SHORT NOTES

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EVIDENCE FOR DIURNALITY FROM AN EYE LENS CRYSTALLIN IN *CNEMASPIS* (REPTILIA, GEKKONIDAE)

BEATE RÖLL

Lehrstuhl für Allgemeine Zoologie und Neurobiologie, Fakultät für Biologie, Ruhr-Universität Bochum, Germany

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Geckos are small to moderate-sized, agile lizards occurring worldwide in tropical and subtropical regions (Gekkonidae *sensu* Kluge, 1967). The majority of gekkonid lizards are nocturnal; however, members of some genera are diurnal. Between the categories "nocturnal" and "diurnal" there are several intermediate conditions, e.g. crepusculo-diurnal geckos like the members of the genus *Sphaerodactylus* or the nocturno-diurnal species *Phelsuma guentheri* of the otherwise strictly diurnal genus *Phelsuma* (Langebaek, 1982).

According to Walls (1942), nocturnal geckos are supposed to have descended from primarily diurnal lizard ancestors whose visual cells had been cones, generally with coloured oil droplets. If so, the rod-like visual cells of extant nocturnal geckos have transmuted from cones. Regarding nocturnality as a derived character, Walls designated geckos as "secondarily" nocturnal. With few exceptions, their visual cells lack oil droplets (Walls, 1942; Underwood, 1970; Röll, 2000*a*). Furthermore, Walls suggested that those gekkonid species which are now diurnal have reverted from nocturnal gecko ancestors and thus are "tertiarily" diurnal. Their visual cells have undergone a second transmutation from rod-like cells back to cones, most of which also lack oil droplets (Walls, 1942; Underwood, 1970; Röll, 2000*a*). In those species that still have oil droplets in some of their visual cells, the oil droplets are transparent. There are no extant "primarily" diurnal geckos (Röll, 2000*b*).

Yellow ocular filters, e.g. yellow oil droplets, are typically associated with diurnality (Lythgoe, 1979). They absorb potentially harmful ultraviolet and shortwave blue radiation. Walls (1942) suggested yellow lenses of some "tertiarily" diurnal geckos to be a substitute for coloured oil droplets. The yellow colour of these lenses is caused by ι-crystallin, a water-soluble eye lens protein which is composed of the retinol-binding protein type 1 (CRBP 1) and the unusual chromophore 3,4-didehydroretinol (Röll, Amons & de Jong, 1996; Röll & Schwemer, 1999). ι-Crystallin occurs exclusively in lenses of diurnal geckos. Neither the retinol-binding protein nor the chromophore 3,4-didehydroretinol, also known as vitamin A_2, have been reported in lenses of other vertebrates.

On the other hand, nocturnal animals (including nocturnal geckos) have colourless eye lenses as it is essential to capture as many photons as possible under dim light conditions. In the daytime, e.g. when basking in the sun, nocturnal geckos close their movable iris, which then forms a characteristic slit pupil. However, diurnal geckos generally have a round pupil, and its diameter is virtually constant.

The gekkonid genus *Cnemaspis* comprises about 35 species which occur in equatorial Africa and from India to south-east Asia. Most species of *Cnemaspis* inhabit rain forests and are found on trees or on rocks near waterfalls or streams (Loveridge, 1947; Manthey & Großmann, 1997). Specimens have been observed basking during the day. When disturbed they quietly slipped into their retreats, which were usually close by (Loveridge, 1947).

When *Cnemaspis* has been described as a diurnal genus, this has often been based simply upon its round pupil, but not verified by behavioural observations, as criticized already by Smith (1935). Nevertheless, at least the Asian species *C. siamensis* appears to be partly diurnal (Taylor, 1963). On the other hand, Loveridge (1947) considered African forms of *Cnemaspis* to be crepuscular or nocturnal. Joger (1981) also reported the West African species *C. occidentalis* and *C. spinicollis* – despite their round pupils – to be nocturnal. However, more recent behavioural observations on Asian species of *Cnemaspis* suggest that these are predominantly diurnal or crepuscular (Denzer, 1996; Manthey & Großmann, 1997).

