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Why colour in subterranean vertebrates? Exploring the evolution of colour patterns in caecilian amphibians

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Abstract

The proximate functions of animal skin colour are difficult to assign as they can result from natural selection, sexual selection or neutral evolution under genetic drift. Most often colour patterns are thought to signal visual stimuli; so, their presence in subterranean taxa is perplexing. We evaluate the adaptive nature of colour patterns in nearly a third of all known species of caecilians, an order of amphibians most of which live in tropical soils and leaf litter. We found that certain colour pattern elements in caecilians can be explained based on characteristics concerning above-ground movement. Our study implies that certain caecilian colour patterns have convergently evolved under selection and we hypothesize their function most likely to be a synergy of aposematism and crypsis, related to periods when individuals move overground. In a wider context, our results suggest that very little exposure to daylight is required to evolve and maintain a varied array of colour patterns in animal skin.

Introduction

Colour patterns in animal skin originate from pigments or structures which reflect specific wavelengths of light (e.g. Bagnara & Hadley, 1973). Colour pigments are transported into the skin via long physiological pathways (Bagnara & Hadley, 1973; Frost-Mason *et al.*, 1994), which require energy and can therefore be considered costly. According to the handicap theory (Zahavi, 1975; Guilford & Dawkins, 1993), colour patterns are most often interpreted as having an adaptive advantage, and thus having evolved under selection. Possible functions of colour patterns include physiological adaptations (e.g. thermoregulation, Guilford, 1988) or visual signals in the context of aposematism, mimicry or crypsis (e.g. Beddard, 1892; Cott, 1940). Like fish and birds (e.g. Galeotti *et al.*, 2003; Salzburger & Meyer, 2004), amphibians provide a variety of colour patterns; furthermore, they are known for their high levels of morphological convergence (e.g. Emerson, 1986; Wake, 1991; Bossuyt & Milinkovitch, 2000), which makes them a well-suited model group for the study of adaptive trait evolution in vertebrates (Vences *et al.*, 2003).

Amphibians which are diurnal, brightly coloured and noxious are considered aposematic, often having specialized on arthropod prey (Daly, 1982; Daly *et al.*, 1987; Santos *et al.*, 2003; Saporito *et al.*, 2006). Several recent studies reflect the opinion that aposematic colour does not match the background by measuring spectra of aposematic amphibians in relation to a supposed ‘natural’ background (Summers *et al.*, 2003; Siddiqi *et al.*, 2004; Uy & Endler, 2004; Endler & Mielke, 2005). Yet, the line that divides aposematism from camouflage (colour patterns that do match the background) is often drawn using subjective criteria, with the consequence of major differences in the putative selective pressures hypothesized to be acting on amphibian colour patterns. There are aposematic species which could alternatively be considered as using camouflage (e.g. metallic-green *Dendobates auratus*), and for many aposematic species toxicity levels can vary (Saporito *et al.*, 2006). On the other hand, classic camouflage-wearing species may appear aposematic when their background is changed (e.g. *Hyperolius marmoratus*). Furthermore, studies on potential diurnal predators tend to focus on those having

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well-studied tetra-chromatic colour vision systems (e.g. birds, Siddiqi *et al.*, 2004; Endler & Mielke, 2005), whereas others with limited or unstudied visual abilities may be far more important but are rarely considered (e.g. snakes, spiders, ants or small mammals; Gower *et al.*, 2004a; Menin *et al.*, 2005; Toledo *et al.*, 2007). Thus, in many cases the predators' colour-vision abilities could not be sufficient to detect the colour that is displayed by their amphibian prey, or they could differ from the classification of colours as 'aposematic' by human observers.

In addition to the various processes of natural selection, other evolutionary mechanisms are also hypothesized to drive the evolution of amphibian colour patterns: polymorphic colour patterns could have evolved under sexual selection (Hayes & Menendez, 1999; Summers *et al.*, 2003; Hagemann & Pröhl, 2007). Alternatively, neutral evolution of polymorphic colour patterns under genetic drift (as unrelated to the evolution of skin toxins and diurnality) could have lead to the presence of both 'aposematic' and 'cryptic' forms within a species (Wollenberg *et al.*, 2006, 2008).

Thus, in many cases it remains unclear whether natural selection, sexual selection or neutral evolution is the driving force in shaping amphibian colour patterns; and even under the relatively simple model of aposematism it is uncertain which trait in such co-evolving trait complexes (colour, noxiousness and related variables such as diurnality or diet specialization) has evolved first. Solving these fundamental questions could facilitate finding explanations for the more complicated systems mentioned above. The evolutionary processes in question are restricted to daylight, because up to now no conspicuously coloured amphibian taxon is known that is a diurnal obligate but not noxious, or toxic but nocturnal obligate (for example, aposematic fire salamanders may be active during daytime after heavy rainfalls). If there were amphibians that were strictly nocturnal, toxic and conspicuously coloured, this could provide a suitable null model for colour pattern evolution in the absence of evolutionary forces operating in daytime. Such examples could be found in the amphibian order Gymnophiona (Caecilians). Caecilians comprise 172 recognized species (AmphibiaWeb 2006, IUCN *et al.*, 2006), are distributed circumtropically east of Wallace's line (Taylor, 1968; Himstedt, 1996) and have been described as an order of elongate, legless animals that live within the soil, with the exception of the aquatic subfamily Typhlonectinae (e.g. Exbrayat, 2000). Although they are mostly subterranean, they display a wide range of colour patterns, with some species being comparable with the most conspicuous frogs and salamanders. Indeed, it has been suggested that the bright colouration of some caecilian species may be aposematic and associated with the presence of skin toxins (Nussbaum, 1998).

