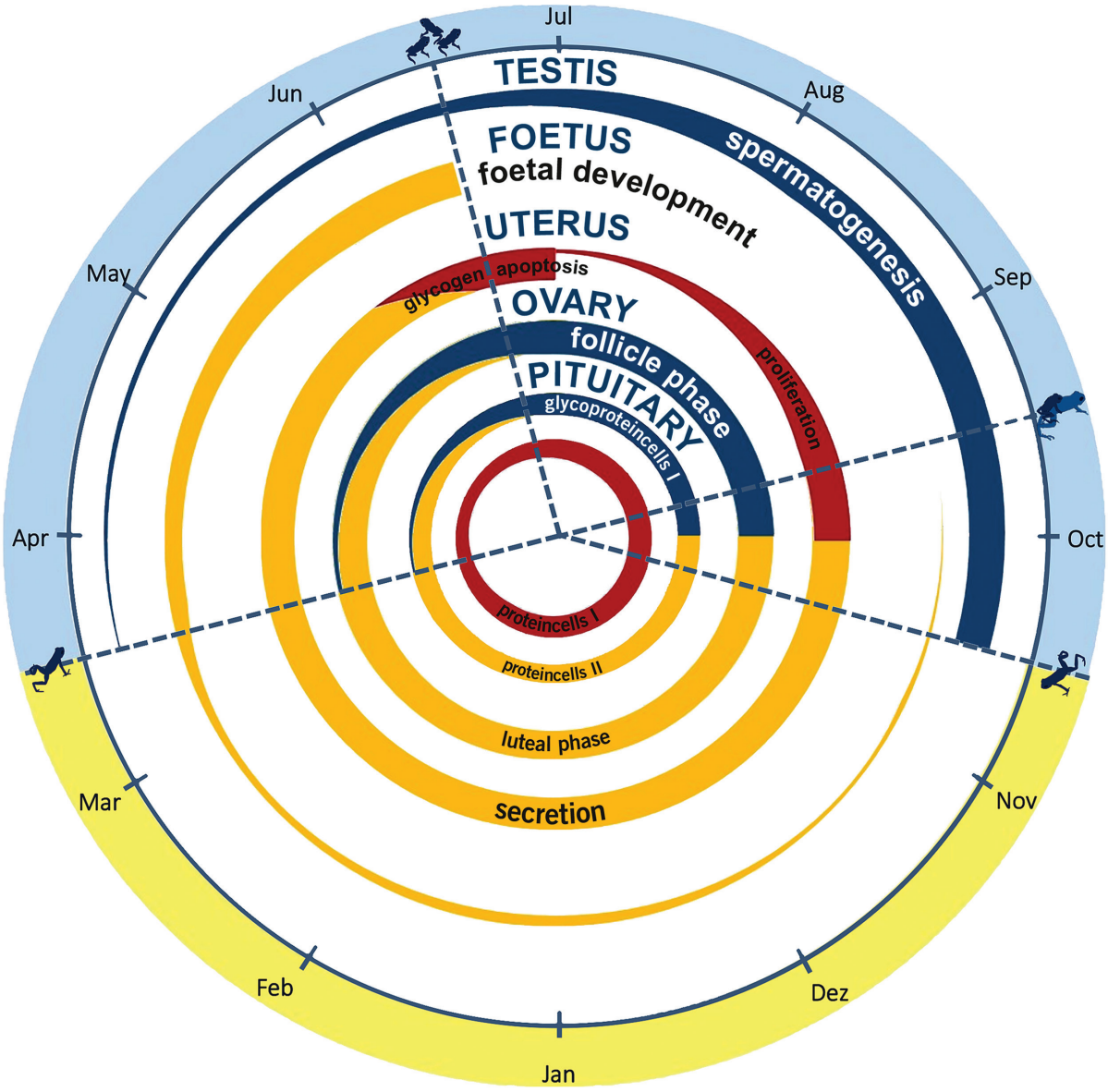

Figure 12. Summary of the temporal development of the foetus and male and female reproductive system. The outer layer gives the months as abbreviations and the seasons by colour (yellow: dry season, blue: rainy season). Toads mate between mid-September and mid-October on average, are going underground mid-October and emerge in mid-March from their dormancy sites. Juveniles are born in mid-June (pictograms). Spermatogenesis shows the development within the male reproductive system. Foetal growth gives the speed of development and growth. The uterus shows three phases: a proliferation phase in which the uterine epithelium develops, a secretion phase during the gestation and an apoptosis phase during which the uterine epithelium is completely exchanged (old one removed and new one built). The ovary has two phases: the follicles phase, characterised by follicle growth and the luteal phase characterised by the presence of corpora lutea. Within the female pituitary three cell types show variation within the annual cycle, the glycoprotein type 1 cells and the protein type 1 and type 2 cells. Within the male pituitary as well three cell types show variation (not shown), glycoprotein type 1 cells and glycoprotein type 2 cells in the same way as those of females, whereas protein type 2 cells are only present in July/ August.

characterised by reproduction at least in females - the end of gestation and intensification of spermatogenesis and intensification of rains (June). In Figure 12 we summarise the temporal changes of the male and female reproductive and foetal development. Most developments can be placed in either of two categories: i) linked to the active above ground life of toads and hence, "environmentally linked", or ii) linked to the gestation period, "reproductively linked". Obviously, these phases are non-exclusive, as both overlap during the first months after emergence and the last months of gestation.

"Environmentally linked" developments start with emergence and end with the beginning of the dormant life underground which coincides with mating (shown in

blue in Figure 12). Within this category fall spermatogenesis, the follicular phase of the ovary, development of the oviductal tube (not shown on Figure 12) and the presence and activity of female and male glycoprotein type 1 cells (gonadotrop cells). The glycoprotein type 1 cells were shown to produce luteinizing hormone (LH), which is generally linked to gonad development and testosterone and oestradiol synthesis. Human