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The Evolution of Defensive Strategies in Cobras

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The Evolution of Defensive Strategies in Cobras

Abstract

Species use multiple defensive strategies aimed at different sensory systems depending on the level of threat, type of predator and options for escape. The core cobra clade is a group of highly venomous Elapids that share defensive characteristics, containing true cobras of the genus *Naja* and related genera *Aspidelaps*, *Hemachatus*, *Walterinnesia* and *Pseudohaje*. Species combine the use of three visual and chemical strategies to prevent predation from a distance: spitting venom, hooding and aposematic patterns. Although the functional morphology and mechanisms behind spitting and hooding are understood, few studies have investigated the evolution and variation of hood size. The aim of this thesis is to reconstruct the evolutionary history of defensive strategies in cobras, investigate the reasons why different strategies are used and to identify trade-offs. Focusing on variation in hood size, X-ray radiography was used to visualize and measure ribs of cobra specimens. Hood morphology and occurrence of hood pattern, ventral bands and spitting were analysed in phylogenetic comparative analyses. A single origin of hooding behaviour in core cobras prompted the evolution of extended ribs multiple times in *Hemachatus* and *Naja*, trending towards a large hood. Reduction in extended ribs occurred multiple times due to specialization to aquatic, subterranean or arboreal habitats. No trade-offs between spitting and hood pattern were uncovered due to the variation in pattern within Asian spitting cobras. Wide hoods were only associated with a hood pattern when phylogenetic signal was not considered, suggesting that correlation may be due to shared ancestry. In different species, size or distinctiveness of aposematic signals may be more important, leading to smaller hoods with bold patterns or large hoods with faint patterns. This study highlights the diverse morphology within cobras and the need for further investigation into frequency, extent of use of displays and predators to determine the triggers for the evolution of spitting.

The Evolution of Defensive Strategies in Cobras

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1.0 Introduction

Anti-predator strategies have evolved due to the constant arms-race between predator and prey, whereby the prey must avoid capture and predator must increase its effectiveness (Dawkins & Krebs, 1979). This is driven by the natural selection of individuals best able to survive predation attempts. However, anti-predator strategies are also influenced by multiple other factors such as the environment in which the animal lives, foraging strategy, other options for escape and many different factors. Advances in recent years in phylogenetic comparative analyses allow us to examine correlations between traits and other potential factors.

Defensive strategies can be categorized into two types of response; primary responses involving avoidance of detection and secondary responses that are utilized after detection to advertise unprofitability of prey (Endler, 1991; Greene, 1969). Primary defence strategies involve cryptic and disruptive colouration and behaviours that decrease detection by predators, for example, foraging at a different time of day. If a primary defence fails and prey is detected, a secondary defence is then deployed (Edmunds, 1974). Secondary defences involve behavioural (e.g. escape, retaliation or bluff), morphological (e.g. spines, armour) or chemical defences that advertise the unprofitability of the prey and negative consequences should the attack continue. This may involve a combination of visual, auditory, olfactory and physical adaptations and behaviours.

Many reptile species have evolved diverse primary and secondary defence strategies due to their ectothermic nature, generally slower movement and lower ability to escape if detected (Caro *et al.*, 2016; Ruxton *et al.*, 2004). Following detection by a predator, lizard species generally use rapid escape as a first line of defence. However, the flight initiation distance depends on multiple factors such as distance to refuge, temperature, season, detectability and running speed. For example, many species show a switch from flight to aggressive defence at low temperatures (e.g. Herrel *et al.*, 2007; Johnson *et al.*, 1993), mainly due to impairment of locomotor capacity (Bennett, 1990). Lizards also use pursuit deterrent signals such as push ups or the exposure of a dewlap, to advertise awareness of a predator and readiness to flee.

Snakes lack legs and therefore display systems are limited to the head, tongue, neck, trunk and tail (Carpenter & Ferguson, 1977). Despite a simple body plan, snakes have evolved diverse antipredator strategies. Venom has a primarily predatory function in snakes. Although a defensive bite may kill a predator, venom can be slow acting and requires the snake to be close to the predator, risking injury. Studies have shown that aggressive and costly responses, such as biting, are infrequently used during interaction with a predator by both non-venomous and venomous species (Bauder *et al.*, 2015; Gibbons & Dorcas, 2002; Shine *et al.*, 2010). Therefore, other

strategies that work from a distance, such as remaining undetected or aposematic displays, are used instead. Many viper species are ambush hunters that remain motionless and vulnerable to attack for a long period of time. Microornamentation of scales of the West African Gaboon Viper (*Bitis rhinoceros*) results in a pattern with areas of alternating reflectiveness, making the pattern indistinguishable from the substrate pattern (Spinner *et al.*, 2013). This camouflage provides further function in preventing detection by potential prey. Common defensive displays used by snakes include protective behaviour and posturing (Carpenter & Ferguson, 1977). Protective behaviour is a passive defence, often aimed at hiding the head under the body (Langkilde *et al.*, 2004). Posturing and bluffing such as a gaping mouth or the neck bent in an S-shape is a common behaviour used as an aposematic signal of readiness to attack and potential consequences in many species of snake (Greene, 1969; Whitaker *et al.*, 2000). Behavioural change often precludes morphological change (Wcislo, 1989). For example, many species of snake use vibrating of the tail when threatened. However, rattlesnakes have evolved modified scales on the tail that increase the sound of vibration (Allf *et al.*, 2016).

1.1 Cobra defensive strategies

Perhaps the most instantly recognizable example of defensive strategies and behaviour exaggerated by morphology is seen in cobras of the genus *Naja*. The core cobra clade is a monophyletic group of Elapids that share behavioural characteristics, containing genera *Aspidelaps*, *Hemachatus*, *Walterinnesia* and *Pseudohaje* as well as the genus *Naja* but excluding the King cobra, *Ophiophagus hannah* (Slowinski & Keogh, 2000). In this thesis, the name cobra is applied only to the genus *Naja* due to the wide range of other snake species referred to as a cobra due to behavioural similarities rather than phylogenetic relationships.

As members of the family Elapidae, all core cobra species are highly venomous, with venom containing a complex mixture of postsynaptic neurotoxins, cytotoxins, cardiotoxins and enzymes which cause neurological symptoms and local necrosis (Chu *et al.*, 2010). Cobras of the genus *Naja* are medically significant in Africa and Asia due to their highly toxic venom and species often occur in anthropized habitats such as cultivated farmland and houses, therefore frequently come into contact with humans (Kularatne *et al.*, 2009). Species delimitation is essential for the production of antivenom and treatment of snakebite, due to the variation in venom composition between and within species (Kularatne *et al.*, 2009; Mukherjee & Maity, 1998; Theakston *et al.*, 1990). In the past, all Asian *Naja* were classified as *Naja naja* (Wüster, 1996). However, in the last 20 years taxonomic revision and species discovery of both Asian and African *Naja* has led to the recognition of around 30 species today (Ceriaco *et al.*, 2017; Wallach *et al.*, 2009). The genus *Naja* is divided into four subgenera: *Uraeus* (African non-spitting), *Boulengerina* (African forest), *Afronaja*

(African spitting) and *Naja* (Asian spitting and non-spitting) (Wallach *et al.*, 2009). Relationships between species are still under debate, particularly within the subgenera *Naja* and *Boulengerina*.

Cobras combine the use of morphological adaptation and behaviour in three main visual and chemical defensive strategies; hooding, hood markings and spitting venom. During confrontation with predators, cobras also hiss and perform false strikes (Nasoori *et al.*, 2016; Rasmussen *et al.*, 1995). These strategies may be used singly or in combination and aim at advertising unprofitability from a distance.

The defensive behaviour of the cobra is ubiquitously recognizable due to the presence of cobras in myths, legends and religions. However, much of our knowledge about cobras comes from anecdotal reports and observations and little is known about their ecology, evolution and the reasons behind their behaviour, highlighting the requirement for intensive scientific studies. In the first section, we look at the three defensive strategies used by cobras, then attempt to summarize potential trade-offs and reasons for the evolution and wide variation in strategies.

1.1.1 Hooding

Dorso-lateral flattening of the body (hooding behaviour) is used as an aposematic display to appear larger and more threatening to predators, reducing the chance of attack. This behaviour is seen in a wide variety of species of snake such as colubrid genera *Heterodon*, *Hydrodynastes* and *Philodryas* (Greene, 1979; Jara & Pincheira-Donoso, 2015; Whitaker *et al.*, 2000). Hooding behaviour is also seen in the elapid genera of *Dendroaspis*, *Pseudonaja* and *Ophiophagus* and in the core cobra genera *Aspidelaps* and *Pseudohaje*.

All species in the core cobra group with the exception of the genus *Walterinnesia* produce a hood by dorso-lateral flattening of the neck region of the body and by raising the upper part of the body, enlarging the appearance to the predator. Instead of performing hooding behaviour, *Walterinnesia* inflates the body and hisses in a similar manner to the defensive posture of American *Crotalus* species (Zinner, 1971). In cobras the characteristic hood is exaggerated by elongated thoracic ribs which are rotated both laterally and dorsally to produce expansion and dorso-ventral compression (Young & Kardong, 2010). The hood is spread and relaxed by the complex interaction of four sets of muscles responsible for lifting the hood, moving the skin, transmitting forces between the ribs and relaxation of the hood (Young and Kardong, 2010).

Within the core cobra group, there is much variation in the size and length of hoods. Young and Kardong (2010) suggested that the difference in shape and size of the hood in cobra species is due to the underlying variation of the length and curvature of the ribs. However, studies looking at the hood morphology of cobras have not looked at the morphology of species that perform hooding

behaviour but appear to lack the extended ribs, focusing instead on species with large hoods such as *N. kaouthia* and *N. haje* (Young & Kardong, 2010) and have not looked at variation between *Naja* and core cobra clade species.

Cobras also raise the front third of their body off the ground while producing the hood. The threat-sensitivity hypothesis predicts that prey animals match their response to the threat level of the predator (Helfman, 1989). Nasoori *et al.* (2016) found that the height of vertical posturing, strike frequency and hissing frequency in *N. oxiana* was closely correlated with stress created by the close proximity of a human, supporting the hypothesis in cobras. Etting & Isbell (2014) found that rhesus macaques responded more strongly to a striking snake than to a coiled snake. Humans also detect snakes in a striking posture significantly faster than snakes in a resting pose (Masataka *et al.*, 2010). Cobras use of an elevated, ready to strike posture during defence suggests that a predator will recognize the high level of threat.

Although hooding is perhaps the most recognizable defensive display in any reptile, there have been no studies on the effectiveness of the hood as a defensive display (Young & Kardong, 2010). Furthermore, although the underlying mechanism and morphology of the hood is understood, no studies have addressed the evolution and interspecific differences of hood morphology between different species.

1.1.2 Hood markings and ventral patterns

Some species of cobra possess distinctive hood patterns on the back of the neck. This can be a spectacle, monocle, heart or spot on the back of the hood that may be linked to a pattern on the throat area. Pigmentation of the pattern is sometimes present on the scales, but is often primarily on the interstitial skin between scales. Therefore, when the hood is spread and the interstitial skin stretched, the pattern appears bolder. Hood patterns are most documented and most distinct in Asian species, particularly *Naja naja*, the spectacled cobra and *Naja kaouthia* the monocled cobra. In the subgenus *Uraeus*, containing African non-spitting cobras, hood patterns have not been reported in *N. nivea*, *N. haje*, *N. anchietae* or *N. arabica*. However, juvenile *N. senegalensis* have a light patch on the back of the neck and in adults a white pattern occasionally remains (Trape *et al.*, 2009). The banded form of *N. annulifera* also occasionally has a light patch on the rear of the hood (Broadley, 1995). Within the forest inhabiting subgenus *Boulengerina*, hood patterns have not been recorded although photos on google images show *Naja melanoleuca* with distinct patterns (personal observation). However, *N. peroescobari* from the island of Sao Tome, formally regarded as *N. melanoleuca*, always has a glossy black dorsal colouration (Ceríaco *et al.*, 2017). In subgenus *Afronaja*, ventral colour is generally plain and no species have hood patterns. However, *N. pallida* and *N. nubiae* have dark or light bands that encircle the neck as well as regular ventral

bands. *N. nigricollis* has variable dorsal colour and may be patterned or variegated and *N. nigricincta* is barred black and white (Wüster & Broadley, 2003). Other members of the core cobra group, *Aspidelaps*, *Walterinnesia*, *Hemachatus* and *Pseudohaje* never have hood patterns. However, species of *Aspidelaps* have an arrow shaped nuchal marking (Broadley & Baldwin, 2006) that may similarly attract attention to the head.

Hood patterns are often thought of as an aposematic signal associated with negative consequences. Aposematic patterns often compromise the species ability to avoid detection by predators, but increase the chance of predators learning to avoid aposematic prey or make decisions based on the provided signal (Mappes *et al.*, 2005; Skelhorn *et al.*, 2016; Speed & Ruxton, 2007). Natural selection acting on predators can also result in innate avoidance of an aposematic signal due to the extreme negative consequences of ignorance. For example, generalist bird species avoid aposematic coral-snake patterns (Smith, 1975; 1977).