Consequently, these animals provide excellent model organisms to study the evolution of conspicuous colour patterns in relation to the exposure to evolutionary forces operating in daylight, and thus their adaptiveness. Our study has two goals: (i) provide the first assessment of colour patterns in the order Gymnophiona in a phylogenetic context and (ii) test the hypothesis of nonadaptive vs. adaptive evolution in caecilian colour patterns. Under adaptive evolution of caecilian colour patterns given a putative function in inter- or intraspecific context, we would expect a phylogenetically independent correlation between caecilian colour patterns and exposure to daylight, reflected by increased aboveground movement or visual abilities. By contrast, under neutral evolution, we would expect no such correlation.

Materials and methods

Colour patterns in caecilian amphibians

We compiled information on caecilian colour and pattern characteristics from the literature, including original descriptions, as well as colour pictures and observations of live caecilians (Barbour & Loveridge, 1928; Taylor, 1968; Nussbaum & Wilkinson, 1987; Nussbaum & Hinkel, 1994; Junqueira *et al.*, 1999; Exbrayat, 2000; Lawson, 2000; Giri *et al.*, 2003; Leong & Lim, 2003; Ravichandran *et al.*, 2003; Vyas, 2003; Wilkinson *et al.*, 2003; Müller *et al.*, 2005; Exbrayat, 2006b; Measey, 2006; G. John Measey, personal observation). We compiled a data matrix for 53 caecilian taxa, representing nearly a third of the 172 known species (AmphibiaWeb 2006, IUCN *et al.*, 2006). Caecilian colour is surprisingly variable and includes most of the range of amphibian colours. Blue was found to be the most common colour, while yellow pigment was also common. The most common pattern characteristics involve a longitudinal lateral stripe or strongly demarcated line between two colours. Other regular patterns were stripes or a serrated lateral change in colour, both of which follow annuli (folds in the skin). Irregular blotches occurred in some species (e.g. *Epicrionops marmoratus*), but no clearly delineated reticulation was present, which is not uncommon in frogs and salamanders. Most caecilians were found to have counter-shading, a trait associated with camouflage of many terrestrial and aquatic species. On this basis, we created a matrix with 10 discrete, multistate colour and pattern characters, which allowed for missing characters (Table S1). Polymorphic character states were also treated as missing. We coded colours in caecilians to reflect the dominant pigment combinations known from other amphibians (Bagnara *et al.*, 1979; Frost-Mason *et al.*, 1994). Yellow is caused by carotenoids and pteridine pigments, whereas melanic tones are either produced by eumelanine, which looks black, or phaeomelanine, which appears reddish-brown or brown-black. Correspondingly, we *a priori* differentiated between animals which appeared to be black (presumed eumelanine) and those which appeared to be brown-black (presumed phaeomelanine, although we acknowledge that this pigment may be rare in Amphibia). Iridophores contain no pigments but guanine crystals, which produce spectral colours ranging from metallic blue to white. These often underlie other pigments to give a hue or tinge of colour. We coded animals that appear to lack any pigment as flesh to pink, even when these animals have iridophores deep in the skin layers which appear to give them a slight blue hue. Lastly, it should be noted that as colours were coded from photographs, descriptions and observations, we were not able to ascertain levels of reflectance in ultraviolet. However, to fit into the framework of aposematism theory, we consider it as sufficient to study colouration from a human observer-based viewpoint first.

Ecological characteristics of caecilian amphibians

To explore the evolution of the above-mentioned colour patterns in caecilians, we constructed a similar matrix for three broad ecological characteristics that we assume to reflect the exposure to evolutionary forces operating in daylight using information taken from reports in the literature or own observations (Table S1).

