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Extended embryo retention, caecilian oviparity and amniote origins

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A recent discussion of the evolution of the amniotic egg (Laurin and Reisz, 1997) was criticized by Wilkinson and Nussbaum (1998), and these criticisms provoked a rebuttal (Laurin *et al.*, 2000). Here we show that the objections raised by Laurin *et al.* (2000) do not substantiate the conclusions of Laurin and Reisz (1997). We also discuss additional evidence on the ancestral ontogeny of caecilians from the literature. This evidence is inconsistent with the view that extended embryo retention is the ancestral condition for caecilians and that it is a parsimonious interpretation of the condition of the ancestral amniote as argued by Laurin and Reisz (1997) and by Laurin *et al.* (2000). The available data are more consistent with the traditional hypothesis that the amniotic egg originated as an adaptation of eggs to the terrestrial environment. We also discuss problems in the definition of ontogenetic characters reflecting variation with respect to extended embryo retention, and we present new observations on the early development of the caecilian *Gegeneophis ramaswamii* Taylor.

KEYWORDS: Evolution, phylogeny, parsimony, Amniota, Gymnophiona, reproductive modes, development, amniotic egg.

Introduction

The origin of the amniotic egg, with its complex of extra-embryonic membranes, has long been considered the key adaptation of the amniotes (Haeckel, 1866). Prime significance has been ascribed to its role in enabling amniotes to sever their reproductive ties with water, thereby enabling more complete adaptation to the terrestrial environment. Thus the amniotic egg usually, though not universally, has been interpreted as a terrestrial egg (see review in Stewart, 1997 and discussion in Skulan, 2000). Following Lombardi (1994), Laurin and Reisz (1997: 33) proposed an alternative to the terrestrial egg hypothesis. In their alternative 'some of the extraembryonic membranes evolved in an embryo-retaining form, as a pathway for fetal–maternal interaction, rather than as protection from a harsh external environment'.

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They did not discuss the nature of the foetal–maternal interaction which presumably might involve exchange of nutrients, respiratory gases, metabolic wastes and/or communication. Laurin and Reisz (1997) used their phylogenetic hypothesis for extant tetrapods (figure 1) to investigate the evolutionary history of an associated binary character, the presence or absence of extended embryo retention (EER). In their analysis, the parsimonious reconstruction of the condition of the ancestral amniote was equivocal with respect to EER. They concluded (p. 34) that 'the scenario that the evolution of the amniotic condition involved the intermediate stage of anamniotic eggs being laid on land is not more parsimonious than the alternative suggested here (that extraembryonic membranes appeared to facilitate extensive embryo retention)'. Thus, they sought support for their alternative hypothesis from their demonstration that it is no less parsimonious than the terrestrial egg hypothesis.

Parsimonious reconstructions of ancestral conditions on phylogenetic trees depend on the relationships in the tree and the coding of the terminal taxa. Wilkinson and Nussbaum (1998) argued that the equivocal reconstruction of the ancestral amniote condition by Laurin and Reisz (1997) was dependent upon an incorrect scoring of caecilians (Gymnophiona) as having EER. When caecilians are correctly scored as lacking EER, the ancestral amniote is unambiguously reconstructed on Laurin and Reisz's tree as also lacking EER. The revised scoring of caecilians renders Laurin and Reisz's preferred hypothesis less parsimonious than the terrestrial egg hypothesis. In the context of caecilians, Wilkinson and Nussbaum (1998) equated EER with viviparity, and oviparity with the absence of EER. They presented a simple analysis of reproductive modes in caecilians that confirmed (p. 1404) 'the