It has been hypothesized that the spectacle marking on the front and rear of the hood of *N. naja* is an imitation of eyes similar to the spots on a butterfly (Langerholm, 1991; Stevens, 2005). In a study looking at the reaction of Bonnet Macaques (*Macaca mulatta*) to different snake species, Ramakrishnan *et al.* (2005) found that even though cobras are not predators of Macaques, a model of *N. naja* elicited the fastest reaction time. Recognition of two facing eyes is an adaptive specialization in primates and therefore two facing eyes and schematic eye spots are both provocative (Emery, 2000). Sudden face-to-face encounters with other primates often lead to an attack. Therefore, a sudden encounter with the eye-spots of a cobra also initiates the same startle response, delaying attack due to confusion. However, many species of cobra lack hood patterns or the hood patterns present have no resemblance to eyes. Experimental studies using avian predators and artificial prey have found that conspicuousness of an aposematic signal is more important than eye mimicry (Stevens *et al.*, 2008).

Colours and patterns may be present on a certain part of the body to attract the attention of a predator to a specific signal, often advertising unprofitability. For example, Zebra-tailed lizards have black and white striped markings on the underside of the tail, which is exposed when detected by a predator, signalling the lizards state of alertness and escape ability (Hasson *et al.*, 1989). Many cobra species have contrasting dark and light ventral bands and lateral throat spots on the upper part of the body that are only exposed when the front of the body is raised and the hood is extended. It has been suggested that ventral patterns attract the predator's attention to the hood and head area, further exaggerating the size of the hood (Nasoori *et al.*, 2016). Ventral bands are seen in both spitting and non-spitting *Naja* species and in related hooding genera *Aspidelaps*, *Hemachatus* and *Ophiophagus*. Furthermore, several species such as *N. mossambica* and *O.*

hannah also have bright yellow/orange markings on the neck (Lim *et al.*, 2011; Marais, 2005), which increases luminance and contrast against background foliage (Stevens & Ruxton, 2012). Related core cobra genera *Walterinnesia* and *Pseudohaje* are uniformly black and always lack ventral bands (Chippaux, 2006; Nilson & Rastegar-Pouyani, 2007) although juveniles of *Walterinnesia morgani* have dorsal reddish crossbars which fade with age.

Lateral throat spots are most noticeable in Asian *Naja* where they are often positioned adjacent to the ventral scales, only visible on the front of the hood when it is expanded. Throat spots are also present in *N. nubiae* (*Afronaja*) (Wüster & Broadley, 2003).

However, there is also much intra- and interspecific variation in hood and ventral patterns. Ontogenetic change in throat bands has been noted in *N. nivea*, *N. anchietae* and *N. annulifera* (Broadley & Wüster, 2004). *N. haje* and *N. arabica* also have highly variable throat bands (Trape *et al.*, 2009), although ontogenetic change has not been reported. Ontogenetic change in hood pattern has been observed in Asian *Naja*. For example, *N. naja* in Pakistan have aposematic patterns as juveniles, but turn black with age, obscuring all patterns (Wüster & Thorpe, 1992a). Reasons for the high degree of variation in pattern are discussed in part 2.

1.1.3 Spitting

Although venom has a primarily predatory function in snakes, the spitting of venom has evolved as a purely defensive adaptation. If venom enters the eyes of the predator it causes instant pain and temporary or permanent blindness if not treated (Chu *et al.*, 2010). This therefore acts as an immediate defence against a predator. Spitting also works at a distance, reducing the risk of damage that may occur to the cobra if it was to bite the predator. Some species can spit up to 50 times in a few minutes (Cascardi *et al.*, 1999). Therefore, spitting can also be used as “covering fire” to keep a predator at a distance by spitting successively providing time for escape or retreat to safety (Chu *et al.*, 2010).

Spitting is the most extensively studied aspect of cobras’ defensive behaviour due to the medical significance of venom and novel use of venom in defence. To be an effective defensive strategy, venom must have a composition that causes immediate pain when venom enters the eyes. The venom of Asian and African spitting cobras is composed of post-synaptic three finger neurotoxins, cytotoxins, cardiotoxins and enzymes (Chu *et al.*, 2010). Cardiotoxins may contribute to the damage seen when venom enters the eye (venom ophthalmia), due to retainment on the cell surface and contribution to membrane leakage and cell death (Wang *et al.*, 2006). Therefore, cardiotoxins may contribute to the damage seen when venom enters the eye, known as venom ophthalmia. In *N. pallida*, the concentration of cardiotoxin decreased after the 20th spit, suggesting

that cardiotoxin is specifically used for defence (Cascardi *et al.*, 1999). However, studies into the specific properties of defensive venom from spitting cobras are still ongoing.

Spitting is achieved by the contraction of skeletal muscles such as the *M. protractor pterygoideus* which releases soft-tissue barriers to allow venom to be ejected through the orifice of the tooth (Young *et al.*, 2004). The degree to which a cobra can spit is determined by the morphological specialization of the teeth; primarily the shape of the orifice. Non-spitting cobras have fangs with long discharge orifices whereas spitting cobras have smaller, shorter orifices (Bogert, 1943). Asian *Naja* have varying degrees of specialization of the teeth whereas spitting morphology is either present or absent in African *Naja* (Berthé, 2011; Wüster and Thorpe, 1992b).

Spitting is believed to have evolved three times independently within the core cobra clade; once in *Hemachatus haemachatus*, once in African spitting cobras and once in Asian spitting cobras (Panagides *et al.*, 2017; Wüster & Thorpe, 1992b). Two Asian cobra species *N. atra* and *N. kaouthia* have been reported to spit in some locations and are therefore regarded as occasional spitters (Santra & Wüster, 2017; Wüster & Thorpe, 1992b). Although three independent origins of spitting are generally accepted, a second hypothesis of a single origin of spitting at the base of the *Hemachatus*-*Naja* clade and 2 losses in the African non-spitting and Asian non-spitting *Naja* is equally parsimonious. However, African *Naja* have a less stereotyped spitting behaviour, enabling them to spit while stationary, from different angles and without the hood expanded (Freyvogel & Honegger, 1965; Rasmussen *et al.*, 1995). In Asian species and *Hemachatus*, spitting is always associated with a forward lunge, similar to a defensive strike (Rasmussen *et al.*, 1995). *Afronaja* also have behavioural modifications, increasing accuracy of spitting and have morphological features that suggest a much earlier evolution of spitting (Berthé *et al.*, 2009; Westhoff *et al.*, 2005; Young *et al.*, 2009).

One theory for the evolution of spitting is as a defence to prevent trampling by large ungulates following the expansion of the savannahs and radiation of ungulate species (Barbour, 1922). However, Wüster *et al.* (2007) found basal divergences in African spitting cobras to date to the early to mid-Miocene and the earliest evidence of increase in grassland coverage in Africa; a pattern very different to that of ungulate expansion. Further divergence within *Afronaja* is due to geological and ecological processes such as volcanism and formation of the Rift Valley. Another proposed hypothesis is the expansion of primates in savannahs who would kill cobras for both food and in defence (pers. comm. Wüster).

However, neither of these explanations would account for the evolution of spitting in Asian cobras which inhabit mostly forested and non-savannah regions. This suggests the potential for different

reasons for the evolution of spitting in African and Asian spitting cobras but provides an ideal situation to study evolution comparatively.

1.2 Variation and trade-offs between strategies

The type of defensive strategy employed depends on multiple factors relating to the predator's ecology, diversity and intensity of attack, the evolutionary history of the prey, environment and available resources and the costs and benefits of each specific defensive strategy (Ruxton *et al.*, 2004).

Although a visual signal such as a hood pattern may be an effective strategy against visual predators such as primates, it is unlikely that aposematic signals will work against predators with poor eyesight. *N. naja* is known to turn its back on a predator, displaying its hood pattern. This puts the cobra at risk of attack. However, hood pattern could be a defence against predators that hunt as a group. A hood pattern can therefore be used to "cover the cobras back" from attack while using the front of the hood to protect the front or while fleeing to prevent pursuit and attacks on the tail. Whilst aposematic colour, pattern and hooding could be aimed at multiple predators or those that hunt in a group, spitting is only effective against solitary predators; venom must be aimed at the eyes of the predator (Berthé *et al.*, 2009).

The habitat in which the cobra lives and foraging strategy may also determine which strategy is used. Greene (1969) suggested that antipredator defences are associated with a shift to active foraging in an open environment where an animal is more vulnerable to predation. Mammals and birds mostly live in groups and therefore, if actively foraging, can share predator vigilance (Caro *et al.*, 2004). Cobras typically actively forage for prey (Radcliffe *et al.*, 1986) and due to their solitary nature, cannot rely on other individuals for vigilance. This suggests that cobras that inhabit more open habitats should display more defensive adaptations than species living in forests.

An evolutionary trade-off occurs when there is a negative correlation between two desirable characteristics. For example, longevity vs. number of clutches in squamates, whereby longer-lived species produce smaller, infrequent clutches (Scharf *et al.*, 2015). An examination of defensive strategies in terms of evolutionary trade-offs may help to understand why species use different strategies.

Aposematic colouration can increase detection and frequency of encounter with predators (Ruxton *et al.*, 2009). The body pattern of cobras is generally plain brown, black or speckled, characteristic of species with a similar active hunting strategy (Allen *et al.*, 2013). Cobra colours sometimes match the substrata of the area (Lin *et al.*, 2008) and hood patterns are most visible when the hood is expanded suggesting an intermediate level of conspicuousness. An intermediate level of

conspicuousness is associated with species with multiple predators with different tendencies to attack and different sensory systems (Endler & Mappes, 2004). This means that a cobra can experience a lower encounter rate with predators, but also has an effective defensive if avoiding detection is not possible.

Ontogenetic colour change in snakes may relate to behavioural changes, differences in diet, habitat and vulnerability. In several species of snake, juveniles are more likely to use defensive behaviour (Bauder *et al.*, 2015; Landová *et al.*, 2013; Roth & Johnson, 2004; Shine *et al.*, 2002). This is because smaller snakes have more predators and are slower to escape, therefore requiring stronger defensive reactions. For example, the racer *Coluber constrictor* has a blotched colouration as a juvenile but changes to uniform as an adult. This correlates with a change in the main defensive strategy of aggressive defence in juveniles, to fleeing in adults (Creer, 2005). Similarly, several species of cobra have more distinctive and aposematic colour patterns as juveniles. For example, juvenile *Naja senegalensis* have a distinctive white spot on the back of the neck, which fades with age (Trape *et al.*, 2009).

Trade-offs also occur due to the allocation of a finite amount of energy to different characteristics such as growth, metabolism and behaviour. It is likely that the production of hooding behaviour is energetically expensive due to the continued muscle activity required. Cobras can maintain the hood in a semi-erect position for over 10 minutes by continued muscle activity (Young & Kardong, 2010). Furthermore, maintenance of the body in an upright position makes cobras liable to hemodynamic imbalances due to gravitational forces. Snakes tilted in a head-up position have increased hydrostatic blood pressure due to blood column formation above the heart which results in restricted blood flow to the head and brain (Lillywhite, 1987; Lillywhite & Gallagher, 1985). However, it is currently unknown how cobras overcome circulatory disturbances (Nasoori *et al.*, 2014).

Venom is made up of complexes of proteins which are energetically costly to produce. Studies have shown that metabolism increases following venom extraction (McCue, 2006). However increasing evidence suggests that the energy needed to produce venom is similar to the energy required for other essential processes such as feeding or shedding (Pintor *et al.*, 2010; Pintor *et al.*, 2011; Smith *et al.*, 2014). However, as venom in cobras is also required for feeding, there may be considerable costs if the cobra is unable to feed or protect itself following depletion of venom stores by spitting (Smith *et al.*, 2014). Differential contraction of the venom gland during spitting and biting means that venom is used with greater efficiency (Hayes *et al.*, 2008). Venom expelled during a single spit represents approximately 1.7% to 3.3% of the total venom yield (Cascardi *et al.*, 1999; Freyvogel & Honegger, 1965) whereas up to 54% of available venom can be expended during a

predatory bite (reviewed in Hayes *et al.*, 1992). Furthermore, highly specialized species of spitting cobra often require very little provocation to spit (Rasmussen *et al.*, 1995), but have behavioural modifications which increase accuracy and therefore reduce the cost of spitting. Some species have been shown to direct their venom at the centre of the body-part of the predator that is closest to them; most often the face and are able to match the venom distribution to the size of the target, independent of the distance (Berthé *et al.*, 2009; Berthé *et al.*, 2013).

Another factor to be considered is the compatibility of defensive strategies with each other and with other morphological traits. For example, spitting requires the cobra to face the predator but aposematic patterns on the back of the hood require the cobra to turn away. This behaviour is seen in species with bold patterns such as *N. naja* which turn away from the predator to display aposematic signals, whereas species that do not have hood patterns such as *N. oxiana* remain facing a predator at all times, displaying contrasting ventral bands (Nasoori *et al.*, 2016). Larger, symmetric and more conspicuous aposematic signals increase learning, memory and avoidance of unprofitable prey (Forsman & Merilaita, 1999; Stevens & Ruxton, 2012). Therefore, a large pattern will be most effective suggesting a positive correlation between hood size and pattern.