Terrestrial caecilians have most often been described as living within the soil (e.g. Exbrayat, 2000). Not surprisingly, recent investigations into the ecology of caecilians have found that this is an oversimplification (see Measey, 2006). Although information about the life history of caecilians is scarce compared with that of frogs and salamanders, some broad patterns regarding reproduction, distribution and vertical and horizontal adult movements have been observed in previous studies (e.g. Wake, 1980; Jared *et al.*, 1999; Rödel & Branch, 2002; Leong & Lim, 2003; Burger *et al.*, 2004; Gower *et al.*, 2004b; Rödel & Ernst, 2004; Kupfer *et al.*, 2005; Measey, 2006; W. C. Funk, personal communication). Based on these studies, we coded the adult habitat (ecological character E1) as a substitute for the probability of the exposure to daylight. Above-ground movement (character state 'fossorial with occasional surface activity') relied on pitfall data. Especially when species occur in sympatry, those methods can give an indication about comparative terrestrial movement. For example, Burger *et al.* (2004) found seven *Geotrypetes seraphini* (coded E1-0) in pitfall traps in Gabon, but sympatric *Herpele squalostoma* (coded E1-1) was never recorded in pitfalls, despite being present at the site (M. Burger, personal communication). Within the aquatic sub-family Typhlonectinae, some species have been found both in water and in soil distant from water. For example, *Chthonerpeton indistinctum* (coded E1-2) is known from a semi-aquatic genus (Nussbaum & Wilkinson, 1989). Another ecological character which can be coded based on literature data is the presence vs. absence of aquatic larvae. We suggest that species with a mode of reproduction involving aquatic larvae are also more likely exposed to daylight than species having fossorial larvae or juveniles hatching through a direct developmental mode (Exbrayat, 2006a, b and references therein; ecological character E2). Whenever caecilian species are associated with water (and have aquatic larvae), they are vulnerable to flooding and hence to movement above-ground (e.g. Péfaur *et al.*, 1987; Kupfer *et al.*, 2005). All ichthyophiids, where reproductive traits are known, have aquatic larvae (e.g. Kupfer *et al.*, 2005), whereas in caeciliids exclusively direct development, viviparity and fossorial larvae are known (Exbrayat, 2006a,b and references therein). The third ecological character we coded was based on information on the eye morphology of caecilians, which possibly indicates visual capabilities (e.g. Taylor, 1968). Although all families have representatives with an eye socket, the caeciliids show a high variety in eye morphology, having examples of eye sockets, eyes covered by roofing bones of the skull, and even protrusible eyes (in scolecomorphids, see O'Reilly *et al.*, 1996; cf. Wake, 1985). We assumed that species having eye sockets and protrusible eyes have better visual capabilities than other caecilian species.

Multiple regression and principal components analysis

To test the influence of ecological characteristics on caecilian colour patterns, we performed: (i) regression analyses between colour pattern and environmental variables, (ii) correlations of colour pattern and ecological principal components as identified using a principal components analysis (PCA, see below) and (iii) their phylogenetic independent contrasts (Felsenstein, 1985).

Multiple regression analyses between colour pattern variables and the three ecology variables were performed using the software DISTLM (distance-based permutational ANOVA; Anderson, 2001; McArdle & Anderson, 2001). We chose this test due to the deviation of the used variables from normal distribution and asymmetry in factor levels (due to an unequal number of taxa with the same character states). To account for possible spatial autocorrelation, we included information on the region of origin for each species as a covariate in the analysis. A euclidian distance matrix of the 10 colour and pattern variables (response variables) was tested against (i) a euclidian distance matrix of the three ecological variables combined (predictors) and (ii) matrices for each ecological factor separately, to find out which of them had the highest univariate effect on the result of all factors combined.

In order to reduce the 10 colour pattern and three ecology variables according to their covariation to facilitate correlation analyses, we transformed them into principal components (PC, using STATISTICA V 7.0; StatSoft, Inc., 1984–2005). The PCA (in Varimax-raw rotated coordinate system) for both the colour pattern and the ecology data set revealed a large amount of

Table 1 Results for principal components analysis of colour pattern data set performed in Varimax-raw rotated coordinate system.

	Factor 1	Factor 2	Factor 3
C1	0.054	**0.824**	0.181
C2	0.378	0.352	−0.672
C3	−0.155	−0.6	0.517
C4	0.015	−0.025	**−0.946**
C5	0.319	−0.343	−0.152
C6	0.241	0.046	**−0.871**
C7	**−0.94**	0.018	0.204
C8	**−0.952**	0.053	0.081
C9	0.065	**−0.71**	0.175
C10	0.332	**−0.714**	0.257
Explained variance (%)	22.34	23.02	25.72
Proportion of total variance (%)	22.34	23.02	25.72

Factor loadings in bold are > 0.7.

Table 2 Results for principal components analysis of ecology data set performed in Varimax-raw rotated coordinate system.

	Factor 1	Factor 2
E1	**0.959**	−0.048
E2	**0.72**	0.583
E3	0.014	**0.971**
Explained variance (%)	1.438	1.284
Proportion of total variance (%)	47.93	42.81

Factor loadings in bold are > 0.7.

covariation among the variables. The 10 variables of the colour pattern data set were reduced to three PCs (CPCs, Table 1), explaining 72.1% of the total variance. The first CPC was significantly explained by C7, C8 and marginally C9, which are pattern characteristics. The second CPC was significantly influenced by C1, C9 and C10, which are also pattern characteristics. The third CPC was determined by C4 and C6, which code for the presence of yellow and the presence of a pattern in general. Two of the ecological variables (E1 and E2), coding adult and larval habitat characteristics, showed covariance, and were placed in a factor together (EPC1, Table 2). The second factor was determined by E3 (coding for estimated visual capabilities). We computed correlations of these PCs with each other for visualizing strength and slope of coherence.