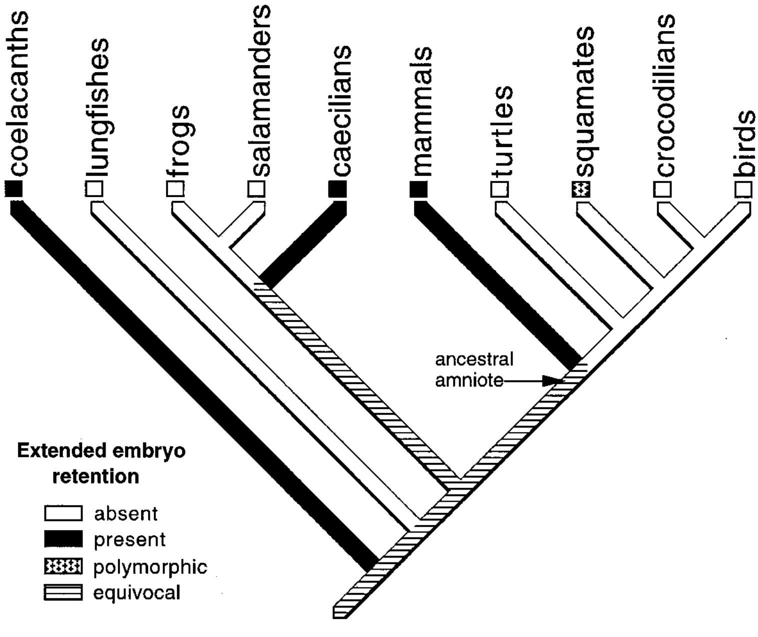

FIG. 1. Tetrapod phylogeny (after Laurin and Reisz, 1997) with the binary character, extended egg retention 'present' or 'absent', mapped on to the tree by MacClade (Maddison and Maddison, 1992). With caecilians coded 'present', as in Laurin and Reisz (1997), the condition of ancestral amniotes with respect to this character is ambiguous.

long held view (e.g. Dunn, 1942) that the primitive reproductive mode in caecilians is oviparity with eggs hatching into free-living larvae that undergo metamorphosis (i.e. the characteristic amphibian bi-phasic life cycle with no extended embryo retention)'.

Laurin *et al.* (2000) accepted Wilkinson and Nussbaum's (1998) conclusion that the ancestral caecilian was oviparous but argued against equating viviparity with EER and oviparity with its absence. They cited Monotremata and some squamates as examples of taxa that are oviparous and have EER. Thus, it is possible that the ancestral caecilian was oviparous, as argued by Wilkinson and Nussbaum (1998) but that, contra these authors, it also had EER. Laurin *et al.* (2000: 311) concluded that Wilkinson and Nussbaum (1998) had misrepresented Laurin and Reisz (1997), and that consequently 'the presence of a significant amount of intrauterine development in the earliest amniotes remains as parsimonious as the alternative'. Laurin *et al.* (2000) also reported a survey of the literature on the stage of development at oviposition in caecilians and found no compelling evidence that oviparous caecilians lack EER. Here we consider the scoring of caecilians with respect to EER and the use of this character in evaluating alternative hypotheses for the origin of the amniotic egg. We show that under any sensible scoring, the ancestral amniote is most parsimoniously interpreted as lacking EER given Laurin and Reisz's (1997) tree, and thus is inconsistent with their hypothesis that the amniotic membranes evolved in an embryo-retaining form. In addition, we demonstrate that the same conclusion holds if caecilians are the sister group of salamanders rather than part of the basal lissamphibian split whatever the scoring of caecilians. A review of the literature and new data supports scoring caecilians as lacking EER, consistent with the terrestrial egg hypothesis.

Scoring of caecilians

We accept the distinction between oviparity and EER and that it is logically possible for the ancestral caecilian to have been both oviparous and to have had EER. We therefore accept that Wilkinson and Nussbaum's conclusions are based on a potentially incorrect premise. However, it is also logically possible that the ancestral caecilian was both oviparous and lacked EER as is believed to be the case for the ancestral anuran and the ancestral urodele (and their last common ancestor), in which case Wilkinson and Nussbaum's conclusions do hold. The key interconnected questions then are 'How reasonable or likely is either of these logical possibilities?' and 'How should caecilians be scored with respect to the EER character?'

Laurin and Reisz (1997) presented no discussion of caecilian reproduction or of their scoring of caecilians with respect to EER. They also gave no operational definition of EER. Laurin *et al.* (2000) claimed that they (i.e. Laurin and Reisz, 1997) had used the occurrence of viviparity 'to argue [*sic*] for extended embryo retention in caecilians'. In a further paper, Laurin and Girondot (1999: 100) also state that Laurin and Reisz's (1997) coding of caecilians was 'largely because information on the early development of gymnophiones is available mostly for viviparous species'. However, that EER occurs in viviparous caecilians does not by itself justify an inference that it occurred in the ancestral caecilian. Viviparity is widely considered derived and oviparity ancestral within caecilians (e.g. Dunn, 1942; Wake, 1977; Nussbaum, 1979; Wilkinson and Nussbaum, 1998; Laurin and Girondot, 1999; Laurin *et al.*, 2000). Thus, it is the extent of embryo retention in oviparous caecilians, particularly those that have the ancestral condition of a free-living larval stage, that is most important in inferring the extent of embryo retention in the ancestral caecilian.