1.3 Comparative evolutionary studies on cobras

In the past, the mechanism and morphological adaptation of spitting and the kinetics of hooding have been examined separately, in individual contexts. However, advances in comparative analysis in the last 20 years allow us to explore and test hypotheses about the comparative evolution of strategies.

So far, only one study has examined cobra defensive strategies in a comparative evolutionary context. Panagides *et al.* (2017) compared cytotoxicity of venom to the evolution of spitting and hooding. No association between spitting and cytotoxicity was found. An association between increased cytotoxicity and hooding display but not with spitting was discovered. However, hooding was treated as a character either present or absent, not accounting for the wide variation seen in size and shape of the hood. Furthermore, *Naja annulata* was classified as not hooding, contrary to numerous photos and reports that *N. annulata* displays a large and impressive hood (O'Shea, 2008).

Panagides *et al.* (2007) suggested that aposematic body banding may be due to higher cytotoxicity in *H. haemachatus*, *O. hannah*, *N. nigricincta*, *N. siamensis* and *N. annulifera*. However, the aposematic function and effect of the banding patterns on a predator has not been tested and the study disregards the wide variability in banding throughout species ranges. For example, *N. annulifera* occurs in a banded and non-banded form (Broadley & Wüster, 2004) and *N. siamensis*

has contrasting black and white markings in central Thailand and a drab, brown pattern in other parts of its range (Wüster & Thorpe, 1994; Wüster *et al.*, 1997).

1.4 Aims and objectives

The aim of this thesis is to reconstruct the evolutionary history of defensive strategies in cobras, investigate the reasons why different strategies are used and identify trade-offs between strategies. We hope to provide the first extensive study into the variation of hood size between species and relate these differences to the evolution of spitting, aposematic patterns and other ecological factors.

Objectives:

- Collect data on hood morphology of 28 cobra species and related genera by x-ray radiography
- To increase understanding of pattern evolution in cobras, focusing on hood pattern, ventral bands and spots in African non-spitting cobras
- Phylogenetic comparative analysis will be used to examine trade-offs and correlations between spitting, hood size and patterns

2.0 Methods

2.1 Hood morphometrics

2.1.1 Specimens

Preserved specimens were sourced from the Natural History Museum (NHM) London for species from the core cobra genera *Naja*, *Aspidelaps*, *Hemachatus*, *Pseudohaje* and *Walterinnesia* and related Elapid genera *Bungarus*, *Dendroaspis*, *Elapsoidea*, *Hemibungarus* and *Ophiophagus* (Supplementary Table 1). Specimens were selected based on their suitability to the study. Wüster (1990) stated that in X-rays of juvenile cobras with a snout vent length (SVL) of less than 40cm, the distal tips of ribs could not be visualized. During trials it was found that ribs could be visualized clearly in specimens greater than 50cm. Therefore, only specimens larger than 45cm SVL were used. In other Elapid species with smaller adult body lengths, half-grown or adult specimens were selected.

Trials discovered that during X-rays, the position of the snake during X-ray can lead to distortion of rib measurements due to the two-dimensional images produced. Wüster (1990) suggested that specimens with straight necks should be used, therefore specimens with straight necks were

selected as best candidates for X-rays. To account for variation and distortion of 2D X-ray images at least 5 specimens per species were used to take averages for a species. A combination of males, females and younger specimens were used due to potential variation between ages and sexes. However, when less than five specimens for a species were available, all specimens were used regardless of preservation position.

2.1.3 Production of X-ray images

Ribs were viewed with the use of X-ray radiography. Specimens were laid as straight as possible on a 35.4 x 43.0cm Fujifilm Imaging Plate Cassette type UR (IP) and secured using string. A Solus-Schall soft tube emitter was used to X-ray the upper third of the body (neck region) to visualize the first 50 ribs. Exposure time and X-ray conditions were adjusted depending on the size of the specimen (12mA and 25kV for small specimens, 30kV for large specimens). X-ray conditions for each specimen can be seen in Supplementary Table 1. The number of ventral scales were counted, and a pin placed in the specimen at the mid-point. A second radiograph was taken of the midbody to visualize mid-body ribs for comparison.

Radiographs were visualized with the use of Fujifilm Computed Radiography (FCR). This method requires no chemicals and is considerably faster than traditional X-ray visualization, resulting in jpeg images which can be stored on a pen drive. FCR digitally processes the image by feeding the IP cassette into a Fujifilm DynamlX HR². The exposure of the resulting images was then adjusted, and the Fujifilm software used to draw a scale bar on the image.

2.1.4 Collection of hood morphological data

The program ImageJ was used to collect data from the radiographs (Schindelin *et al.*, 2012; Schindelin *et al.*, 2015). Firstly, the scale for measurements was set using the scale bar. Points were placed on the image using the Point Picker tool (ImageJ plugin) starting from the superior articular facet as 1, furthest distal point as 2, superior articular facet of second rib as 3 etc. The points could then be moved independently to adjust measurements. As placement of the points requires some subjective judgement, the same person carried out all measurements to remove any differences due to observer bias. A macro was written in java to measure the length of the first 50 ribs. Measurements were plotted in Microsoft Excel with rib number against rib length to detect any abnormally short or long ribs and any anomalous measurements were repeated.

To standardize individual specimen data to account for size difference and enable comparison between species, two methods were compared. Young & Kardong (2010) used the average length of 20 midbody ribs to standardize the length of neck ribs removed from skeletons. Therefore, we trialled this method using the average length of fifteen midbody ribs (no individual rib varying from

the mean by more than 5%). The 50 neck-rib measurements were then divided by the average mid-body rib length to produce a measure of how many times greater neck ribs are compared to body ribs. Young and Kardong (2010) used individual ribs removed from the skeleton. However, in this study, X-rays were used, providing a two-dimensional view of the skeleton. Furthermore, due to the large number of species used in our study, there was considerable variation in mid body size.

The second standardization method used the average length of neck ribs after the end of the hood. The extended ribs that make up the hood in all species examined ended by approximately the 30th rib. Therefore, each individual neck rib was divided by the average length of ribs 40 to 50. As ribs 40-50 were visualized from the same X-ray as the neck ribs, errors that may have occurred during the manipulation and movement of the specimen for midbody X-ray were removed. Only specimens where ribs 1- 40 could be visualized were included in the analysis.

Following standardization, the length of the longest rib in the hood as a percentage of a body rib was calculated by the standardized length of the longest rib multiplied by 100, hereafter referred to as maximum rib length. Maximum rib length provides a measure of the maximum width of one side of the hood.

The area of the hood as a percentage of an equal section of body was calculated to determine the increase in area when the hood is expanded. The standardized length of ribs 1 to 40 were plotted against rib number and the area under the curve (AUC) calculated using the package MESS version 0.4-15 in R version 3.4.1 (R-Development-Core-Team 2017) (Ekstrøm, 2017). AUCs were then divided by 40: the area of an equal section of body (standardized mid body=1, $1 \times 40 = 40$) and multiplied by 100. The resulting percentage is hereafter referred to as hood area.

Average hood area and average maximum rib length were calculated for each species to control for individual variation, differences between adults, juveniles and sexes and 2D distortion from X-rays. and.

To determine whether species had extended ribs in the hood, Paired *t*-tests were used on the raw rib length data to look for difference between the length of the longest rib (mm) and the average length of ribs 40 to 50 (used for standardization) for all species with greater than 4 specimens.

2.2 Colour and pattern

2.2.1 Pattern definitions

Hood pattern: a well differentiated mark on the back of the neck, but not a continuation of the body pattern (Wüster, 1990).

Ventral band: Bands on the ventral scales of the body, displayed when the hood is extended and the body raised. The band can be broad or narrow and there may be single or multiple bands.

Lateral spots: Anteriorly positioned paired dark spots on the light background of the throat. May extend onto dorsal scales in some species.

2.2.2 Pattern in the core cobra group

To look at presence, variation and ontogenetic changes in pattern in the subgenus *Uraeus* and the *melanoleuca* species complex (*Boulengerina*), all available preserved specimens at the NHM (Supplementary Table 2) were examined for presence, absence or possibility of:

1. Hood pattern (Figure 1)

a. Definite- differentiated marking

b. Possible- any faint or obscure pattern on the back of the neck. May be a continuation of the body pattern which is differentiated beyond a simple band.

c. Absent- no indication of marking. Bands on hood not different to those on the body.

2. Paired lateral throat spots

3. Dark throat band

a. Definite- Clear dark bands

b. Possible- band faded or obscured by pigment or possibly due to preservation of specimen

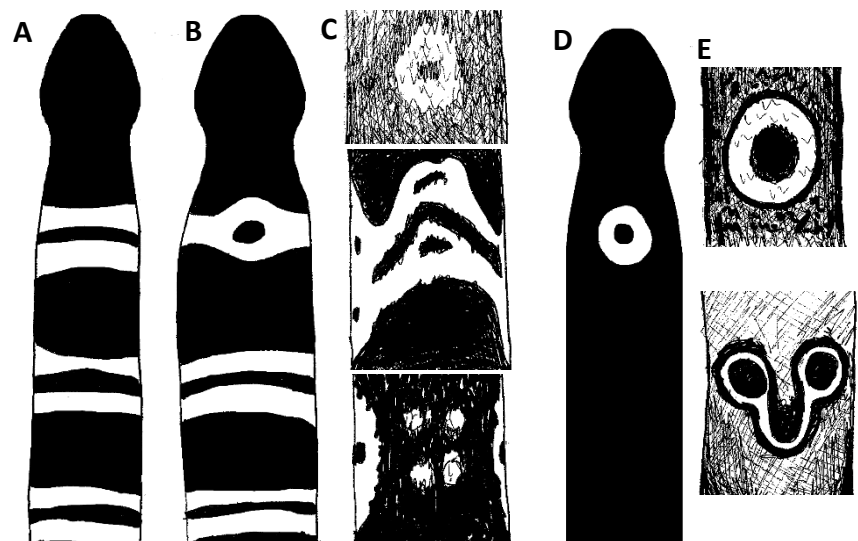


Figure 1. Hood pattern examples

A. No hood mark: pattern on neck is a continuation of body pattern

B. Possible hood mark: pattern on hood is a continuation of body pattern but further differentiated

C. Other examples of possible hood marks (indistinct monocle, differentiated bands connected to throat, indistinct spots)

D. Definite hood mark

E. Examples of definite hood marks (monocle e.g. *N. kaouthia*, spectacle e.g. *N. naja*)

- c. Absent- No dark bands on neck, throat may be dark or light

For analysis of ontogenetic changes in pattern in *Uraeus*, the approximate age of the specimen was noted as juvenile, half-grown or adult. Chi-squared tests of independence in R were undertaken to look for differences in ventral pattern between ages in *N. haje* and *N. arabica*. The number of specimens examined for *N. annulifera*, *N. anchietae* and *N. nivea* were too small to statistically analyse.

Pattern in other *Naja* subgenera *Naja* and *Afronaja* and core cobra genera *Hemachatus*, *Aspidelaps*, *Walterinnesia* and *Pseudohaje* were assessed from literature and summarized.

2.3 Comparative analysis and ancestral state reconstruction

For all comparative analyses and ancestral state reconstructions a maximum clade credibility tree from Bayesian analysis in *BEAST, constructed from 2 mitochondrial (cytb & ND4) and 5 nuclear (NT3, PRLR, UBN1, c-mos and RAG1) genes was used (Von Plettenberg Laing, 2017) (Figure 2). This is the most comprehensive phylogeny to date including all currently accepted *Naja* species except for *N. christyi* and *N. sputatrix*, which has allowed previously disputed relationships within subgenera *Naja* and *Boulengerina* to be re-evaluated. Within subgenus *Boulengerina*, possible species of *N. melanoleuca*: the West African banded form and the West African black form are included along with *N. melanoleuca*, *N. subfulva* and the newly described *N. peroescobari* (Ceríaco *et al.*, 2017). Also included is *N. miolepis* from the island of Borneo previously considered synonymous with *N. sumatrana* due to morphological similarity (Wüster, 1996). The phylogeny also includes *Pseudohaje*, which has not been included in a phylogenetic study and species from related core cobra genera *Hemachatus*, *Aspidelaps* and *Walterinnesia*. More distantly related genera *Hemibungarus*, *Ophiophagus*, *Dendroaspis* and *Bungarus* were also included. As relationships between *Ophiophagus*, *Hemibungarus*, *Bungarus* and *Dendroaspis* are still under discussion and have low support, Australian Elapids *Micropechis ikaheka*, *Oxyuranus scutellatus*, *Acanthophis praelongus* and *Pseudechis australis* were included to root the tree.

2.3.1 Categorisation of discrete data for all species

Information on lateral spots, ventral bands and hood patterns in all species examined were collated from specimens (section 2.2.2), literature search and personal observations. Hood pattern, ventral bands and lateral throat spots were classified as discrete multistate characters using the following criteria:

- Present: most individuals in the species have a clear or well-defined pattern

- Possible: only some individuals in the species have a pattern, pattern is poorly defined (see pattern classification in section 2.2.2, Figure 1) or ontogenetic variation means that pattern is not consistent throughout lifetime
- Absent: most individuals in the species have no indication of a pattern

Presence and absence of spitting adaptation was assessed from Wüster *et al.* (2007) and Santra and Wüster (2017). Habitat type (open, closed or mixed habitat) was assessed from literature or personal experience. Presence of hooding behaviour was assessed from personal experience and presence of extended ribs was assessed from the outcome of *t*-tests for difference between the length of ribs in the hood and body (section 2.1.4 Collection of hood morphological data) and personal experience for species not included.