Phylogenetic correction and ancestral character state reconstruction

Principal components pairs showing significant correlations were phylogenetically corrected using a molecular phylogeny (with midpoint rooting). To obtain this molecular phylogeny, sequences from 41 caecilian species containing representatives of 21 genera were extracted from GenBank (for sequence sources, see Frost *et al.*, 2006; Roelants *et al.*, 2007; Table S2). Our data set contained approximately 24% missing data. However, recent simulations (Wiens, 2003; Philipe *et al.*, 2004) and analyses of empirical data sets (Driskell *et al.*, 2004; Phillipe *et al.*, 2004) suggest that incomplete taxa can be accurately placed in phylogenetic analyses if the overall number of characters is high (cf. Smith *et al.*, 2007). Bayesian analyses were performed using a data set of 365 bp of the mitochondrial 12SrRNA gene, 538 bp of the mitochondrial 16SrRNA gene (excluding the hypervariable region) and 546 bp of the nuclear Rag1 gene (1465 base pairs in total). We added new 16SrRNA sequences for *Boulengerula taitanus* and *B. uluguruensis* (submitted to GenBank, see Table S2). We then constructed unrooted Bayesian phylogenies by running four chains for two million generations under four different partitioning strategies. For each character set used in these partitions, best-fit models of evolution were constructed with MrModeltest (Nylander, 2004; Table S3). Subsequently, the optimal partitioning strategy was determined using *a posteriori* computing Bayes factors (cf. Brandley *et al.*, 2005). Phylogenetic independent contrasts were then computed using the software caic (Comparative Analysis by Independent Contrasts V.2.6.9, Purvis & Rambaut, 1995) both under gradual (including branch lengths) and punctual models of evolution (all branch lengths set equal). Note that independent contrasts could only be computed for 31 of 53 taxa due to missing molecular, ecology or colour pattern data. Furthermore, we used the Bayesian tree topology for ancestral character state reconstruction (using maximum likelihood framework under the Mk1 model of character evolution) of some colour pattern and ecology variables with the Mesquite system for phylogenetic computing (v2.5, Maddison & Maddison, 2008). For selected nodes of C4, we inferred proportional likelihood scores for each possible ancestral character state to test hypotheses of convergent evolution.

Results

Multivariate regression analyses

Our results indicate that, while limited, the chosen set of environmental variables together explains a significant proportion of the multivariate variation in colour pattern data, even after the variation that could have been caused by geographical constraints (i.e. spatial autocorrelation) is taken into account (Table 3). As the significant *P*-level for $n = 4$ tests under Bonferroni correction is 0.0125, only the effects of E1 and E2 on the colour pattern data set are significant, with E1 (adult surface activity) and E2 (larval habitat) explaining most of the total variance in the colour variables C1–C10. Therefore, the combined significant effect of all ecology variables on the colour pattern data set is due to the significant univariate effects of E1 and E2 (adult surface activity and larval habitat).

Table 3 Results for multivariate multiple regression analysis of colour pattern and ecology data sets with DISTLM.

Predictor variable	Response variable	Covariate	*P*	pseudo-F	Expl. prop. of total variance (%)
E1–E3	C1–C10	Geography	**0.0001**	5.61913	26.69
E1	C1–C10	Geography	**0.0003**	9.80396	16.93
E2	C1–C10	Geography	**0.0002**	9.68487	16.76
E3	C1–C10	Geography	0.0247	3.58908	6.97

Nontransformed nonstandardized data, analysis based on Euclidean distances. For determination of significant effects, 9999 permutations were used. Significant regressions (after Bonferroni correction) are in bold.

Principal components correlations and phylogenetic correction

Complementing the multivariate regression analysis using the raw data, some colour pattern and ecology PCs were largely correlated (significant correlations were found between EPC1 and CPC1 and CPC3 and between EPC2 and CPC2, not shown). However, after Bonferroni correction only the correlation between EPC1 and CPC3 remained significant (Fig. 1a, $P < 0.0001$).

Because taxa in comparative phylogenetic studies are not independent due to shared common ancestry, we repeated the significant correlation between EPC1 and CPC3 with phylogenetic independent contrasts computed from the raw data using the Bayesian tree topology. The preferred partitioning strategy with the highest Bayes factors compared with all other partitions was the most complex partition (Table 4). The Bayesian phylogeny yielded a well-supported topology (Fig. 2); the phylogenetic position of all included taxa was identical to that in Frost *et al.* (2006) and Roelants *et al.* (2007), with *Ichthyophis* being paraphyletic with respect to *Uraeotyphlus* and *Caudacaecilia*, as previously suggested by the taxonomic distinction between the subfamilies Uraeotyphlidinae and Ichthyophidinae (Frost *et al.*, 2006).

Phylogenetic correction using independent contrasts and the Bayesian phylogeny did not affect the significant correlation of CPC3 (presence of yellow pigment and presence of a pattern) and EPC1 (adult and larval habitat), under either gradual or punctual models of evolution (Fig. 1b and c).