These contradictory descriptions of the activity cycle of *Cnemaspis* are probably due to the secretive habits of several species. Under laboratory conditions with a 12 hr light/dark cycle, *C. africana* remains extremely shy and hides most of the time in a hollow branch from where it watches for passing prey. When undisturbed during the day, it may sit outside its retreat – especially in the morning – but it will disappear immediately when slightly aroused. This extremely secretive behaviour is in marked contrast with that of members of other diurnal genera, e.g. *Lygodactylus*, *Phelsuma* or *Pristurus*. However, the behaviour of *C. africana* is quite different from a "typical" nocturnal gecko just basking in the sun. The pupil of *C. africana* is circular – as it is in its congeners and in typical diurnal geckos as well – and it is fully dilated during daytime (Fig. 1). Its narrow iris is light brown with a golden reflection.

Correspondence: B. Röll, Lehrstuhl für Allgemeine Zoologie und Neurobiologie, Fakultät für Biologie, Ruhr-Universität Bochum, D-44780 Bochum, Germany. *E-mail*: roell@neurobiologie.ruhr-uni-bochum.de

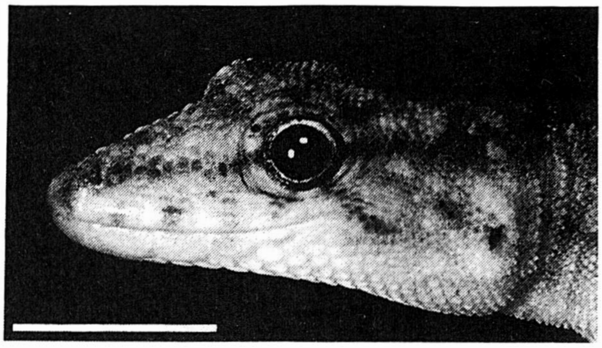

FIG. 1. Portrait of *Cnemaspis africana*. Note the large round pupil. The narrow iris is of golden-brown colour. Scale bar = 0.5 cm.

Nocturnal and diurnal gekkonid species differ in the biochemical compositions of their eye lenses. Nocturnal species have uniform crystallin compositions made up of the ubiquitous α–, ß- and γ-crystallins and the taxon-specific δ- and τ-crystallins (the latter occur in all lizards). Further taxon-specific crystallins have been utilized by diurnal genera, e.g. π-crystallin by *Phelsuma*, *Rhoptropella* and *Rhoptropus*, ε-crystallin by *Phelsuma* and ι-crystallin by e.g. *Lygodactylus*, *Pristurus* and *Quedenfeldtia* (Röll, 1995; Röll & de Jong, 1996; Röll & Schwemer, 1999). The latter crystallin is especially interesting because of its physiological role as yellow ocular filter (see introductory paragraphs). A screening of the lenses of *Cnemaspis* for its crystallin composition in general and especially for the presence of any of the just mentioned taxon-specific crystallins seemed promising.

For examination, two eye lenses of *C. africana* were available. The eye lenses are definitely yellow, comparable with lenses of *Lygodactylus* and *Pristurus*. This colouring of the lens immediately suggests diurnal habits (see above).

For examination of the crystallin composition, lenses were homogenized in distilled water. Insoluble fractions were removed by centrifugation at 4 °C for 15 min at 15 000 x *g*. Samples for gel electrophoresis were prepared in 50 mM Tris-HCl buffer, pH 8.8. After determination of the protein concentration, the samples were denatured in a solution containing 5% SDS, 2% mercaptoethanol and 10% glycerol with bromphenol blue and boiled for 3-5 min. Aliquots were run on 14% polyacrylamide gels containing 0.1% SDS. Protein bands were stained with Coomassie Brilliant Blue R-250 and scanned densitometrically. They were compared with those of species of the diurnal genera *Gonatodes*, *Lygodactylus* and *Pristurus* (Fig. 2). Additionally, Western blots of lens extracts were performed as described in Röll & Schwemer (1999). For identification of ι-crystallin, the blots were incubated with a monoclonal antibody against CRBP I.