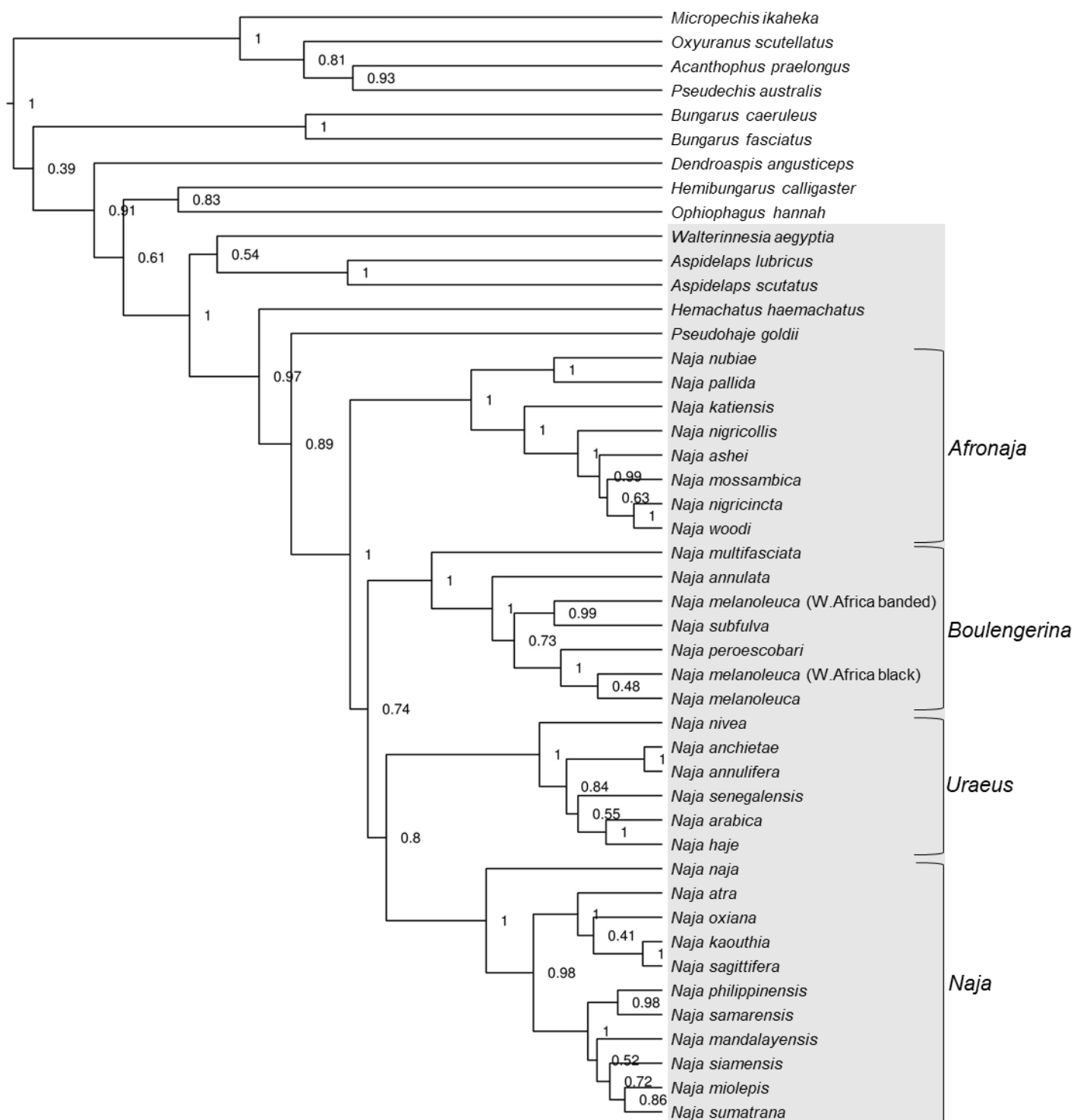


Figure 2. Maximum clade credibility tree from Bayesian analysis of two mitochondrial (cytb & ND4) and five nuclear (NT3, PRLR, UBN1, c-mos and RAG1) genes. Values at nodes represent posterior probabilities. Grey box represents core cobra species (von Plettenberg Laing, 2017).

2.3.2 Comparative analysis

Phylogenetic Generalized Least Squares (PGLS) uses linear models to fit statistical models to comparative data while taking phylogeny into account and incorporating both discrete and continuous variables (Symonds & Blomberg, 2014). Due to a lack of hood size data for some species, the consensus phylogeny (Figure 2) was pruned to contain all species with hood size data from the genera *Naja*, *Aspidelaps*, *Hemachatus*, *Walterinnesia* and *Pseudohaje*. Species used in analysis are summarized in Supplementary Table 3. Relationships between hood size (area and maximum rib length), markings on the rear of the hood, spitting, ventral bands, lateral throat spots and habitat were examined using PGLS analyses, implemented through the program *caper* version 0.5.2 in R (Freckleton *et al.*, 2002; Orme, 2013). PGLS uses the scaling parameter lambda that estimate phylogenetic correlation between species. A Lambda of 1 suggests correlation between species is equal to Brownian evolution, whereas a lambda of 0 suggests that there is no correlation between species. Lambda was set to “ML” to calculate the maximum likelihood value for the lambda parameter within set bounds.

As *N. atra* and *N. kaouthia* are regarded as possible spitters, all models were run twice with the species coded as spitters then non-spitters to assess differences. *N. sagittifera* was also regarded as a possible spitter due to similar fang morphology and former classification as a subspecies of *N. kaouthia* (Wüster, 1990).

For each model, residuals were checked for normality using Shapiro-Wilk tests and the likelihood profile for the branch length transformation was examined. However, when a small number of species (20-30) are used in a PGLS analysis, lambda has low power to detect phylogenetic signal (Freckleton *et al.*, 2002). Our dataset for PGLS analysis has 31 species and following examination of lambda profiles for models, it was clear that overparameterization was an issue for some models. Therefore, results are reported from an Ordinary Least Squares (OLS) regression which does not take phylogeny into account, and a PGLS model with lambda set to 1 (equivalent to an independent contrasts) for all models except habitat for predicting maximum rib length. All models for hood area were run using lambda set to ML following examination of lambda profiles.

Phylogenetic logistic regressions (Phyloglm) were used to examine relationships between discrete characters (Ho & Ané, 2014; Ives & Garland, 2010). The original consensus phylogeny was pruned to contain all species in the core cobra group: all *Naja* species, *Hemachatus*, *Aspidelaps*, *Walterinnesia* and *Pseudohaje* (species used summarized in Supplementary Table 3). Phyloglms were conducted through the phyloglm function in the R package phyloglm v.2.5 (Ho & Ané, 2014) using the method logistic MPLE which maximizes the penalized likelihood of the logistic regression. Firstly, spitting predicted by lateral throat spots, ventral bands and habitat was investigated using

spitting coded as a binary variable with 0 (non-spitting) or 1 (spitting). Analysis was repeated with ambiguous spitters *N. atra*, *N. kaouthia* and *N. sagittifera* coded as spitting and non-spitting to assess differences between models.

It was predicted that spitting species will lack hood marks due to the need to face predators. Therefore, hood pattern predicted by spitting was analysed using hood pattern as a binary variable. As there are only three species with a definite hood mark, species with possible or indistinct hood marks were coded as having a hood mark.

2.3.3 Ancestral state reconstruction

For ancestral reconstruction of continuous and discrete characters, the complete consensus phylogeny was used containing genera *Naja*, *Pseudohaje*, *Hemachatus*, *Walterinnesia*, *Aspidelaps*, *Dendroaspis*, *Ophiophagus*, *Hemibungarus* and *Bungarus* and trimmed to contain only species with hood size data (Supplementary Table 3).

The contMAP function in *phytools* version 0.6-20 (Revell, 2012) was used to visualize evolutionary change in maximum rib length and hood area. ContMAP produces maximum likelihood estimations of the states of internal nodes using the function 'fastAnc' (fast estimation of ML ancestral states) and interpolates ancestral states along each edge using equation (2) of Felsenstein (1985), assuming a Brownian model of evolution (Revell, 2012). Character evolution was visualized as a colour gradient and error bars were added using the function `errorbar.contMap` (Revell, 2013).

Ancestral states were reconstructed for hood pattern, spitting (ambiguous species coded spitting and non-spitting), hooding behaviour and extended ribs (from results of *t*-tests). Using the discrete data from section 2.2.2, the fit of macroevolutionary models to the discrete data (section 2.2.2) was tested using 'ace' in the program *ape* version 4.1 (Paradis, 2012; Paradis *et al.*, 2004; Popescu *et al.*, 2012). Models tested included equal-rates (ER), symmetrical (SYM) and all-rates-different (ARD). Models were compared using AIC and AICc values, and the best fitting model for each character was used for subsequent analyses. ER models best fitted spitting (both ambiguous coded yes and no), hooding behaviour and extended ribs. An All Rates Different model was used for hood pattern. Stochastic mapping was used to infer character histories for the characters hood pattern, ventral banding, lateral spots, spitting and habitat. This was carried out using the 'make.simmap' function in *Phytools* (Revell, 2012) which implements the stochastic mapping method by Huelsenbeck *et al.* (2003).

Although attempts were made to include phylogenetic uncertainty by running stochastic simulations over 100 random trees from the Bayesian output. Attempts were unsuccessful due to low support for subgenera relationships (e.g. posterior probability of 0.74 for ancestor of *Boulengerina*, *Naja*

and *Uraeus*) and unresolved ancestry of genera such as *Ophiophagus* (Figure 2). Therefore, analysis was undertaken without phylogenetic uncertainty and the maximum clade credibility tree was used.

The appropriate model for each character was fitted to the transition matrix and run through 1000 stochastic simulations. Posterior probabilities were computed and mapped onto the consensus tree with maximum rib length reconstructed to enable visualization of discrete and continuous character evolution.

3.0 Results

Species included in the different analyses are summarized in Supplementary Table 3. Some species were excluded from analysis due to lack of specimens for hood size data and some were not included in the phylogeny. Table 1 provides a summary of the discrete data collected on spitting, hood pattern, ventral bands, lateral throat spots, habitat, hooding behaviour and extended ribs for comparative analysis.

Table 1. Discrete data collected on all species included in comparative analysis.

Species	Spitting	Hood pattern	Ventral bands	Lateral throat spots	Habitat	Hooding behaviour	Hood pattern (all)	Extended ribs
<i>Naja (Afonaja) ashei</i>	yes	No	Yes	no	Mixed	yes	no	yes
<i>Naja (Afonaja) katiensis</i>	yes	No	yes	no	mixed	yes	no	no
<i>Naja (Afonaja) mossambica</i>	yes	no	yes	sometimes	mixed	yes	no	yes
<i>Naja (Afonaja) nigricincta</i>	yes	no	yes	no	open	yes	no	yes
<i>Naja (Afonaja) nigricollis</i>	yes	no	yes	no	mixed	yes	no	yes
<i>Naja (Afonaja) nubiae</i>	yes	no	yes	yes	open	yes	no	no
<i>Naja (Afonaja) pallida</i>	yes	no	yes	no	open	yes	no	no
<i>Naja (Afonaja) woodi</i>	yes	no	no	no	open	yes	no	yes
<i>Naja (Boulengerina) annulata</i>	no	no	yes	no	closed	yes	no	no
<i>Naja (Boulengerina) melanoleuca</i> (W. African black form)	no	sometimes	yes	yes	closed	yes	yes	yes
<i>Naja (Boulengerina) melanoleuca</i>	no	sometimes	yes	yes	closed	yes	yes	yes
<i>Naja (Boulengerina) multifasciata</i>	no	no	no	no	closed	yes	no	no
<i>Naja (Boulengerina) melanoleuca</i> (W. African banded form)	no	sometimes	yes	yes	closed	yes	yes	yes
<i>Naja (Boulengerina) subfulva</i>	no	sometimes	yes	yes	closed	yes	yes	yes
<i>Naja (Boulengerina) peroescobari</i>	no	no	yes	yes	closed	yes	no	yes
<i>Naja (Naja) atra</i>	possible	yes	yes	yes	closed	yes	yes	yes
<i>Naja (Naja) kaouthia</i>	possible	yes	yes	yes	closed	yes	yes	yes