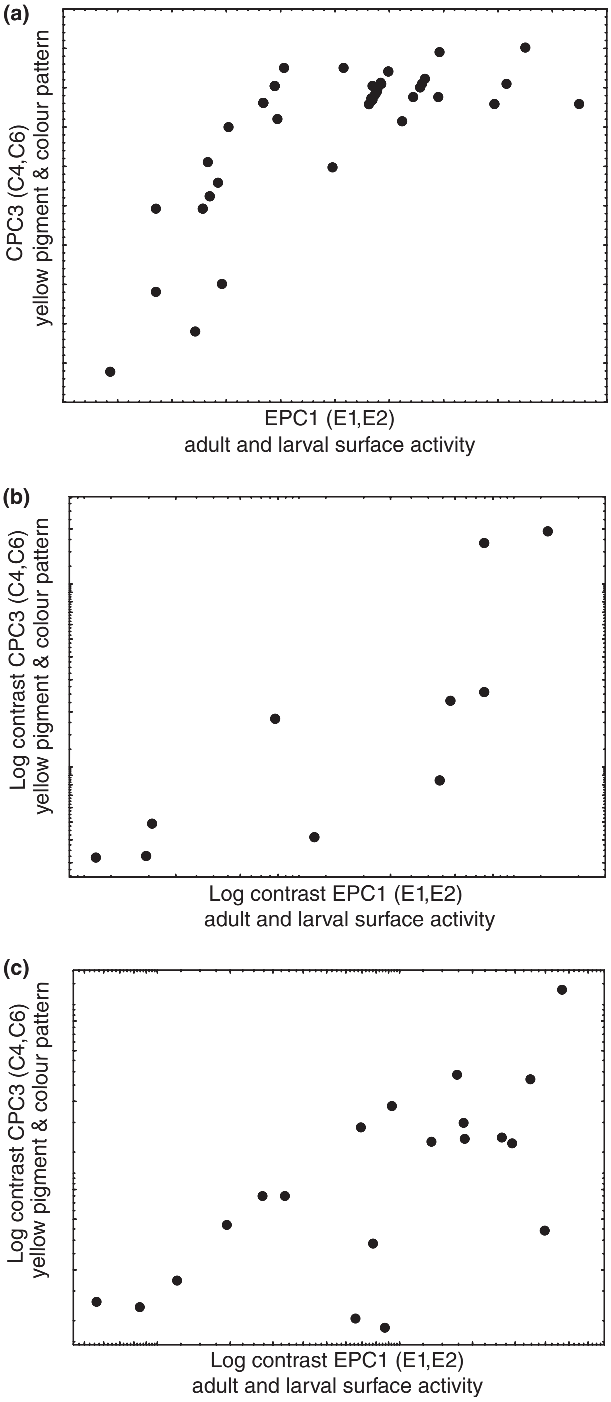

Fig. 1 Significant correlations of colour pattern PC 3 with ecology PC1 (a), and of their phylogenetic independent contrasts (displayed as logarithms) under punctual (b) $P = 0.003$ and gradual (c) $P = 0.0006$ (as obtained using CAIC) model of evolution.

Ancestral character state reconstruction

Maximum likelihood character tracing of the colour pattern variables contributing to CPC3 (C4 and C6) revealed that yellow pigment has evolved in three convergent events: in the two investigated morphs of *Schistometopum thomense* (freckled and immaculate), in Ichthyophidinae and in the clade containing *E. marmoratus* and *Rhinatrema bivittatum* (Fig. 3). As proportional likelihood values in the nodes joining Ichthyophidinae, Uraeotyphlidinae and the clade containing *E. marmoratus* and *R. bivittatum* indicate that the ancestral character

Table 4 Bayes factor calculations.

2 ln BF	P1	P2	P3	P4
P1	−15 268.64			
P2	−133.22	−15 202.03		
P3	462	595.22	−15 499.64	
P4	883.58	1016.8	421.58	−15 710.43

In the lower triangle are the 2 ln Bayes factors in support of H1 (the next more complex partitioning strategy). P1 = unpartitioned data set; P2 = 12S16S + RAG1; P3 = 12S + 16S + RAG1; P4 = 12S + 16S + 1stposrag + 2ndposrag + 3rdposrag. Preferred partitioning strategy is P4; diagonal values show harmonic means of negative log likelihood (−lnL).

state was most likely without yellow pigment, the convergent evolution hypothesis is to be favoured over a scenario where the ancestor of Uraeotyphlidinae and Ichthyophidinae lost the yellow pigment (cf. Fig. 3). Furthermore, the presence of yellow colouration coincides with the evolution of a longitudinal stripe in Ichthyophidinae. *Scolecomorphus vittatus* shows a similar longitudinal stripe pattern, but with a flesh/pink colouration instead of yellow on the flanks and *B. taitanus* and *Siphonops paulensis* exhibit contrasting annulation together with a blue hue. *Epicrionops marmoratus* and *R. bivittatum* display irregular blotches of dark pigment together with the yellow pigment and one of the two *S. thomense* morphs exhibits freckling. With the exception of the immaculate *S. thomense*, all yellow caecilian species also have evolved some kind of pattern, which already hints at adaptive evolution of these phenotypes (unless those traits, yellow pigment and pattern, were genetically linked).