Laurin *et al.* (2000) attempted to summarize the 'meagre' data on the early stages of development in oviparous caecilians. They reported that, with a single exception, all caecilian eggs that have been reported include well-developed embryos (see below). The noted exception comes from the work of Brauer (1897) on the direct developing Seychellean caecilian *Hypogeophis rostratus* (Cuvier). He reported that eggs are laid at the blastula stage implying that there is no EER. Laurin *et al.* (2000) note that there has been no confirmation of Brauer's work, but we have no reason to doubt the accuracy of his meticulous observations. Thus, according to the review of Laurin *et al.* (2000), the only information available on the early development of any oviparous caecilian comes from a single species in which eggs are laid in the blastula stage without any EER. They note (p. 313) that 'If evidence about the early development of all but one species of caecilian is lacking in the scientific literature, the arguments of Wilkinson and Nussbaum (1998) are invalid and they have not falsified our hypothesis'. Their view is that the available information 'does not provide a safe basis to infer the primitive reproductive pattern for caecilians. Therefore, until more data about the early development of oviparous caecilians become available it is still justifiable to consider that caecilians could perform extended embryo retention'.

For the moment, we accept both parts of their argument. Ideally, we would like more data for inferring the ancestral condition of caecilians (see below) because, given the limited evidence uncovered by Laurin *et al.* (2000), EER in the ancestral caecilian remains a possibility. At least equally, however, the absence of EER in the ancestral caecilian also remains a possibility, and the evidence from *Hypogeophis* supports, albeit weakly, this possibility. Thus, the information at Laurin *et al.*'s (2000) disposal indicates that there is no more basis for coding caecilians as showing EER than there is for coding them as lacking EER. Laurin *et al.* (2000: 313) note that 'the literature does not appear to provide decisive evidence regarding the amount of intra-uterine development in most oviparous caecilians'. However, they did not explore the consequences of the logical conclusion that caecilians should be scored as equivocal (i.e. with a missing entry) with respect to EER. Importantly, scoring caecilians as equivocal has the same effect upon the parsimonious reconstruction of the ancestral amniote (figure 2) as does Wilkinson and Nussbaum's (1998) scoring of caecilians as lacking EER and it demonstrates the validity of their conclusions regardless of any misrepresentation of the distinction between oviparity and EER. Only if caecilians are scored as having EER, for which there is no good evidence, is the parsimonious reconstruction of the ancestral amniote ambiguous on the Laurin and Reisz (1997) tree.

Laurin and Girondot (1999) also discussed the available literature on the early development of caecilians and its consequences for coding caecilians with respect to EER. They pointed out that Brauer (1897) attributed to Sarasin and Sarasin (1887–1890) the observations that the eggs of *Ichthyophis glutinosus* (Linnaeus) are also laid at the blastula stage. In contrast, however, Laurin and Girondot (1999: 101) reported that 'when reading Sarasin and Sarasin, we were unable to find this statement in this publication' (but see below). They went on to conclude that 'If we assume that *Hypogeophis rostratus* (and perhaps, *Ichthyophis glutinosus*) is representative of most oviparous gymnophiones, and if we accept the relatively

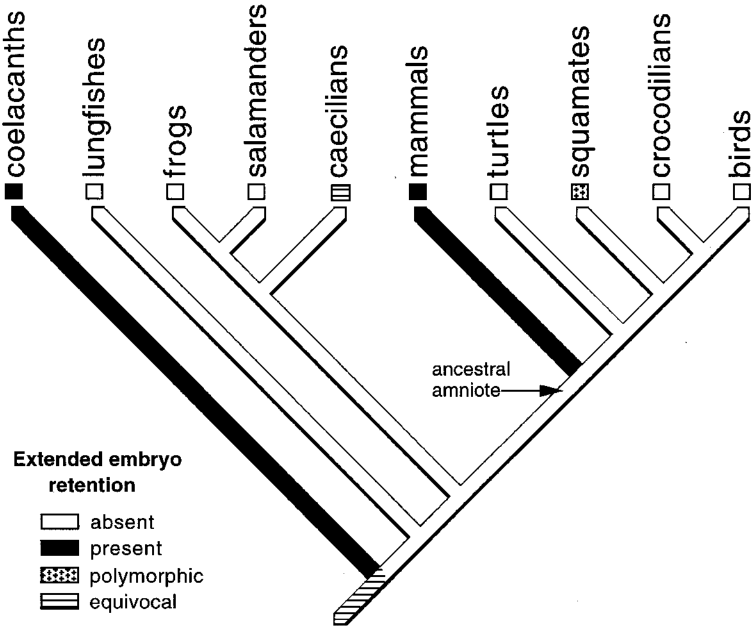

FIG. 2. Tetrapod phylogeny (after Laurin and Reisz, 1997) with the binary character, extended egg retention 'present' or 'absent', mapped on to the tree by MacClade (Maddison and Maddison, 1992). With caecilians (Gymnophiona) coded as equivocal, the parsimonious interpretation is unambiguous and indicates the absence of EER in the ancestral amniote.

well-established hypothesis that gymnophiones were primitively oviparous, this group has to be coded as not performing extended embryo retention'. Confusingly, Laurin and Girondot (1999) reach a very different conclusion from Laurin *et al.* (2000) regarding the coding of caecilians with respect to EER, but neither contribution refers to the other or provides any explanation of the obvious discrepancy. We agree with Wilkinson and Nussbaum (1998) and Laurin and Girondot (1999) that caecilians should be coded as lacking EER.