gonadotrop cells produce as well follicle stimulating hormone (FSH), which in turn stimulates the granulosa cells, which produce progesterone in Nimba toads (Xavier 1971). Oestradiol and progesterone together trigger oviduct development (Xavier 1971). Hence, all these processes lead to ovulation, mating and finally successful establishment of the gestation. In males, the glycoprotein type 1 and 2 cells are linked to spermatogenesis and nuptial pad development; both are most developed in July and August, just prior to the mating season. As all these processes start with emergence, an environmental trigger for this development could be assumed.

"Reproductively linked" developments start with mating and the establishment of the gestation and end with the birth of juveniles (shown in yellow on Figure 12). This category focusses mainly on females. The uterine secretion phase, the ovarian luteal phase, the presence and activity of protein type 2 cells and obviously foetal development are within this category. The female protein type 2 cells were classified as somatotropic cells. During the dry season, foetal development is slow, the uterus is secreting and stretching slowly and within the ovary corpora lutea are producing progesterone. The developments within this category are nevertheless as well influenced by the environment, as most reproductive organs start slowly decreasing their presence and activity after emergence, including progesterone levels, with the exception of foetal development, which increases in speed after emergence.

The uterine apoptosis and proliferation phases and the presence and activity of protein type 1 cells (shown in red in Figure 12) do not fit in either of these categories. The uterine apoptosis phase starts slowly at the end of gestation with the production of glycogen, which is linked to foetus development (stage III) but not with emergence. During apoptosis the whole uterine mucous layer is replaced, hence this is the phase with the most immediate impact on the uterus. The uterine proliferation phase fits into the environmentally linked definition in that it leads to ovulation and mating at the end of the rainy season, but nevertheless, contains the uterus foetuses until parturition, and preparations for a new gestation can only start after the epithelial cells are replaced during the apoptosis phase. Hence, the secretion and the proliferation phase cannot overlap, as they do in other organs. The presence and activity of the protein type 1 cells were linked to prolactin secretion.