Regarding the ecological variables contributing to EPC1 (E1 and E2), the above-mentioned taxa that have coevolved yellow pigment and pattern also show increased above-ground movement compared with those caecilians lacking yellow pigment or a pattern (Fig. 3).

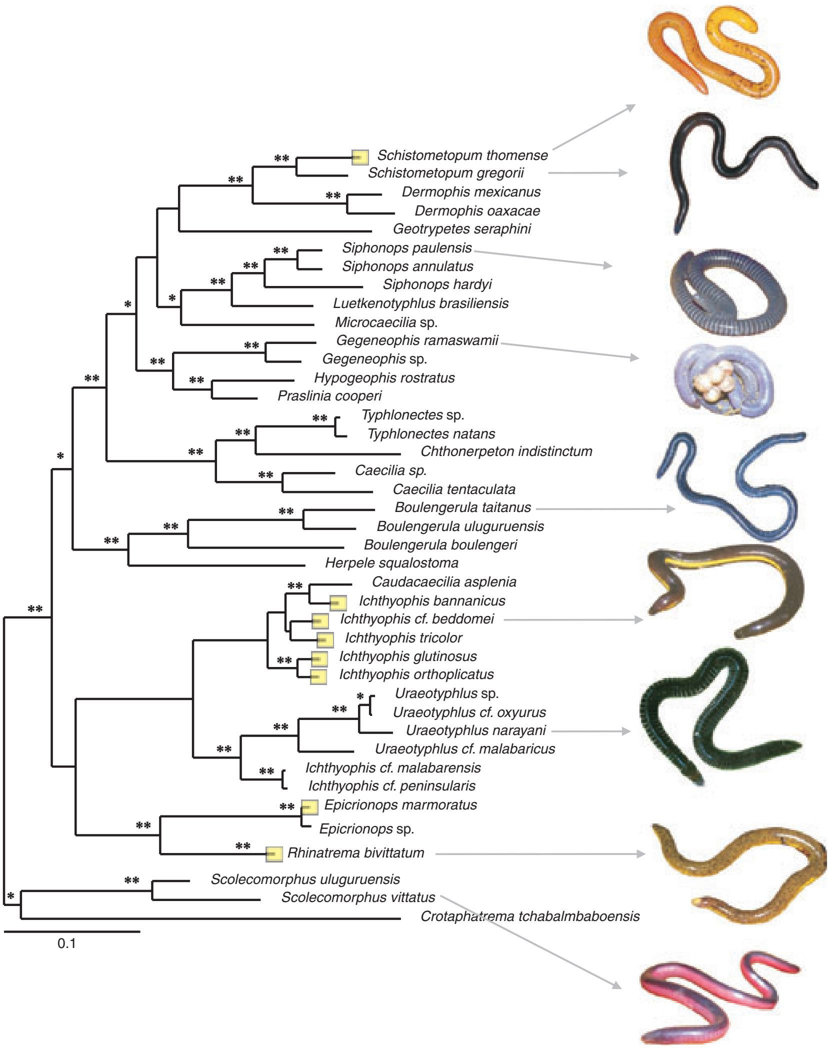

Fig. 2 Midpoint-rooted Bayesian phylogeny obtained under the optimal partitioning strategy for the 12S, 16S and RAG1 genes data set. Node support < 95% (asterisks), node support < 99 (double asterisks). Yellow boxes on branch tips: taxa where conspicuous yellow coloration is present.