The most abundant proteins in the lens of *C. africana* are δ-, τ- and ß-crystallins (Fig. 2A). The subunits of the tetrameric δ-crystallin and the dimeric

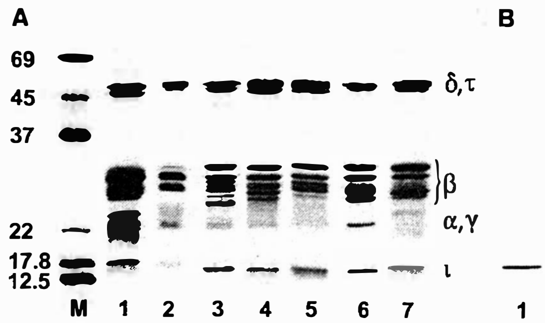

FIG. 2. A, Crystallin compositions of crude lens extracts of *Cnemaspis africana* and of other geckos with yellow lenses. SDS gel electrophoresis (14% polyacrylamide) of water-soluble proteins (about 10 μg per lane). Lane M: marker proteins (molecular weights in kDa); lanes 1-7: lens extracts of *Cnemaspis africana* (1), *Gonatodes albogularis* (2), *Lygodactylus capensis* (3), *L. chobiensis* (4), *L. tuberosus* (5), *Pristurus celerrimus* (6), *P. sokotranus* (7). Composite of lanes from different gels. Greek letters mark the crystallins.
B, Western Blot stained with peroxidase using a monoclonal antibody against CRBP I. Lane 1: lens extract of *C. africana*, lane 2: lens extract of *L. chobiensis* (positive control).

or monomeric τ-crystallin have molecular weights of about 52 and 50 kDa, respectively. ß-Crystallin consists of 4 different subunits in the range of 30-25 kDa. The monomeric γs-crystallin and the two subunits of α-crystallin cannot be recognized as clearly separated protein bands because of their similar molecular weights in the range of 19 to 22 kDa. These five crystallins are typical for nocturnal gekkonid and other squamate lizards (Röll, 1995). As in other diurnal geckos the expression of α- and γs-crystallin is reduced.

In addition to these typical crystallins, the lens extract of *C. africana* contains ι-crystallin which induces the yellow colour of the lens (Fig. 2B). Depending on genus, ι-crystallin has an apparent molecular weight between 17 and 16 kDa. The different mobilities on the gel seem to be due to different amino acid compositions; e.g., the crystallin of *Gonatodes vittatus* deviates from that of *Lygodactylus picturatus* in seven amino acids out of 58 residues which have been sequenced (nearly one half of the whole protein) (Werten, Röll, van Aalten & de Jong, 2000).

In lenses of *C. africana* the content of ι-crystallin reaches about 6% of the total amount of crystallins. This is comparable with the amount of ι-crystallin in lenses of most species of *Lygodactylus* (Röll & Schwemer, 1999). In lenses of *Gonatodes* the amount of ι-crystallin reaches only 2-4% (Röll & Schwemer, 1999), but because of the high absorption coefficient of CRBP I this amount is sufficient to cause a yellow lenticular colour.

The yellow colour of the lens of *C. africana*, which is caused by the taxon-specific ι-crystallin, definitely points to *C. africana* being diurnal. This is based on two lines of argument: all other genera possessing ι-crystallin are diurnal, and it has been argued that ι-crystallin is an autapomorphy of this group of genera because of its complex structure (Röll & Schwemer, 1999). Secondly, yellow ocular filters are characteristic for diurnal vertebrates and should be selected against in nocturnal animals.

So far, the crystallin compositions of eye lenses within a given gekkonid genus have proved to be very uniform (Röll, 1995; Röll & de Jong, 1996; Röll & Schwemer, 1999). Therefore, it is predicted that other species of *Cnemaspis* will also possess yellow lenses with ι-crystallin. Thus, the genus *Cnemaspis* as a whole is regarded as diurnal.

On the other hand, the monophyly of the genus is sometimes doubted because of the disjunct distribution of its members. Therefore, it would be interesting to examine Asian members of *Cnemaspis* for their lens crystallin compositions.

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