<i>Naja (Naja) mandalayensis</i>	yes	sometimes	sometimes	sometimes	mixed	yes	yes	yes
<i>Naja (Naja) miolepis</i>	yes	no	sometimes	no	closed	yes	no	yes
<i>Naja (Naja) naja</i>	no	yes	yes	yes	mixed	yes	yes	yes
<i>Naja (Naja) oxiana</i>	no	no	yes	no	open	yes	no	yes
<i>Naja (Naja) philippinensis</i>	yes	no	no	no	closed	yes	no	yes
<i>Naja (Naja) sagittifera</i>	possible	sometimes	sometimes	sometimes	closed	yes	yes	yes
<i>Naja (Naja) samarensis</i>	yes	no	yes	no	closed	yes	no	yes
<i>Naja (Naja) siamensis</i>	yes	sometimes	yes	no	closed	yes	yes	yes
<i>Naja (Naja) sumatrana</i>	yes	no	sometimes	sometimes	closed	yes	no	yes
<i>Naja (Uraeus) anchietae</i>	no	no	sometimes	no	open	yes	no	yes
<i>Naja (Uraeus) annulifera</i>	no	sometimes	yes	sometimes	open	yes	yes	yes
<i>Naja (Uraeus) arabica</i>	no	no	sometimes	no	open	yes	no	yes
<i>Naja (Uraeus) haje</i>	no	no	sometimes	sometimes	open	yes	no	yes
<i>Naja (Uraeus) nivea</i>	no	no	sometimes	no	open	yes	no	yes
<i>Naja (Uraeus) senegalensis</i>	no	sometimes	sometimes	no	open	yes	yes	yes
<i>Pseudohaje goldii</i>	no	no	no	no	closed	yes	no	no
<i>Walterinnesia aegyptia</i>	no	no	no	no	open	no	no	no
<i>Aspidelaps lubricus</i>	no	no	yes	no	open	yes	no	no
<i>Aspidelaps lubricus</i>	no	no	yes	no	open	yes	no	no
<i>Aspidelaps scutatus</i>	no	no	yes	no	open	yes	no	no
<i>Hemachatus haemachatus</i>	yes	no	yes	no	open	yes	no	yes
<i>Ophiophagus hannah</i>	no	no	yes	no	closed	yes	no	yes
<i>Bungarus caeruleus</i>	no	no	no	no	mixed	no	no	no
<i>Bungarus fasciatus</i>	no	no	yes	no	closed	no	no	no
<i>Dendroaspis angusticeps</i>	no	no	no	no	closed	no	no	no
<i>Hemibungarus calligaster</i>	no	no	yes	no	closed	no	no	no

3.1 Markings

3.1.1 Subgenus *Uraeus*

There was a significant difference in ventral banding between juvenile, half-grown and adult *N. haje* (X^2 (2, $n=34$) = 12.719, $p=0.0127$), but not *N. arabica* (X^2 (4, $n=18$) = 8.56, $p=0.073$). Most Juvenile *N. haje* had ventral bands, whereas approximately 60% of adults lacked bands (Figure 3).

Overall, potential hood patterns were identified in 20.6% of *N. haje* specimens. Patterns were only observed in juvenile specimens, of which 63.6% had hood marks. Hood marks were observed in specimens from across the whole of the species range: Somalia, Algeria, Egypt, Kenya and Nigeria.

The shape of hood marks varied from a continuation of light ventral markings across the back of the neck differentiated to form chevrons (possible mark), to a white monocle independent of the ventral markings, similar to *N. kaouthia* (Figure 4).

3.1.2 Subgenus *Boulengerina*

All specimens of *N. melanoleuca*, *N. subfulva*, *N. peroescobari*, *N. melanoleuca* (West African

black form) and *N. melanoleuca* (West African banded form) had multiple contrasting black ventral bands and at least one pair of lateral throat spots, often positioned on an extension of the light ventral pattern onto the dorsal scales. An indistinct hood pattern was observed in between 40% and 67% of *N. melanoleuca* complex specimens, excluding *N. peroescobari* (Table 2). The pattern commonly consisted of bands across the back of the hood differentiated to form chevrons or diamonds (Figure 4). Occasionally an indistinct hood pattern in the form of spots appeared in isolation from the banding pattern. All three *N. melanoleuca* (West African banded) specimens also had bands that continued for at least half of the body. The dorsal pattern of *N. annulata* has between 21 and 23 bands (Chippaux, 2006). Although these bands cross the hood, they are not differentiated. *N. annulata* also has a banded ventral.

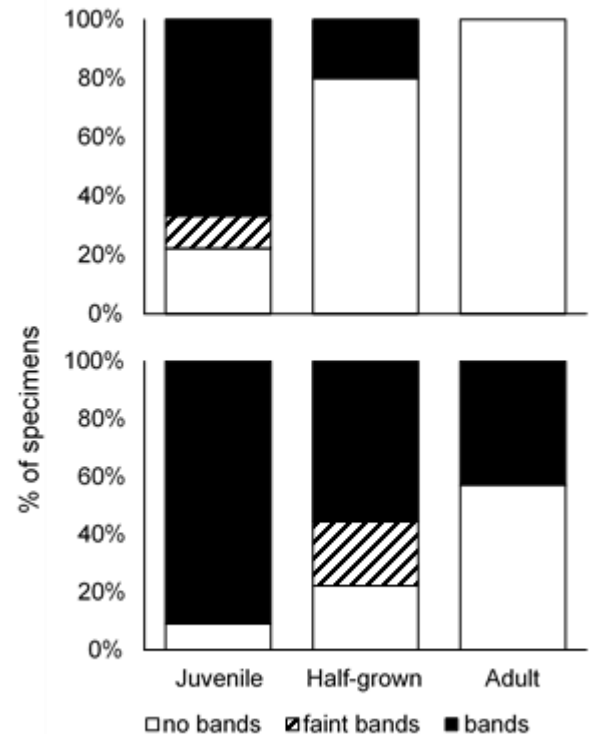


Figure 3. Occurrence of ventral markings in *Naja arabica* and *Naja haje*.

Table 2. Presence of hood patterns in *Naja melanoleuca* species complex.

Species	Number of specimens examined	Indistinct Pattern	No pattern
<i>N. melanoleuca</i> (West African black form)	6	0.67	0.33
<i>N. melanoleuca</i>	22	0.59	0.41
<i>N. melanoleuca</i> (West African banded form)	3	0.67	0.33
<i>N. subfulva</i>	28	0.39	0.61
<i>N. peroescobari</i>	1	0.00	1.00



Figure 4. Hood markings in juvenile *Naja haje* (A, B & C) and *Naja melanoleuca* species complex (D, E & F). A- *N. haje* (1897.10.28.618, Giza (below pyramids), Egypt) light double chevron on dark neck band. B- *N. haje* (BMNH1966.161, Athi River near Nairobi, Kenya), bold monocle hoodmark similar to *Naja kaouthia*, three pairs of lateral throat spots. C- *N. haje* (BMNH1951.1.5.38, Lira Langa, Uganda) diamond connected to ventral white patch that extends onto dorsal scales. D- *N. melanoleuca* (BMNH1968.50, Bota, Cameroon) double chevron joined to white neck band. E- *N. subfulva* (BMNH1954.1.12.51a, Jinja, Uganda) four light spots, not connected to neck pattern. F- *N. melanoleuca* (West African banded form) (BMNH1911.5.29.12, Gold Coast, Ghana) double chevron joined to light ventral band. Note the undifferentiated bands lower down neck.

3.1.3 Pattern in subgenus *Afronaja*

No species in subgenus *Afronaja* have hood patterns. Ventral bands are present in all species.

Notably *N. pallida*, *N. nubiae* and *N. katiensis* have regular bands on the throat, whereas *N. mossambica* has irregular bands that do not cross the neck (Wüster & Broadley, 2003). *N. nubiae* is the only *Afronaja* species to have lateral throat spots.

3.1.4 Pattern in subgenus *Naja*

Hood pattern, lateral throat spots and ventral bands found in subgenus *Naja* are summarized in Table 3. Hood pattern is variable throughout the subgenus *Naja*. Hood pattern is most obvious in *N. atra*, *N. kaouthia* and *N. naja* which represent the main shapes of hood marks in other species: mask, monocle and spectacle. However, in *N. naja* the hood mark is often faded or obscured by black pigment in Northern India or Pakistani populations. Ventral bands and throat spots are generally present in most species although they may be obscured by dark pigmentation and mottling.

Table 3. Hood patterns and markings in subgenus *Naja*.

Species	Hood pattern	Regularity of occurrence	Shape of marking	Lateral throat spots	Ventral bands	Reference
<i>N. siamensis</i>	Yes	Frequently absent or indistinct	Spectacle, U, V, H	Absent or ill defined	Absent or ill defined	Wüster <i>et al.</i> (1997)
<i>N. sputatrix</i>	Yes	Sometimes present	Chevron or heart shaped	Indistinct or missing	Yes	Wüster (1990)
<i>N. sumatrana</i>	No	Never	-	Sometimes	Sometimes	Wüster (1990)
<i>N. philippinensis</i>	No	Never	-	No	Sometimes	Wüster (1990)
<i>N. mandalayensis</i>	Yes	Very rare	Faint spectacle in some juveniles only	Obscured by dark mottling in adults	Obscured by dark mottling in adults	Slowinski & Wüster (2000)
<i>N. oxiana</i>	No	Never	-	No	Yes- usually fades in adults	Wüster (1990)
<i>N. kaouthia</i>	Yes	Almost always	Monocellate or mask	Yes, well defined	Yes, well defined	Wüster (1990)
<i>N. atra</i>	Yes	Almost always	Variable- spectacle, mask, horseshoe, connected to throat	Clearly defined	Single band	Wüster (1990)
<i>N. naja</i>	Yes	Usually in most of range often absent in Pakistani	Spectacle	Yes	Yes	Wüster (1990)

		and Northern India				
<i>N. samarensis</i>	No	Never	-	No	Single broad band	Wüster (1990)
<i>N. sagittifera</i>	Yes	Most juveniles, fades in adults	Monocle	Yes- fade in adults	Sometimes	Whitaker & Captain (2004)

3.1.5 Pattern in *Aspidelaps*, *Walterinnesia*, *Hemachatus* and *Pseudohaje*

Both *Pseudohaje* and *Walterinnesia* lack ventral bands, throat spots and hood patterns.

Pseudohaje goldii and *P. nigra* are uniformly black dorsally and pale white or yellow ventrally (Bogert, 1942; Chippaux, 2006). *Walterinnesia aegyptia* and *W. morgani* are black dorsally and ventrally although juvenile *W. morgani* have dorsal reddish crossbars which fade with age (Rastegar-Pouyani, 2007).

Hemachatus has a dark plain or banded body with multiple ventral bands on the front of the neck. However, they lack lateral throat spots (personal observation).

Aspidelaps lubricus has a banded body as a juvenile, the first 2 to 3 bands extend around the neck to form ventral bands. *Aspidelaps scutatus* has a blotched body colour but also has a black collar that circles the neck and forms a ventral band (Broadley & Baldwin, 2006).

3.2 Hood size

Paired *t*-tests between the length of the longest rib and the length of the mean body rib (ribs 40-50) for each species, revealed that all species in subgenera *Naja* and *Uraeus* included in this study have neck (hood) ribs that are significantly longer than the body ribs (Table 4). However, in subgenus *Afronaja*, *N. mossambica* and *N. nigricollis* had significantly longer neck ribs, but *N. pallida* and *N. katiensis* had neck ribs that were of similar length to the body. Similarly, in subgenus *Boulengerina* neck ribs of *N. annulata* were similar length to the body ribs, whereas *N. melanoleuca* and *N. subfulva* had significantly longer neck ribs. Related core cobra *Hemachatus* also had significantly longer ribs in the hood, whereas *W. aegyptia*, *W. morgani*, *P. goldii*, *Aspidelaps lubricus* and *A. scutatus* lacked extended ribs. Out of all other related Elapids examined, only *Ophiophagus hannah* had significantly longer neck ribs.

Differences in rib length (following standardization) and hood area between all species studied are displayed in Figure 5, showing the high variability throughout all species studied. *N. naja* had the longest neck ribs out of all species (Mean length: 151.75% \pm 20.60). However, *N. melanoleuca*

(Mean area: $113.17\% \pm 9.27$) followed by *N. nivea* (Mean area: $110.74\% \pm 8.038$) had the largest hood area.

Following standardisation of the neck ribs, there was a significant relationship between hood area and maximum rib length over all *Naja* and related Elapid species (Pearson correlation: $t=13.489$, $df=43$, $p<0.001$). This shows that species with a larger hood width have a larger hood area.

Table 4. Results of Paired Samples t-tests between the length of the longest rib and the mean length of body ribs for core cobra and Elapid genera. Significant results highlighted in bold.