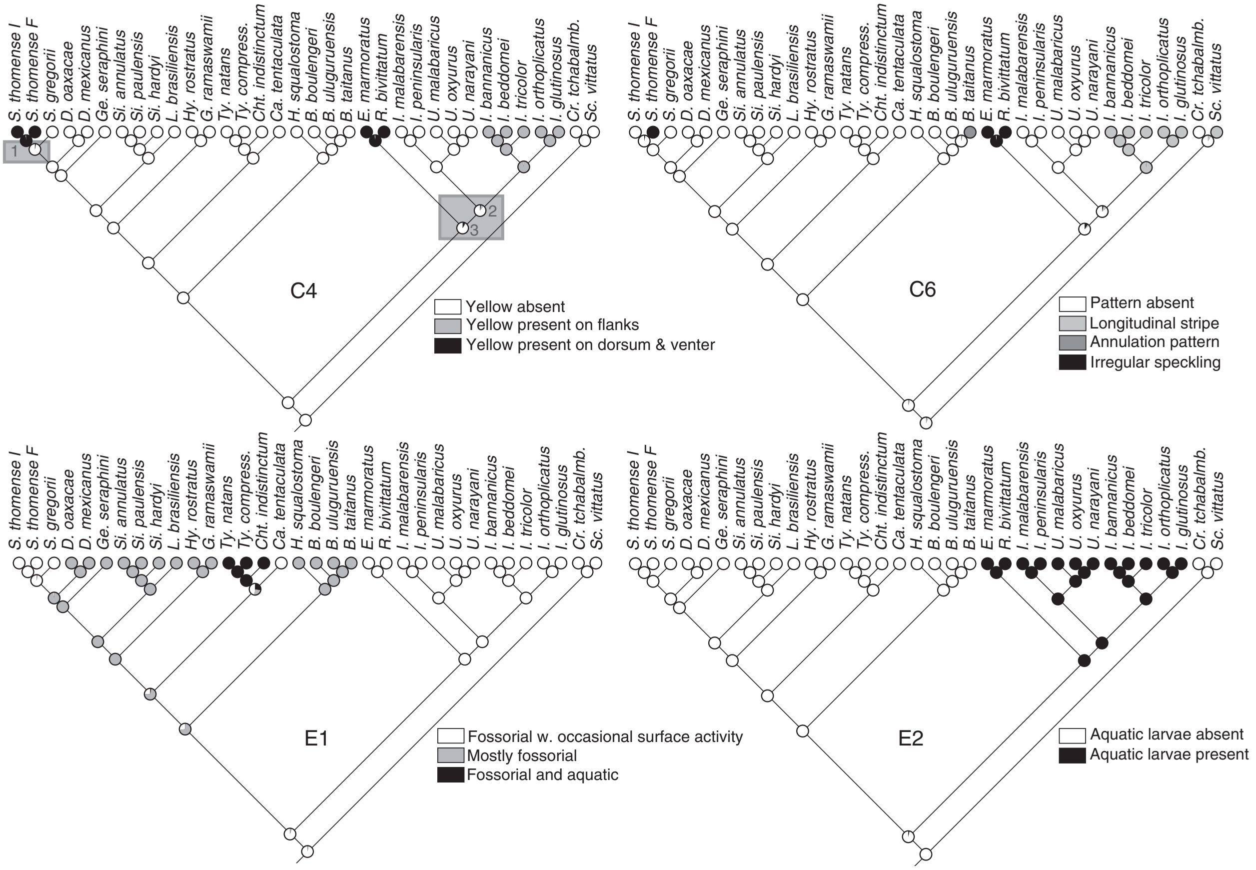

Fig. 3 Results of maximum likelihood character tracing of the colour pattern variables C4 (yellow pigment) and C6 (pattern) and the ecological variables E1 (adult habitat) and E2 (larval habitat). Nodes represent pie charts with proportional likelihoods of each character state. Preferred ancestral character states at numbered nodes (with supporting proportional likelihood values in brackets) are 1 = yellow absent (0.974), 2 = yellow absent (0.905), 3 = yellow absent (0.905), which supports the hypothesis of three events of convergent evolution of yellow coloration.

With the exception of the two *Schistometopum* morphs, those furthermore have aquatic larvae. The absence of aquatic larvae in *Schistometopum* may have developmental constraints linked to their phylogenetic placement in a clade with nonaquatic larvae.

Discussion

Our results suggest that the evolution of specific colour pattern characteristics in caecilians is correlated with an increase in above-ground movement, even when corrected for phylogenetic constraints and spatial autocorrelation. These are: (i) yellow colouration and (ii) a pattern in general. Under the hypothesis of neutral evolution of caecilian colour patterns we would have expected no clear correlation of colour patterns with those environmental parameters. We can therefore reject the null hypothesis that colour patterns in this amphibian order are a product of neutral evolution or caused by physiological constraints. Furthermore, we can conclude that the rather coarse approach of human-based colour definitions was sufficient to identify a presumably adaptive trait component in this group. Ancestral character state reconstruction showed that conspicuous yellow colouration has evolved in three convergent events, and seems to have coevolved with clearly defined pattern characteristics (stripes or speckling) in caecilian species which are likely to be active during daylight (Fig. 3). We therefore conclude that these colour patterns in caecilian skin evolved under either natural selection (camouflage or aposematism) or sexual selection (cf. Wollenberg *et al.*, 2008).

We did not find a significant relationship between the evolution of a colour pattern with the retention of an eye socket or the protrusion of eyes in scolecomorphids (see O'Reilly *et al.*, 1996; cf. Wake, 1985), which we had assumed to indicate better visual capabilities in them than in other caecilians. Caecilians in general show a high degree of morphological eye modifications (Wake, 1985) and their visual abilities are little studied. However, we consider it extremely unlikely that sexual selection produces any of the colour patterns observed in caecilians. The reasons for this are: (i) the position of the eyes, (ii) the very poor vision in caecilians (cf. Wake, 1985) and (iii) the apparent importance of olfactory cues to caecilians (Himstedt & Simon, 1995; Himstedt, 1996). Although sexually dimorphic characters have been recorded for caecilians (Delêtre & Measey, 2004 and references therein), there is no record of any colour or pattern dimorphism between the sexes, lending further support to the lack of any intraspecific interactions related to caecilian colour patterns. This leaves natural selection operating in daylight as the most probable explanation for the evolution of conspicuous colour patterns in caecilians, which is congruent with the findings of a concerted evolution of bright colour patterns and diurnality in Neotropical poison frogs (Dendrobatoidea, Summers & Clough, 2001) and Malagasy poison frogs of the genus *Mantella* (Schaefer *et al.*, 2002). To us, it seems equally likely that natural selection may operate under a model of camouflage or aposematism; the bright yellow *S. thomense* bear the classic aposematic colouration yellow, but are hard to discern from the background when moving in leaf litter (G. John Measey, personal observations, Fig. 4).