Alteration of the relationships of the lissamphibians in the Laurin and Reisz (1997) tree also affects the interpretation of the ancestral amniote. For example, if caecilians are placed as the sister group of salamanders as suggested by some molecular data (e.g. Feller and Hedges, 1998), then the parsimonious reconstruction of the ancestral amniote again is unambiguous as lacking EER whatever the scoring of caecilians (figure 3). The sensitivity of reconstructions of the ancestral amniote to alternative interpretations of phylogenetic relationships was also noted by Laurin and Girondot (1999).

To summarize this section, Laurin and Reisz (1997) concluded that their preferred hypothesis of the origin of the amniotic egg is no less parsimonious than the alternative. This hypothesis depends on two conditions, the coding of caecilians as having EER ancestrally and a sister group relationship between frogs and salamanders. The first is not supported by Laurin *et al.*'s (2000) interpretation of the literature and is contradicted by Laurin and Girondot (2000), and the second has not been clearly established. Under other plausible relationships, or under scorings of caecilians that are the most plausible (absence of EER in the ancestral caecilian)

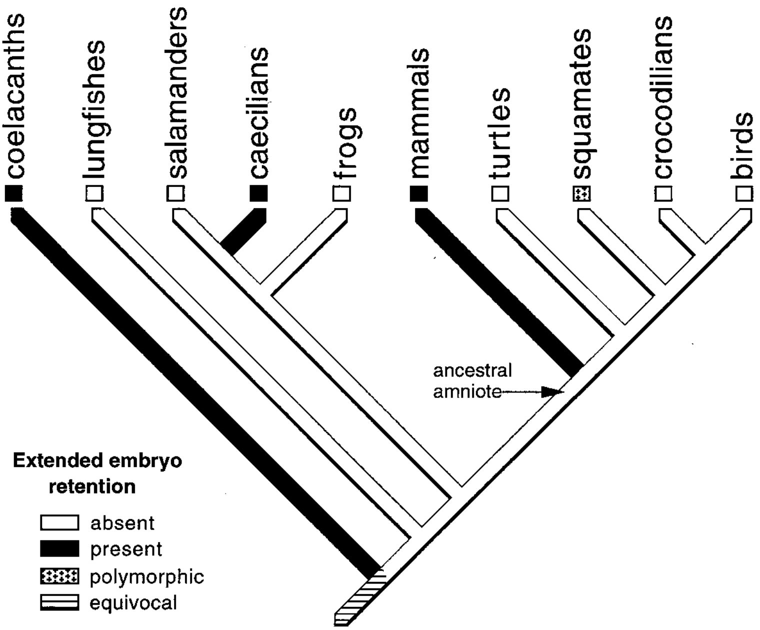

FIG. 3. Alternative tetrapod phylogeny with the binary character, extended egg retention 'present' or 'absent', mapped on to the tree by MacClade (Maddison and Maddison, 1992). With caecilians coded present as in Laurin and Reisz (1997) but placed as the sister group of the salamanders (Urodela), the parsimonious interpretation is unambiguous and indicates the absence of EER in the ancestral amniote.

or simply equivocal, the condition of the ancestral amniote is unambiguous: it lacks EER. Thus, despite the objections of Laurin *et al.* (2000), the alternative hypothesis of Laurin and Reisz (1997) is less parsimonious than the terrestrial egg hypothesis as an explanation for the origin of the amniotic egg.

Additional data from the literature

Laurin *et al.* (2000: 312) and Laurin and Girondot (1999: 100) reported conducting a 'thorough literature review' and 'an extensive literature search', respectively, but found no evidence on the early embryology of oviparous caecilians other than Brauer's (1897) study of *Hypogeophis rostratus*. However, additional data do exist. Firstly, Brauer (1897) conducted a comparative study of two oviparous caeciliid caecilians, *Hypogeophis rostratus* and *Grandisonia alternans* (Stejneger), both of which are reported as lacking EER. Recognition that *G. alternans* lacks EER is important because this species has a larval stage, and in this respect is more similar to the assumed ancestral caecilian than the direct developing *H. rostratus*.