Species	N	Mean maximum rib length (mm) ± SD	Mean length of body ribs (mm) ± SD	T value	p-value
<i>Naja (Afronaja) katiensis</i>	6	18.61 ± 4.33	17.40 ± 4.64	2.540	.052
<i>Naja (Afronaja) mossambica</i>	6	22.00 ± 2.73	16.51 ± 2.18	19.879	<.001 ***
<i>Naja (Afronaja) nigricollis</i>	5	29.71 ± 9.21	20.35 ± 5.37	4.353	.012 **
<i>Naja (Afronaja) pallida</i>	4	18.31 ± 2.10	17.12 ± 1.10	1.337	.274
<i>Naja (Boulengerina) annulata</i>	7	18.19 ± 6.41	18.84 ± 7.02	-1.328	.232
<i>Naja (Boulengerina) melanoleuca</i>	7	32.41 ± 7.16	21.89 ± 6.37	9.630	<.001 ***
<i>Naja (Boulengerina) subfulva</i>	7	23.48 ± 4.02	17.80 ± 3.64	8.946	<.001 ***
<i>Naja (Naja) atra</i>	6	24.10 ± 4.13	20.73 ± 4.06	8.112	<.001 ***
<i>Naja (Naja) kaouthia</i>	10	34.05 ± 4.64	23.26 ± 3.82	8.412	<.001 ***
<i>Naja (Naja) miolepis</i>	6	20.97 ± 1.42	15.75 ± 1.64	10.387	<.001 ***
<i>Naja (Naja) naja</i>	11	28.33 ± 7.42	18.94 ± 5.74	8.968	<.001 ***
<i>Naja (Naja) oxiana</i>	5	18.52 ± 5.96	15.68 ± 5.04	4.607	.010 **
<i>Naja (Naja) siamensis</i>	4	34.42 ± 3.43	24.64 ± 3.14	11.142	.002 **
<i>Naja (Naja) sumatrana</i>	6	22.81 ± 4.54	17.73 ± 2.35	3.741	.013 *
<i>Naja (Uraeus) arabica</i>	6	22.60 ± 8.49	18.97 ± 6.45	3.594	.016 *
<i>Naja (Uraeus) haje</i>	6	28.84 ± 10.53	23.69 ± 7.87	3.326	.021 *
<i>Naja (Uraeus) nivea</i>	5	29.99 ± 2.64	22.33 ± 4.26	8.871	<.001 ***
<i>Hemachatus haemachatus</i>	4	28.44 ± 9.28	20.14 ± 6.96	5.179	.014 **
<i>Pseudohaje goldii</i>	4	17.42 ± 3.57	18.24 ± 4.43	-1.011	.386
<i>Walterinnesia aegyptia</i>	3	20.23 ± 2.46	20.75 ± 1.90	-1.571	.257
<i>Aspidelaps lubricus</i>	6	11.47 ± 1.42	11.25 ± 1.84	0.522	.624
<i>Aspidelaps scutatus</i>	4	14.30 ± 1.89	13.13 ± 2.36	1.544	.220
<i>Walterinnesia morgani</i>	4	12.84 ± 1.09	13.59 ± 1.79	-1.785	.172
<i>Bungarus caeruleus</i>	5	11.91 ± 2.40	11.56 ± 2.06	0.799	.469
<i>Bungarus fasciatus</i>	4	15.94 ± 4.10	16.51 ± 5.16	-1.013	.386
<i>Dendroaspis angusticeps</i>	4	17.88 ± 1.38	18.43 ± 1.99	-1.468	.238
<i>Dendroaspis jamesoni</i>	6	17.48 ± 2.31	16.79 ± 2.65	1.018	.355
<i>Dendroaspis viridis</i>	5	16.57 ± 1.82	16.64 ± 2.06	-0.555	.608
<i>Elapsoidea boulengeri</i>	5	10.76 ± 0.94	10.24 ± 1.25	1.316	.258
<i>Ophiophagus hannah</i>	12	27.65 ± 13.53	21.04 ± 10.15	6.082	<.001 ***



Figure 5. Comparison of standardized maximum rib length (left) and hood area (right) in core cobra genera *Naja*, *Aspidelaps*, *Hemachatus*, *Pseudohaje* and *Walterinnesia* and related elapid genera *Dendroaspis*, *Bungarus*, *Elapsoidea*, *Hemibungarus* and *Ophiophagus*. Lines at 100% represent that the maximum rib is the same size as the average body rib. Error bars are standard deviation.

3.3 Comparative analysis

Longer ribs were significantly associated with a hood pattern and ventral bands during OLS analysis but not when phylogenetic signal was taken into account during PGLS analysis (Figure 6)(Table 5). Species that used spitting (both ambiguous species coded spitting and non-spitting) or had lateral throat spots were not associated with wide hoods in PGLS or OLS analysis (Table 5).

Table 5. Results of PGLS and OLS analysis of maximum rib length predicted by hood pattern, spitting, ventral bands and lateral throat spots.

Response variable	OLS (lambda=0)		PGLS (lambda= 1)	
	T	P-value	T	P-value
Hood pattern sometimes	2.716	.011	1.843	.078
Hood pattern always	2.185	.038	1.613	.120
Spitting no	0.928	.361	0.535	.596
Spitting yes	1.550	.132	0.916	.369
Ventral bands sometimes	3.100	.004	1.166	.256
Ventral bands always	3.052	.005	1.428	.167
Lateral throat spots sometimes	1.350	.188	-0.209	.836
Lateral throat spots always	1.643	.112	1.547	.136

Habitat type was not associated with longer ribs (PGLS ($\lambda=0.927$): Mixed T=1.224, p=0.234, Open T= -0.839, p=0.410).

A large hood area was significantly associated with ventral bands (PGLS ($\lambda=0.801$): bands sometimes T=2.1711, p=0.040, bands always T= 2.277, p=0.032). However, hood area was not associated with hood pattern (PGLS $\lambda= 0.940$, Sometimes T=1.358, p=0.188; Always T=1.137, p=0.267), spitting (Ambiguous coded non-spitting: PGLS ($\lambda= 0.960$) T=0.576, p= 0.57; Ambiguous coded spitting: PGLS ($\lambda= 0.971$) T=0.757, p=0.457), habitat (PGLS ($\lambda= 0.955$), Mixed T=1.076, p=0.293; Open T=-0.321, p=0.751) or lateral throat spots (PGLS ($\lambda= 1$), Sometimes T=-0.827, p=0.417; Always T=1.307, p=0.204).

Phylogenetic logistic regression models found no significant associations between spitting and lateral spots, ventral bands or habitat (Table 6). There was no relationship between spitting and any hood pattern despite the differential coding of the possibly spitting species (Ambiguous non-spitting: Z=-1.813, p=0.070, Alpha= 4878.427; Ambiguous spitting: Z=-0.565, p=0.572, Alpha= 1837.653) (Figure 7).

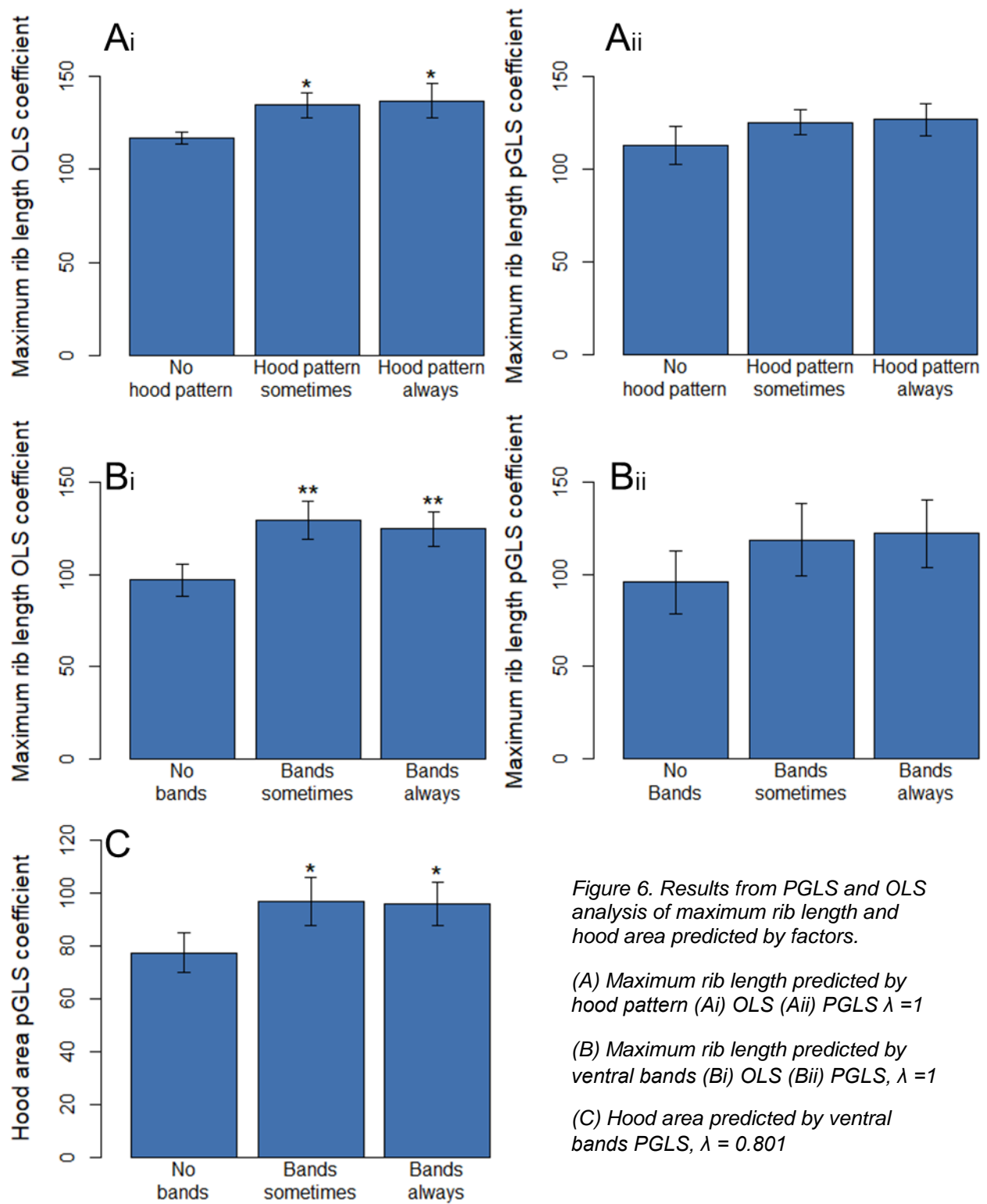


Figure 6. Results from PGLS and OLS analysis of maximum rib length and hood area predicted by factors.

(A) Maximum rib length predicted by hood pattern (A_i) OLS (A_{ii}) PGLS $\lambda = 1$

(B) Maximum rib length predicted by ventral bands (B_i) OLS (B_{ii}) PGLS, $\lambda = 1$

(C) Hood area predicted by ventral bands PGLS, $\lambda = 0.801$

Table 6. Phyloglm models for spitting predicted by lateral spots, ventral bands and habitat type.

Model	Estimate	S.E.	z- value	p- value	Alpha
Spitting predicted by lateral spots and ventral bands (ambiguous species non-spitting)					
(Intercept)	0.097	1.500	0.065	0.948	65.052
Lateral spots sometimes	-0.012	0.285	-0.042	0.966	
Lateral spots always	-0.006	0.434	-0.014	0.989	
Ventral bands sometimes	0.011	0.483	0.023	0.982	
Ventral bands always	-0.002	0.393	-0.004	0.997	
Spitting predicted by lateral spots and ventral bands (ambiguous species spitting)					
(Intercept)	-0.449	1.160	-0.387	0.699	147.746
Lateral spots sometimes	0.002	0.403	0.006	0.995	
Lateral spots always	-0.003	0.622	-0.004	0.997	
Ventral bands sometimes	0.003	0.687	0.004	0.997	
Ventral bands always	0.003	0.576	0.006	0.996	
Spitting predicted by habitat (ambiguous species non-spitting)					
(Intercept)	-0.184	1.429	-0.129	0.898	82.552
Habitat mixed	0.000	0.568	0.001	0.999	
Habitat open	0.000	0.559	0.000	1.000	
Spitting predicted by habitat (ambiguous species spitting)					
(Intercept)	-0.501	1.219	-0.411	0.681	150.162
Habitat mixed	0.294	0.870	0.337	0.736	
Habitat open	-0.123	0.795	-0.155	0.877	

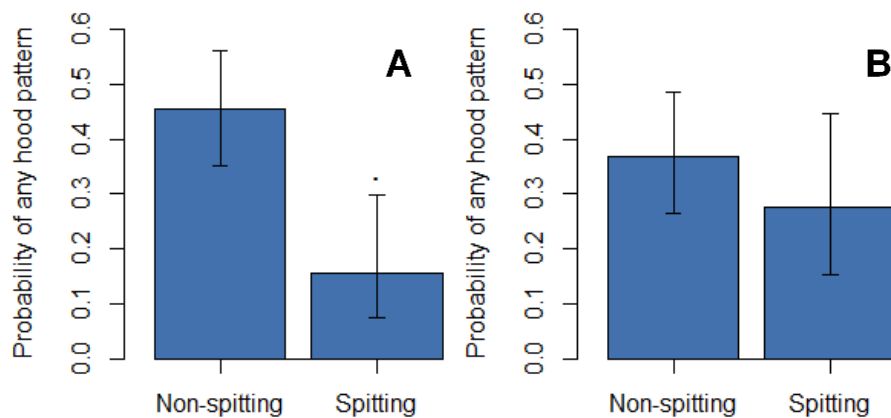


Figure 7. Phyloglm models for spitting predicted by any hood pattern. A- Ambiguous spitting species (N. kaouthia, N. atra and N. sagittifera) non-spitting. B- Ambiguous spitting species spitting.