Interestingly, colour patterns in some species suggest differential visual signals towards terrestrial and aerial predators: many Ichthyophiids possess characteristic yellow longitudinal lateral stripes combined with a dark dorsal colouring, where the yellow stripe may serve as an aposematic adaptation (Merilaita & Tullberg, 2005; Tullberg *et al.*, 2005) to deter diurnal visually orientated predatory snakes (see Sillman *et al.*, 1997; Gower *et al.*, 2004a), whereas the darker dorsum may serve as a camouflage against predatory birds (see Greeney *et al.*, 2008).

What remains to be tested in order to know whether conspicuously coloured caecilians are cryptic or aposematic is the level of toxicity in their skin. In previous studies, the abundance of large granular glands in the caecilian integument has been equated with the production of a defensive toxin (Toledo & Jared, 1995), whereas others dispute this (Moodie, 1978; Fox, 1983; Jared *et al.*, 1999; and references therein but see Measey & Turner, 2008), but no comprehensive toxicity assays exist for caecilians yet. Here, we present a hypothetical framework for future studies on this subject, containing three possible evolutionary scenarios: (i) the independent evolution of conspicuous colour patterns in several convergent events under an aposematic model would require the same convergence of defensive skin toxin evolution in these animals. (ii) If no toxins in caecilians are identified, this would leave a crypsis model as an explanation for conspicuous colour patterns in caecilians, which would require the aposematism hypothesis to be confirmed in other conspicuously coloured, daylight-exposed amphibians. (iii) If a study of caecilian skin toxins finds equally high toxicity levels among all taxa, this would mean that, at least in this amphibian order, toxicity has evolved first, followed by concerted evolution of colouration and exposure to daylight. We hypothesize that a likely explanation is the early evolution of defensive skin toxins in all caecilians (iii): any predators deterred by bright colouration of caecilians would themselves have to detect these under suitable light conditions not normally present in a subterranean environment inhabited by nonconspicuous caecilians. Caecilian predators have most commonly been described as snakes (e.g. Barbour & Loveridge, 1928; Gower *et al.*, 2004b), although some authors argue that invertebrate predators exert a much more important predatory effect (Measey, 2004). Although understudied, tetra-chromatic colour vision is known in some species of diurnal snakes, but absent in invertebrate predators and nocturnal snakes (Sillman *et al.*, 1997). It therefore seems likely for caecilians to evolve skin toxins first, in order to deter colour-blind predators or predators in subterranean environments (see Measey & Turner, 2008). A subterranean lifestyle presents great

Fig. 4 Cryptic (a) vs. aposematic (b) impression of a conspicuously coloured caecilian species (*Schistometopum thomense*) in relation to background.

difficulties for practitioners due to the high energetics of locomotion through soil by burrowing (Gans, 1974; Ducey *et al.*, 1993; O'Reilly *et al.*, 1997, 2000; Navas *et al.*, 2004; Measey & Herrel, 2006). By contrast, overground movement represents an opportunity to move greater distances with less energetic cost at the expense of increasing risk of predation, which could be compensated by the evolution of a colour which can serve as camouflage in the leaf litter in most cases, but on the other hand deter colour vision-abled predators (such as birds, Greeney *et al.*, 2008) by exhibiting a general defence colour towards which predators could have innate aversions (see Merilaita & Tullberg, 2005; Tullberg *et al.*, 2005; Exnerová *et al.*, 2007).

Contrasting the general picture of caecilians being dull, subterranean vertebrates, recent investigations report a large ecological diversity (see Measey, 2006). In this study we show that this ecological diversity is mirrored by the adaptivity in morphological characters (colour patterns). Further interpretation, however, is stymied by our lack of knowledge about caecilian behaviour, ecology and functional morphology. However, it seems most remarkable that animals which are extraordinarily adapted for subterranean life (e.g. O'Reilly *et al.*, 1996, 1997) and which are so rarely found on the surface (Rödel & Branch, 2002; Gower *et al.*, 2004b) have also evolved and maintained a wide array of colour patterns for use under diurnal conditions. This conclusion provides evidence that evolution of (presumably) costly colour patterns may not require much exposure to daylight.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Characters used in colour pattern and ecology matrix. C, colour pattern variable; E, ecology variable. All characters were treated as unordered with equal weights.

Table S2 Species used in the phylogenetic reconstruction, colour and ecology analyses and GenBank Accession numbers.

Table S3 Best-fit models of evolution for the various character sets used in partitioned Bayesian analysis obtained with MrModeltest.

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