The most important additional evidence is to be found in the Sarasin cousins' magnificent work on the Sri Lankan ichthyophiid caecilian *Ichthyophis glutinosus* (Sarasin and Sarasin, 1887–1890). This species, like *Grandisonia alternans*, is oviparous with a free-living larval stage (the assumed reproductive mode of the ancestral caecilian), and is a member of a more basal assemblage of caecilians than are *Hypogeophis* and *Grandisonia* (Nussbaum, 1979; Duellman and Trueb, 1986; Hillis, 1991; Hedges *et al.*, 1993; Wilkinson and Nussbaum, 1996; Wilkinson, 1997). The Sarasins figured very early stages of the development of *I. glutinosus*, including cleavage, blastula, gastrula and neurula, and some of these have been reproduced in textbooks (e.g. Noble, 1931: figure 10; Duellman and Trueb, 1986: figures 5–20; Himstedt, 1996: Abb. 48). Their 'Tafel II' is reproduced here as figure 4.

Laurin *et al.* (2000: 312) reported of the Sarasins' work that they 'could not, after careful reading, find the mention of when the eggs were laid'. Unfortunately, they overlooked several passages that make this information plain. The Sarasins conducted extensive investigations aimed at documenting the reproduction and development of *Ichthyophis glutinosus* in the course of which they examined thousands of specimens. The Sarasins reported (p. 13) that there appears to be a rapid passage of eggs through the female after fertilization and that only cleavage, the earliest stage of embryonic development, occurs in the oviducts prior to egg laying. They went on to state (p. 15) that 'Gleich nach Ablauf des eben beschriebenen Stadiums wird der Laich in die Erde abgelegt; denn kurz nach dem Funde in den Oviducten erhielten wir aus der Erde ein Klümpchen Eier, die in ihrer Entwicklung sich unmittelbar an die Obigen anreihten. Nicht alle Eier dieses Klümpchens waren gleich weit vorgeschritten, so dass wir für diese frühen Stadien eine hübsche Serie gewinnen konnten, Taf. II, 10–16' and that 'Das jüngste Stadium dieses Eierhäufchens giebt Fig. 10 wieder; man erkennt daran nichts als eine graue, durch die Behandlung unregelmässig geschrumpfte Keimscheibe, umgeben von einem helleren Gürtel, dem Keimwall'. Thus the early stages illustrated here in figure 4 are from eggs that had been laid in earth. Any thorough reading of the Sarasins' studies shows that they demonstrated conclusively that EER does not occur in *I. glutinosus*.

Laurin and Girondot (1999: 101) also failed to find any reference to the stage of development at oviposition in the Sarasins' works but, as noted earlier, they found reference to this in Brauer (1897). They offered a possible explanation of the discrepancy between Brauer's report and their own findings, namely that 'Brauer obtained this information by personal communication from Sarasin and Sarasin (they were all German scientists)'. Clearly, this fanciful explanation is not needed. Furthermore, given the aims of their study, we consider it implausible that the Sarasins might have made the pertinent observations but not published them. Laurin and Girondot's notion that the Sarasin cousins were German is incorrect, they were Swiss. We also note that, like many modern scientists, many 19th-century scholars corresponded with colleagues in several languages. Hence, shared nationality does not necessarily make personal communication more plausible.

Laurin *et al.* (2000) also briefly discussed other reports on the development of *Ichthyophis glutinosus* and *I. malabarensis* Taylor by Breckenridge and Jayasinghe (1979) and Balakrishna *et al.* (1983), respectively. They summarized (Laurin *et al.*, 2000: 312–313) that these studies were of 'accidentally found eggs that had been laid for an unknown period of time' and that 'the smallest observed caecilian embryos in laid eggs measured 6 mm and possessed at least 21 somites'. However, this is a rather incomplete and misleading summary of these important works.

Breckenridge and Jayasinghe (1979) reported on five different collections of eggs with no indication that they were found accidentally. Of greatest interest to the present discussion are the eggs collected in July 1974 because these represent the earliest stages of development described by these authors. Breckenridge and Jayasinghe (1979: 191) reported that these eggs had 'a minute embryo visible as a thin grey streak on the yolk surface'. For the first 3 days of observation, these embryos had an 'irregular outline and rudiments of eyes, gills and heart are not visible'. It was only after 9 days of observation that about 21 somites could be