3.4 Ancestral state reconstruction

Ancestral reconstruction of maximum rib length and hood area showed a clear trend towards a larger rib length and greater area of hood throughout the genus *Naja* (Figure 8). *Ophiophagus* and *Hemachatus* show independent increases in both rib length and area. Maximum rib length and hood area generally show a similar image. However, the ancestor of *Uraeus* likely had a large hood area but smaller extended ribs.

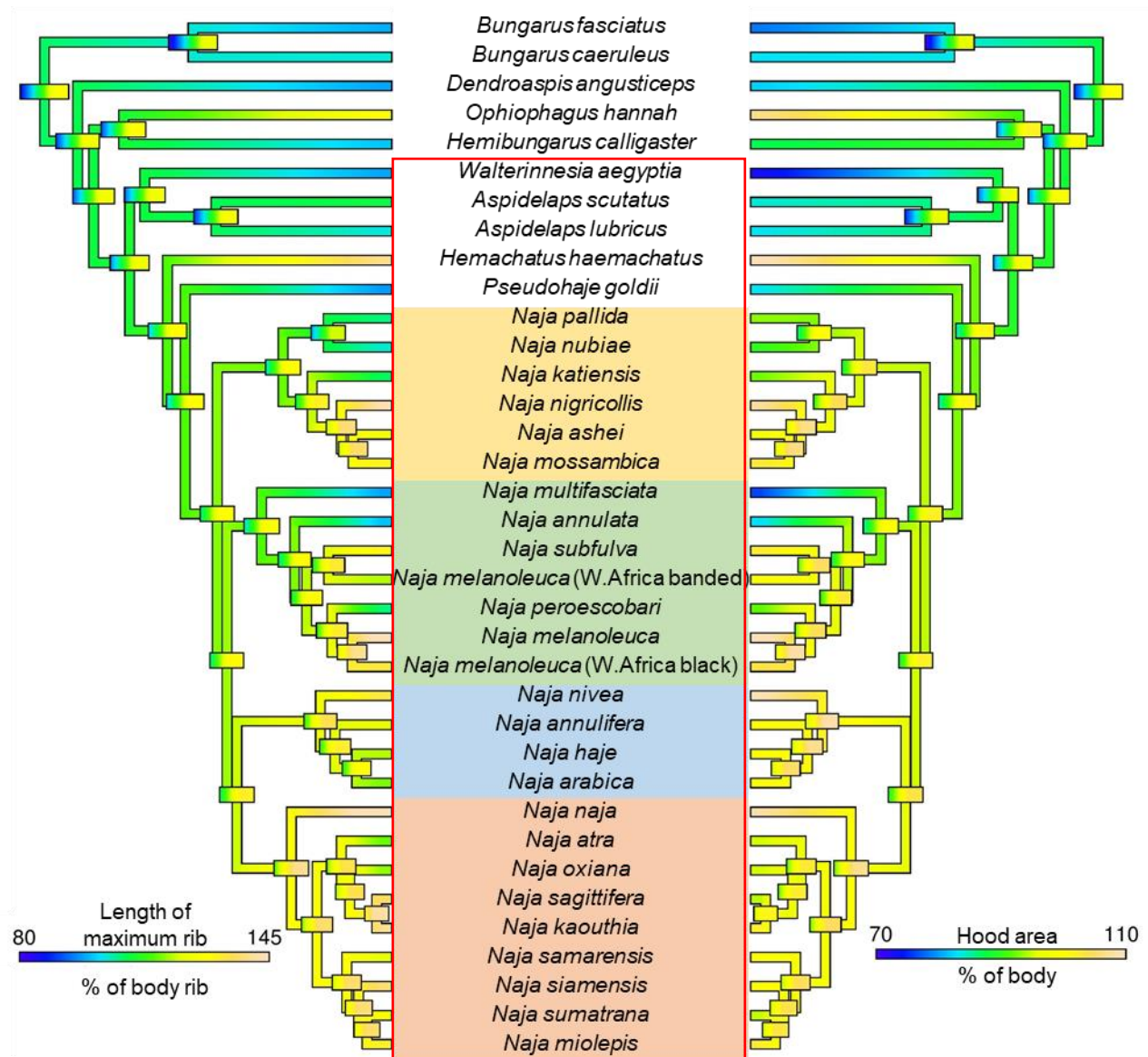


Figure 8. Ancestral state reconstruction of maximum rib length (left) and hood area (right) for Genus *Naja* and related genera. Coloured bars represent 95% confidence intervals for nodes. Colours behind species name represent *Naja* subgenera: yellow- *Afronaja*, green- *Boulengerina*, blue- *Uraeus*, red- *Naja*. Red box represents core cobra group.

Stochastic mapping of discrete characters for hood pattern and spitting onto a phylogeny (Figure 9) suggested that the ancestor of the core cobras was non-spitting and lacked a hood pattern.

Evidence for hood pattern evolution is inconclusive. A definite or indistinct pattern may have been present in the common ancestor of subgenera *Naja*, *Uraeus* and *Boulengerina* leading to a loss in most *Uraeus* species, *N. annulata* and *N. multifasciata* in subgenus *Boulengerina*, and subsequent reductions and losses in pattern in subgenus *Naja*. However, hood pattern may have evolved multiple times: once in *Boulengerina*, once in subgenus *Naja* and in *Uraeus*.

When possible spitters (*N. kaouthia*, *N. atra* and *N. sagittifera*) were regarded as non-spitting, ancestral state reconstructions suggested three independent origins of spitting in the core cobras: once in *Hemachatus*, once in subgenus *Afronaja* and once in the ancestor of the spitting adapted Asian cobras (*N. miolensis*, *N. siamensis*, *N. samarensis* etc.). When possible spitters were regarded as spitting, the origin of spitting in Asian *Naja* was in the ancestor of all Asian cobras with the exclusion of *N. naja* and spitting was lost once in *N. oxiana* (Figure 9).

The ancestor of the core cobras most likely displayed hooding behaviour, but lacked extended ribs (Figure 9). However, hooding behaviour was lost once in *Walterinnesia*. Ancestral reconstructions suggest that extended ribs evolved multiple times within the core cobra group, after the evolution of hooding behaviour. Although inconclusive, reconstructions suggest three independent evolutions of extended ribs once in *Hemachatus*, once in the ancestor of the subgenera *Naja*, *Boulengerina* and *Uraeus* and once in the ancestor of large hooded *Afronaja* (*N. nigricollis*, *N. mossambica* and *N. ashei*). Reconstructions suggest that extended ribs have been lost in *N. annulata* and *N. multifasciata* in subgenus *Boulengerina*. However, another possibility is two independent evolutions of extended ribs: once in *Hemachatus* and once in the ancestor of the genus *Naja*, followed by losses in small hooded *Afronaja* and *N. annulata* and *N. multifasciata* in subgenus *Boulengerina*. Both hooding behaviour and extended ribs evolved independently in *Ophiophagus*.

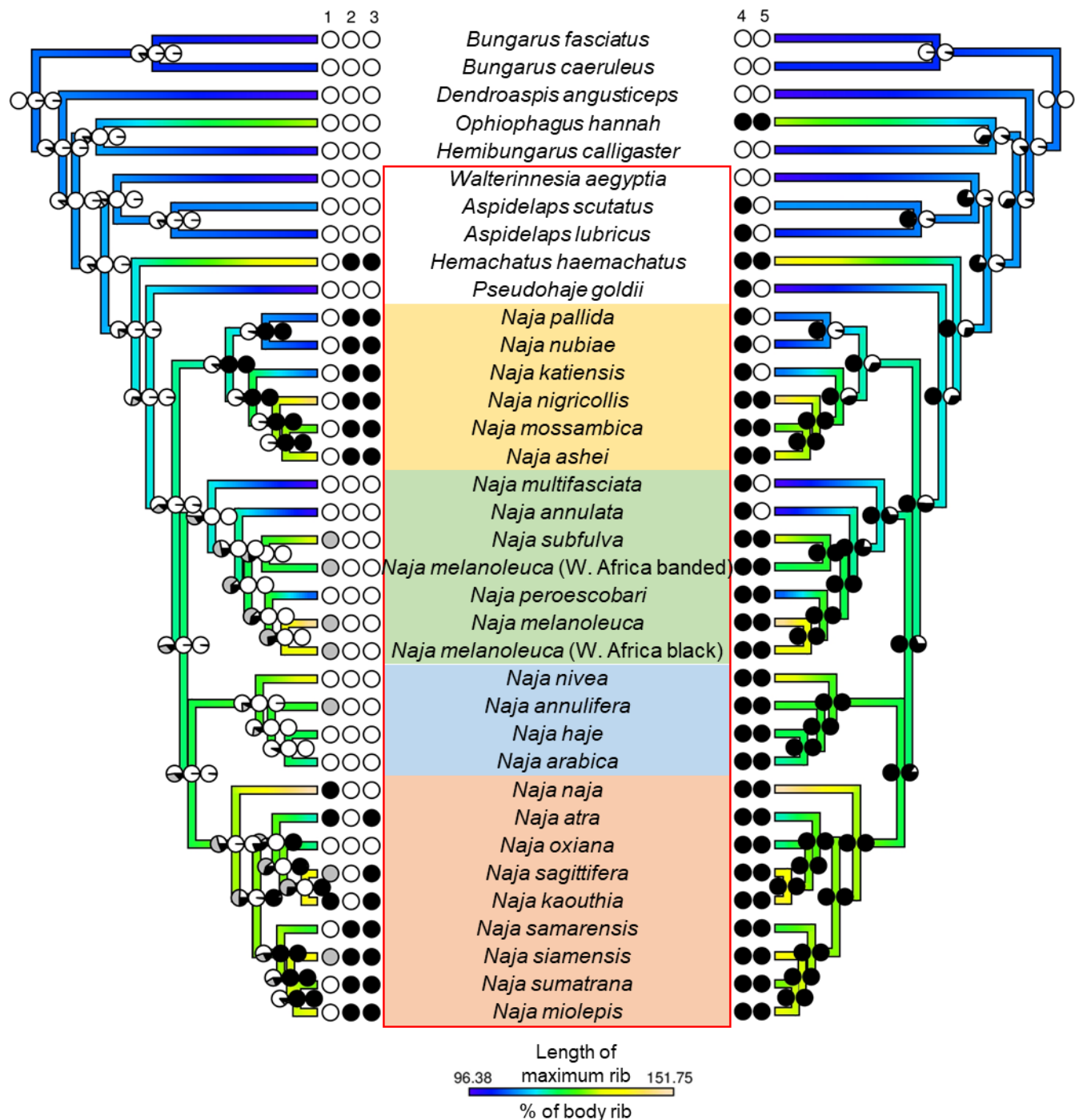


Figure 9. Ancestral reconstruction of maximum rib length using ContMap with make.simmap reconstructions of discrete characters: 1- Hood pattern, 2- Spitting (ambiguous non-spitting), 3- Spitting (ambiguous spitting), 4- Hooding behaviour and 5- Extended ribs. Pies at nodes represent posterior probabilities. Black- present, white- absent, grey- possible or indistinct. Colours behind species name represent Genus *Naja* subgenera: yellow- *Afronaja*, green- *Boulengerina*, blue- *Uraeus*, red- *Naja*. Red box- core cobra group.

4.0 Discussion

Our study suggests that the ancestor of the core cobra group used hooding behaviour but lacked extended ribs, hood pattern and spitting. Extended ribs, spitting and hood pattern have evolved multiple times within the group. It was expected that wide hoods would be associated with hood patterns. Although a hood pattern was associated with longer hood ribs, when phylogenetic relationships were taken into account, there was no association. Both wide hoods and hoods with a large area were associated with ventral bands, but not when relationships were considered. However, there were no relationships between wide hoods and habitat or lateral throat spots.

Spitting in the core cobra group was not associated with hood patterns, wide hoods, ventral bands and spots or a large hood area in any analysis. Although a negative association was expected between hood pattern and spitting due to the requirement for a cobra to face a predator during spitting and turn away in order to display the hood pattern. However, no association between hood pattern and spitting was found, although there was a lower probability of hood pattern when ambiguous spitting species were coded as non-spitting.

4.1 Hood size and hooding behaviour

The evolution of hooding behaviour facilitated the evolution of extended ribs. A larger aposematic signal increases avoidance learning in predators (Forsman & Merilaita, 1999), therefore extended ribs are the obvious next step in evolution. Young and Kardong (2010) suggested that the ability of the cobra to erect a hood is due to neuromuscular control rather than morphological specialization and elongation of the ribs. Our study supports this view because multiple species such as *N. pallida* and *N. annulata* clearly produce a hood, but do not have extended thoracic ribs.