In Nimba toads the reproductive cycle is tightly linked to the seasons. In most other viviparous amphibians an environmental dependency is assumed, but detailed data rarely exist (Wake 2015b). For oviparous caecilians it is known that they may retain the fertilised eggs within the oviduct until a suitable breeding site is found and timing seems to be correlated with the onset of the rainy season (Gomes et al. 2012, Wake 2015a, 2015b). In *S. atra* the duration of gestation is longer at higher altitudes (Wunderer 1910) and is further prolonged by unsuitable environmental conditions (Vilter 1986). In squamate reptiles it was hypothesised that the cold climate at high elevations and high latitudes (Tinkle and Whitfield Gibbons 1977, Watson et al. 2014) or the less variable female body temperature, compared to the surroundings (Shine 1995), favours a viviparous reproduction. Nimba toads occur in an environment with fluctuating temperatures and at high elevation (>1,200 asl). Likewise all *Nectophrynoides* occur between 800 and 2700 m asl (Clarke 1988, Menegon et al. 2004, 2007, Channing et al. 2005, Channing and Howell 2006, Loader et al. 2009), with lower temperatures compared to the lowlands. Nevertheless, the possibly viviparous Central American *C. laticeps* occurs between 10 and 1,500 m asl, and *E. jasperi* and *L. larvaepartus* occur at low elevations (Wake 1978, Iskandar et al. 2014), and most viviparous caecilians are lowland species (Wake 1980b, 1993, Gomes et al. 2012). In caudates, high as well as low elevation species with a viviparous reproduction are known (Wake 2015b). For *L. larvaepartus* it was hypothesised that viviparity might have evolved due to competition avoidance (Iskandar et al. 2014). In pueriparous, adelphophagous subspecies of *S. salamandra* it is hypothesised that scarcity of open water and harsh environments promoted viviparity (García-París et al. 2003, Buckley et al. 2007, Velo-Antón et al. 2012, Escoriza and Ben Hasssine 2014). Within the Nimba mountains high altitude grasslands standing open water is only present during some months during the rainy season at two locations, temperature fluctuations are large and competition quite likely scarce as only two other anuran species occur within the same area (Guibé and Lamotte 1958, L. Sandberger-Loua 2016a). It is likely that scarcity of open water and harsh environments promoted as well viviparity in Nimba toads, or supported the survival of this unique reproductive mode in these special and isolated conditions.

Conservation

Most amphibian species with derived reproductive modes are threatened (Wake 2015b). This is particularly true for Nimba toads, which are listed under the IUCN red list as critically endangered (IUCN SSC Amphibian Specialist Group January 11, 2017/2016). Across a total range of 4 km² the total toad population was estimated to comprise 16 million individuals in August in the 1950s (Lamotte 1959) and 14 million in 1966 (Xavier 1971). A calculation based on an assumed equal range size, our annual monitoring data (2007–2016; 1,160 examined plots of 5 × 5 m², of those 178 in August, and 88 in high density areas in August compare Sandberger-Loua et al. 2016a), would result in 2.8 million toads in August (population size oscillates throughout season). This translates into an 82% decrease in toad numbers since 1959, which quite likely underestimates the decrease

as our distribution estimate is smaller than 4 km², and we only included numbers from areas with high toad abundances to calculate the average number of toads per 1 m². The excellent studies, summarised in this review, are based on > 3,000 females, several hundred foetuses and an unknown number of males (F. Xavier included about 3,000 females and several hundred foetuses and newborns in her doctoral thesis alone, Xavier 1971). During our ten years of field work we recorded less than 61% of the number of females sacrificed to understand the toad's reproduction (1,844 females in total recorded within 29,000 m² of high altitude grasslands searched for 1,740 person hours). Between the 1950s and 2007 two mining exploration campaigns were carried out in the area (Poilecot and Loua 2009), the Nimba mountains were first declared a World Heritage Site (1981/ 1982) and 10 years later (1992) listed as World Heritage Site in Danger (UNESCO 1992). Some anuran species with derived and unique reproductive modes are already considered extinct (*Rheobatrachus silus*, *R. vitellinus*, *E. jasperi*, Wake 2015b) and hence, protection of the reproductive diversity within anurans is important. Considering their complex life cycle, in which reproductive and seasonal cycles are tightly linked, understanding and protecting the Nimba toad's threatened environment is of utmost importance.