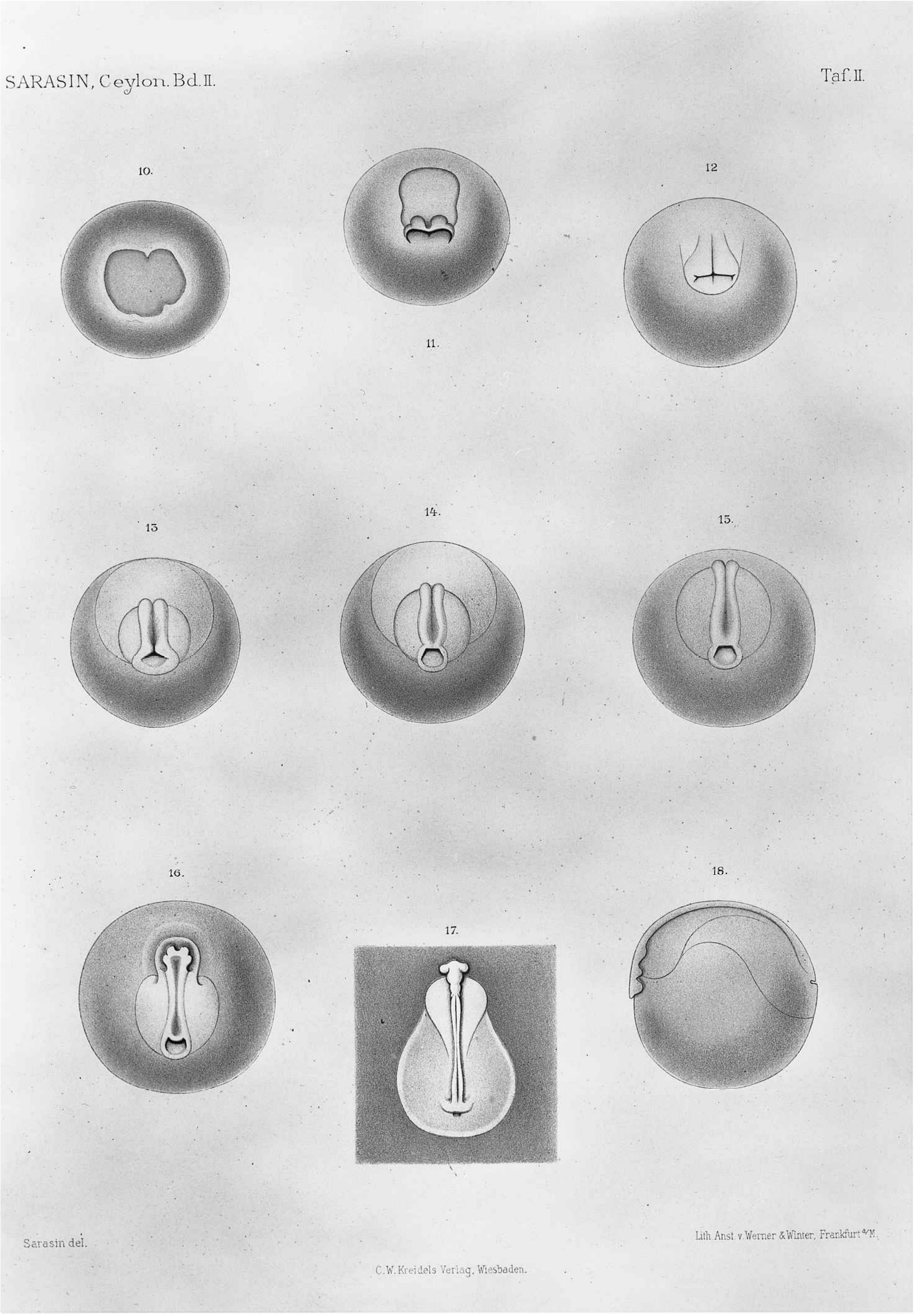

FIG. 4. Stages in the early development of the oviparous Sri Lankan caecilian *Ichthyophis glutinosus* (reproduced from Sarasin and Sarasin [1887–1890]). All of the stages illustrated were observed in eggs that had been laid.

counted on an enlarged (8 mm) embryo. This report provides strong independent evidence that *Ichthyophis glutinosus* lays eggs at a very early stage of development and that it lacks EER.

The report by Balakrishna *et al.* (1983) follows an earlier report by Seshachar *et al.* (1982) on reproduction in the Indian caecilian *Ichthyophis malabarensis* that also has the ancestral life history of oviparity and a free-living larva. Both studies and other papers by these authors were the result of concerted efforts to find and study caecilians rather than of accidental discoveries. Seshachar *et al.* (1982) reported finding a female *I. malabarensis* coiled around about 100 eggs. No embryos could be detected in the yolky eggs and the authors suggested that either the eggs were laid at a very early stage of development or that the eggs were infertile. Unfortunately, histological sections which might have distinguished between these alternatives were not prepared. Balakrishna *et al.* (1983) reported on a second clutch in which embryos measured 8 mm and had 21 somites, a well-differentiated cephalic region, optic vesicles and a tail bud. Given that eggs had been laid an unknown period of time before their collection, the early stage of development (early tailbud) of the latter embryos is consistent with, and the potential lack of any discernible embryonic differentiation in the former clutch is suggestive that, *I. malabarensis* lacks EER. This conclusion is further strengthened by the observations of Bhatta (1999) who reported finding a clutch of *I. malabarensis* in which no embryos were visible on the day of collection but in which embryos became apparent after 3 days.

Laurin *et al.*'s (2000) view of the literature is that it is ambiguous with respect to EER in oviparous caecilians. However, as we have shown, the primary literature includes good evidence that a number of oviparous caecilian species lack EER and no clear evidence that any oviparous caecilian has EER. Two of us (M.W., D.J.G.) have examined numerous (*ca.* 50) gravid specimens of a number of species of *Ichthyophis* and have never observed embryos, further supporting the view that EER is absent in this genus. Although these data are far from comprehensive, they are unopposed and they favour the hypothesis that the ancestral caecilian was oviparous and lacked EER. As shown by Wilkinson and Nussbaum (1998), if the ancestral caecilian is scored so as to reflect this more likely hypothesis then, contra Laurin and Reisz (1997), the parsimonious reconstruction of the ancestral amniote is also as lacking EER.

Defining EER

We do not wish to criticize Laurin and Reisz's (1997) intention of investigating an ancestral condition by parsimoniously mapping a character on to a phylogenetic hypothesis. However, we are concerned about the definition of the character states of their EER character and how these relate to the alternative explanatory hypotheses for the origin of the extra-embryonic membranes of amniotes that they sought to test. In cladistic analyses, character states used to infer relationships represent similarities that are taken as putative homologies. Ideally, they are defined sufficiently clearly and non-arbitrarily that alternative workers would recognize the same character states and score individual taxa identically. In our view, the same ideals apply to characters used for reconstructing ancestral character states rather than for inferring phylogeny. It is unfortunate then that Laurin and Reisz (1997) provided no explication of their character, EER present or absent. As Laurin *et al.* (2000:

312) point out, ‘Egg retention in tetrapods exhibits continuous variation’. This suggests that determining a non-arbitrary division of this variation into a dichotomous presence or absence of EER may not be simple. From Laurin and Reisz (1997) it is not clear to us where, or how, the distinction between the alternative character states has been drawn. Laurin *et al.* (2000) equated EER with embryos being retained ‘for a significant portion of their development’ without defining ‘significant’ in this context and they ‘explained’ their usage only incompletely through limited examples. Consequently, the reader is left not knowing whether ‘significant’ refers to the proportion of the embryonic period, to a stage in development or to something else.

The definition of cladistic characters and character states drawn from ontogeny may be complicated by variation in both absolute and relative developmental timing. For example, there is considerable variation within amphibians in the temporal duration of embyrogenesis. In the model anuran *Xenopus laevis* (Daudin), neurulation begins after just 12 h (Nieuwkoop and Faber, 1994) whereas it does not begin until 14 days in the urodele *Necturus maculosus* (Rafinesque) (Eycleshymer and Wilson, 1910). Thus, there is no absolute temporal scale that can underpin meaningful cross-taxonomic comparisons of developmental stages of disparate taxa. In the absence of a temporal scale, what constitutes ‘significant’ EER must be defined in relation to some temporally discrete and putatively homologous developmental event(s), that can be independently documented and verified for any taxon. However, the definition of EER by reference to major stages in organogenesis is complicated by developmental heterochronies and major differences in the sequence of appearance of characteristic vertebrate embryonic features and stages (Richardson, 1995). For example, somitogenesis tends to proceed more rapidly in embryos of species with elongate body forms and high numbers of somites (Richardson *et al.*, 1998; see also Raynaud, 1994). Thus the stages at which the embryos of different species reach a given number of somites or a given proportion of the adult number of somites may not correspond in any other meaningful way. Laurin *et al.* (2000) did not consider *Ichthyophis* eggs containing embryos with 21 somites as evidence of a lack of EER. However, adult *Ichthyophis* have more than 100 vertebrae (Taylor, 1968) and some caecilians have in excess of 300 vertebrae (M.W., personal observation). Caecilian embryos with only a couple of dozen somites are likely to be less well developed than many other vertebrate embryos with a similar somite number (see below). This suggests that if ‘significant’ EER is defined in terms of particular developmental events, these events need to be chosen carefully and should not be subject to major developmental heterochronies.

Given that Laurin and Reisz (1997) argue that some extra-embryonic membranes evolved through EER, and that this is the hypothesis they seek to test, one might naturally consider the retention of embryos until at least the development of the extra-embryonic membranes as the *sine qua non* of the kind of ‘significant’ EER required of the ancestral amniote by their scenario. Only if amniotes retain embryos until the development of their extra-embryonic membranes would they show ‘significant’ EER. From this perspective, Laurin and Reisz’s (1997) coding of monotremes as having EER seems questionable. At oviposition, after an intrauterine stage of development, the embryonic Platypus, *Ornithorhynchus anatinus* (Shaw), lacks both a chorion and allantois and is only in the early stages of amniogenesis (Stewart, 1997).