Future work

The strong link between the Nimba toad's reproductive and life-cycle with the Nimba mountains seasonality and other environmental factors indicates that these conditions might have favoured the evolution or at least the maintenance of viviparity. Hence, ecological studies on *N. occidentalis* and the other species within the Nimba toad's clade (*Didynamipus*, *Altiphrynoides*, *Schismaderma*, *Nectophrynoides*), several of which have derived reproductive modes as well, may give insights into the evolutionary drivers of viviparity in African bufonids. Comparatively little is known about the other species with and without derived reproductive modes within this clade. For example, the reproductive mode of *D. sjostedti* is only assumed to be direct development (Grandison 1981, Gonwouo et al. 2013) and little else is known (Gonwouo et al. 2013). Of the two Ethiopian *Altiphrynoides* species, some information exist on the reproductive mode (Wake 1980b), but little on the ecology. For *A. osgoodi* it may be impossible to study the ecology as it is feared to be extinct (Gower et al. 2013). Within *Nectophrynoides*, most ecological information exists for *N. asperginis* (Channing et al. 2006), which is extinct in the wild. This emphasises two reasons why ecological studies are needed for all species in this clade: first, they may give insights into the evolution of viviparity and second, they may help to protect these threatened species from extinction.

Conclusion

Viviparity is rare in anurans and the only known matrotrophic anuran is the Nimba toad. In Nimba toads three observations support matrotrophy: first, newborn Nimba toads are 15 times larger and > 200 times heavier than the egg. Second, amino acids injected into the mother were recorded first within the digestive system and liver and later in other areas of the foetuses. Third, foetal size at birth is linked to environmental conditions during the last third of the gestation period, during which females are active and most of the foetal growth occurs. We have identified six characteristics linked to viviparity specific of Nimba toads, which provide strong evidence that viviparity evolved independently in this species. These characteristics comprise three traits which are common in other amphibians, but not usually found in other viviparous species (small and yolk poor eggs, mucoprotein secretion by oviduct/ uterus epithelium, labial papillae of the foetuses), two behavioural or ecologically important traits ("behavioural birthing" and the developmental break during the dry season) and one characteristic which to our knowledge is not known from any other amphibian, but from mammals (complete apoptosis and rebuilding of the uterine epithelium after parturition). Apart from the mucoprotein secretion of the oviduct/ uterus which is found in all amphibians before fertilisation - but in Nimba toads additionally after fertilisation - these traits are more likely present in oviparous species with free-swimming tadpoles than in direct developing and lecitotrophic viviparous species. The other three unique traits can be regarded as adaptations to an unpredictable environment and time constraints due to the environments seasonality. Most reproductive developments can be placed in either of two categories: i) linked to the gestation period, "reproductively linked", or ii) linked to the active above ground life of toads and hence, "environmentally linked". Hence, it is likely that the harsh unpredictable environment and scarcity of open water promoted viviparity in Nimba toads, or supported the survival of this unique reproductive mode in these special and isolated conditions. Considering their complex life cycle, in which reproductive and seasonal cycles are tightly linked, understanding and protecting the Nimba toad's threatened environment is of utmost importance.

Acknowledgements

For support to receive rare and difficult to access publications we thank Martina Rißberger and Hans-Ulrich Raake from the MfN library. Johannes Penner organised access to the doctoral thesis of Françoise Xavier. Nèma Soua Loua provided one photograph in Figure 2. Joseph Doumbia took the photograph of the male nuptial pads (Figure 11). Thomas Schmid-Dankward assisted in preparing Figure 12. This support is very much appreciated! We thank Marvalee H. Wake and an anonymous reviewer for their valuable comments.

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