Similarly, the appropriate coding of crocodilians is not completely clear. According to Ferguson (1985: 381): 'The amniotic primordium, derived from somatopleure around the trunk, arises in continuity with that of the head ... this combined head-trunk amnion reaches the level of the blastopore about the time of egg laying'. Although amniogenesis is under way at oviposition (stage 1) the allantois does not appear until stage 4. Thus, the formation of the extra-embryonic membranes is a protracted process and this raises further questions about how much development of these membranes is 'significant'. Laurin and Reisz (1997) scored crocodilians as lacking EER whereas Laurin and Girondot (1999) recoded them as having EER (see below).

Importantly, this coding framework is not directly applicable to anamniotes, such as caecilians, that lack the extra-embryonic membranes characteristic of Amniota. Thus, instead we might seek to identify developmental events that co-occur with the development of the extra-embryonic membranes in amniotes and use these to define 'significant' EER in anamniotes. Unfortunately, we have not been able to identify any such events.

Laurin and Girondot (1999) reported a bimodal distribution of developmental stages at oviposition in sarcopterygians and used this as a basis for a more explicit redefinition (p. 100) of the EER character: 'taxa that lay eggs at a gastrula stage or earlier do not perform extended embryo retention. On the other hand, taxa that lay eggs in which the embryo already has somites (somites appear in the neurula stage, in squamates) perform embryo retention'. This definition is an advance over that of Laurin *et al.* (2000) in that it is more precise and uses early developmental stages that we might hope are less dramatically affected by developmental heterochronies (but see Richardson, 1995). However, gastrulation is a very early developmental event and there appears to be considerable variation in the relative timing of the onset of somitogenesis, the onset of the development of extra-embryonic membranes and the development of functional extra-embryonic membranes (Keibel, 1897–1938). Somitogenesis does not appear to be tightly coupled to the development of extra-embryonic membranes. Laurin and Girondot's revised formulation of the EER character is therefore somewhat disconnected from the kind of 'significant' EER that might underpin the development of extra-embryonic membranes to facilitate foetal–maternal interaction. Consequently, its use in evaluations of the alternative hypotheses for the evolution of the amniotic egg is correspondingly weakened. In other words, an inference that the ancestral amniote laid eggs after the gastrula stage does not entail the opportunity for extensive foetal–maternal interaction in the ancestral amniote.

Our recent observations on the early development of the oviparous caeciliid *Gegeneophis ramaswamii* Taylor are illuminating here. This species is widely distributed and locally abundant in southern Kerala, India (Oommen *et al.*, 2000). Seshachar (1942) briefly reported on eggs and embryos of this species (reported as *G. carnosus* Beddome). Seshachar's report establishes that *G. ramaswamii* is oviparous but the embryos he observed were well-developed and tell us little of the stage of development at oviposition and thus the extent of any embryo retention.

Eggs of *Gegeneophis ramaswamii* at an earlier stage of development were collected at Cheeranikkara, Kerala (08°39′08.8″N, 76°57′38.7″E), on 30 June 1999. The eggs were fixed in formalin and stored in ethanol (70% aqueous solution). Embryos were not clearly discernible in these eggs, and the quality of preservation was rather poor, but routine histology (dehydration through graded ethanols, embedding in technovit, and staining with haematoxylin and eosin) reveals some useful detail (the slides are maintained in the collections of the Natural History Museum, London). One of the eggs contains an early embryo with approximately 35 somites. According to Laurin and Girondot (1999), if these eggs had been laid at this stage then *G. ramaswamii* would be considered to have extended embryo retention. However, by several criteria this embryo is at a very early stage of development. The number of somites is only about one-third of the number of adult vertebrae, the somites are still epithelial, some of the more cranial somites have nephric tubules, the notochord is vacuolar with some yolk platelets. The endoderm is heavily yolked, the neural folds are closed in most of the trunk although a caudal neuropore is still open. Pharyngeal arches and possibly pericardiac coelom are present but no heart tube is visible. We suggest that these embryos are at a sufficiently early stage of development to provide no evidence that *G. ramaswamii* has any 'significant' embryo retention. We predict that *G. ramaswamii*, like all other oviparous caecilians for which there is any pertinent information, lacks EER.

The above example illustrates that there is as yet scope for disagreement regarding the scoring of particular taxa with respect to the presence or absence of EER. This will only be resolved through further consideration of how these character states are defined. Such resolution will need to facilitate meaningful comparisons across diverse taxa subject to many developmental heterochronies. Simultaneously, it will need to ensure that the character states relate to differences that could be of real significance for enhanced foetal–maternal interaction. However, irrespective of how these character states are defined, our review suggests that neither the ancestral caecilian nor the ancestral amniote had EER. With respect to this character and Laurin and Reisz's (1997) tree, the terrestrial egg hypothesis is more parsimonious.

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