Our study found substantial variation in hood morphology between different species. Although the trend throughout all cobra subgenera is towards extended ribs and large hoods, secondary reduction of rib length has been seen in multiple species potentially linked to habitat specialization. In subgenus *Boulengerina*, species are adapted to a large diversity of habitats and therefore show a similarly high diversity in morphology. Actively foraging species in the *N. melanoleuca* species complex have very large hoods, with both a large area and width. In contrast aquatic specialists *N. annulata* and *N. christyi* (not included in comparative analysis) lack extended ribs which may negatively affect swimming and underwater foraging. Being highly proficient swimmers also gives *N. annulata* and *N. christyi* another avenue for rapid escape and avoidance of predators on land. *N. multifasciata* is a small, semi-fossorial leaf-litter forager (Chippaux, 2006; O'Shea, 2008) that lacks extended ribs and uses a reduced hooding behaviour in the form of flattening of the neck, but does not raise its upper body from the ground (personal comm. Wüster). Similarly, the small size of *N.*

multifasciata and ecology suggests different predators, lower predation risk and other options for escape e.g. underground.

Pseudohaje similarly displays hooding behaviour but lacks extended ribs. *Pseudohaje* is arboreal and semi-aquatic, inhabiting forests related to waterbodies and feeds on anurans and fish (Akani *et al.*, 2005; Chippaux, 2006). Therefore, extended ribs may interfere with an arboreal lifestyle and again, different predators and options of escape into water or trees may have led to reduction in extended ribs.

Although the common ancestor of *Walterinnesia* and *Aspidelaps* probably displayed hooding behaviour, differences in habitat and niche have likely caused the loss of hooding behaviour in *Walterinnesia*. *Walterinnesia* is a nocturnal, slow active forager with poor eyesight that feeds on sleeping lizards and toads (Zinner, 1971). Similar to *Pseudohaje*, *Walterinnesia* is unlikely to come into contact with the same predators as large hooded *Naja* species due to its ecology. In the habitat and range that *Walterinnesia* occupies, there are few nocturnal predators that prey on snakes (Zinner, 1971). When a predator is encountered, *Walterinnesia* produces an s-shaped posture, inflates its body and hisses (Amr & Disi, 2011), in contrast to visual displays such as hooding and patterns which are likely less affective in the dark.

In the African spitting subgenus *Afronaja*, *N. pallida*, *N. nubiae* and *N. katiensis* produce hooding behaviour but lack extended ribs, whereas *N. mossambica*, *N. nigricollis* and *N. ashei* have large hoods with extended ribs. Reasons for extended ribs in *Afronaja* are likely to be related to the evolution of spitting in the subgenus, discussed in section 4.2.

Extended ribs evolved independently in genera *Naja*, *Hemachatus* and also in the distantly related *Ophiophagus*. The main feature that hooding species share in the genera *Naja*, *Hemachatus* and *Ophiophagus* is a terrestrial active foraging strategy (Bhaisare *et al.*, 2010; Radcliffe *et al.*, 1986; Shine *et al.*, 2007), suggested by Greene (1969) to be associated with antipredator defences. Species often forage diurnally or during the day time (e.g. Alexander & Marshall, 1998; Bhaisare *et al.*, 2010; Broadley & Cock, 1989) and their preoccupied foraging strategy and lack of group vigilance leaves them vulnerable to predation.

4.2 Spitting evolution

Our study strongly supports the theory of three independent origins of spitting proposed by Wüster *et al.* (2007) when *N. kaouthia* and *N. atra* are regarded as non-spitting. As in Panagides *et al.* (2017), our study found a single evolution of spitting within the *Naja* subgenus at the base of the highly adapted spitters (*N. mandalayensis*, *N. miolepis*, *N. samarensis* etc.) as well as single origins in *Afronaja* and *Hemachatus*.

However, when *N. kaouthia*, *N. sagittifera* and *N. atra* are regarded as spitting, the origin of spitting in the subgenus *Naja* is different. Panagides *et al.* (2017) suggested that spitting evolved in the ancestor of all Asian cobras and was then lost once in the ancestor of *N. oxiana* and *N. kaouthia*. Panagides *et al.* (2017) used the phylogeny of Lee *et al.*, (2016), who grouped *N. naja* as a sister species to *N. atra*, whereas the phylogeny used in our study has placed *N. naja* as sister species to all other Asian cobras (von Plettenberg Laing, 2017). Therefore, our study suggests that spitting evolved in the ancestor of Asian cobras minus *N. naja* and was lost once in *N. oxiana*, in support of Wüster *et al.* (2007).

Recent research into spitting in *N. kaouthia* confirms that populations in Eastern India spit (Santra & Wüster, 2017). Furthermore, Santra and Wüster (2017) stated the possibility of multiple non-spitting and spitting adapted cryptic species within *N. kaouthia*. Therefore, the theory of three origins and one loss of spitting should be the more accepted. *N. kaouthia* shows a similar degree of spitting adaptation to *N. philippinensis*, an accomplished spitter (Wüster & Thorpe, 1992b). Bogert (1943) suggested that a lack of selective disadvantage for reduction of orifice size could mean that spitting fang morphology could persist in a species, even if the species lost the behaviour of spitting. Therefore, it appears that spitting behaviour has been lost in some populations. For example, spitting behaviour has not been observed in *N. sagittifera* (previously a subspecies of *N. kaouthia*) which has similar fang morphology to *N. kaouthia* (Wüster, 1990). *N. sagittifera* is a species endemic to the Andaman Islands, which has few predatory species compared to mainland Asia, lacking primates, mongoose and other potential cobra predators. Birds of prey may be the main predators of cobras on the Andaman Islands such as the Andaman serpent-eagle, *Spilornis elgini* and Crested Serpent-eagle, *Spilornis cheela*, which has been reported to feed on *N. naja* in India (Gokula, 2012). However, *N. sagittifera* is a poorly studied and rare species so spitting behaviour may be present but rarely used or unobserved.

Compared to the visual defences of aposematic patterns and a large hood, spitting is a purely chemical defence due to the colourlessness of the venom, affect only if venom enters the eyes and speed at which it is expelled (Nasoori *et al.*, 2016). A positive association between spitting and a large hood were expected due to a large hood being a signal of readiness to spit. However, our study found no associations between spitting and hood size. In subgenus *Afronaja*, *N. pallida*, *N. nubiae* and *N. katiensis* lack extended ribs and *N. pallida* often spits without the hood extended, from various angles and allowing defence in multiple habitats (Rasmussen *et al.*, 1995). In contrast, *N. mossambica*, *N. ashei* and *N. nigricollis* have extended ribs. *N. mossambica* is capable of spitting from multiple angles whereas *N. nigricollis* often spits with the hood extended (Rasmussen *et al.*, 1995). This suggests that a large hood may be a warning of spitting in *Hemachatus* and subgenus *Naja*, but only in large hooded species of *Afronaja* such as *N. mossambica*, *N. nigricollis*,

N. ashei, *N. woodi* and *N. nigricincta*. Ancestral state reconstructions suggested a possible reduction in extended ribs in the ancestor of *Afronaja*, followed by an increase in rib length in the ancestor of *N. nigricollis*. However, a hood is still produced by *N. pallida*, *N. nubiae* and *N. katiensis*, despite the lack of extension to the ribs. Behavioural modifications that increase the accuracy of spitting (e.g. Berthé *et al.*, 2013; Westhoff *et al.*, 2010; Young *et al.*, 2009) and hooding behaviour alone may provide a strong enough aposematic signal to stop attack by predators. Extended ribs may have evolved after spitting in *Afronaja* as an aposematic signal of readiness to spit, reducing the need to expend venom and increasing the chance of escape and survival. In comparison, the ancestor of Asian *Naja* had extended ribs and hooding behaviour and therefore spitting evolved after extended ribs.

Panagides *et al.* (2017) suggested that the upright posture and hooding display was essential for the evolution of spitting. Our study agrees with the hypothesis; hooding behaviour evolved before spitting.

The independent evolution of spitting along with extended ribs in *Hemachatus* may be due to the evolution of viviparity (Neill, 1964). *Hemachatus* have a stocky body plan enabling high fecundity (Shine *et al.*, 2007). However, gravidity reduces locomotor capacity (Seigel *et al.*, 1987) and escape ability and predation on the mother will result in the loss of all offspring. In other reptiles, gravid females display a shift in antipredator strategies towards aggressiveness or crypsis rather than flight (Bauwens & Thoen, 1981; Brodie III, 1989; Creer, 2005; Jayne & Bennett, 1990). Coupled with an active foraging strategy, these factors lead to a requirement for greater defensive ability.

4.3 Evolution of patterns

Hood pattern appears to have evolved at least twice within the genus *Naja*. However, a distinct, differentiated pattern has only evolved in Asian cobras. Pattern in the *N. melanoleuca* complex and *N. annulifera* appears to have evolved from banding across the back of the neck. This also gives an idea of how the highly differentiated pattern of *Naja naja* and other Asian species may have evolved.

To our knowledge, this is the first time that hood markings have been observed in *N. haje*. Hood markings were recorded in over half of all juvenile *N. haje* examined. This may be because of sampling bias by collectors who selected conspicuous individuals due to personal preference or convenience/ease of encounter (Ponder *et al.*, 2001; Shine, 1994). The presence of hood markings in around half of specimens examined from the *N. melanoleuca* species complex may similarly be due to collection bias although it has been mentioned that light markings are common on the dorsal neck area of *N. melanoleuca* (Ceriaco *et al.*, 2017).

In our study, it was uncertain whether a correlation between hood pattern and the size of hood exists. Without taking phylogenetic signal into account, species with a definite or indistinct hood pattern tend to have longer extended ribs. However, it is important to account for shared ancestry due to the resulting phenotypic similarity between closely related species (Felsenstein, 1985; Hansen & Martins, 1996) and in our study when phylogenetic signal was accounted for, there was no association between hood pattern and extended ribs. However, the solution used to overcome the issue of overparameterization used extremes of phylogenetic signal: no phylogenetic signal or $\lambda=1$, a Brownian model. As the issue was mostly due to a small number of species included in the study, if all known core cobra species could be used in future analysis, increasing the number of species included from 31 to 42, the overparameterization problem may be overcome allowing λ to be estimated by maximum likelihood for all models and leading to more conclusive results.

In the absence of further data for species missing from this analysis, behavioural studies could provide valuable evidence for correlation between hood size and pattern. For example, the effect on predators by large hoods and large hood patterns vs. small hoods and small hood patterns.

This also calls into question which strategy is more effective: a large hood, a large hood pattern or a highly contrasting hood pattern. The size and conspicuousness of an aposematic signal is important for predator avoidance and learning (Alatalo & Mappes, 1996; Forsman & Merilaita, 1999; Gittleman & Harvey, 1980; Roper, 1990). Both hood size and hood pattern are aposematic signals. *N. naja* has the largest hood width and a very distinctive hood pattern across much of its range, providing evidence for a large hood and large pattern. However, *N. atra* has a comparatively small hood, despite almost always having a distinctive pattern, providing evidence towards pattern conspicuousness being effective.

Although a negative relationship between hood pattern and spitting was expected due to the incompatibility of the two strategies, no relationship was found although the probability of spitting was slightly lower when a hood pattern was present and ambiguous spitting species coded as non-spitting. Although all *Afronaja* lack hood patterns, in subgenus *Naja*, spitting species *N. siamensis* and *N. mandalayensis* are polymorphic. *N. siamensis* sometimes has a pattern and a hood pattern is sometimes seen in juvenile *N. mandalayensis* but not in adults (Slowinski & Wüster, 2000). Hood pattern has been lost in *N. samarensis*, *N. sumatrana*, *N. philippinensis* and *N. miolepis*.

When possible spitters *N. kaouthia*, *N. atra* and *N. sagittifera* were regarded as spitting, there was no relationship between spitting and hood pattern. *N. atra* and *N. kaouthia* always have highly contrasting hood patterns and *N. sagittifera* often has a pattern as a juvenile that may fade with age. A potential reason for the presence of hood marks in Asian spitting cobras is the lower level

and variation in spitting adaptation compared to *Afronaja* (Bogert, 1943; Wüster & Thorpe, 1992b). All *Afronaja* species are highly specialized spitters with further behavioural modifications that increase accuracy and efficiency of spitting. *N. kaouthia* and *N. atra* have larger fang orifices than highly adapted Asian *Naja* such as *N. samarensis* and *N. sputatrix* (Wüster & Thorpe, 1992b). Unreliable spitting ability in Asian *Naja* mean that other more reliable strategies for defence are also required. For example, *N. siamensis* spits venom as a mist at a range of around 1 metre (Rasmussen *et al.*, 1995) but also has a tendency to present the rear of its hood when annoyed (Berthé, 2011).

No associations were found between hood pattern and habitat. Cryptic colouration restricts movement to certain times of day and specific backgrounds (Stevens & Ruxton, 2012). By having an aposematic pattern, an individual can forage in multiple habitats, despite being in sight of predators (Speed *et al.*, 2010). A potential reason for the lack of significance is the ignorance of microhabitat structure, for example, *N. melanoleuca* species live in closed habitats but may need to move between patches of forest. Although *N. philippinensis*, *N. atra* and *N. kaouthia* were classified as inhabiting closed habitat due to the occupancy of mostly forest habitats, along with multiple other Asian *Naja* such as *N. naja* and *N. mandalayensis*, these species are highly adaptable to anthropogenically disturbed habitats such as paddy fields, houses and agriculture (Slowinski & Wüster, 2000; Watt *et al.*, 1987). A reason for this highly adaptable nature may be the presence of multiple strategies including aposematic pattern that protect from a wide variety of predators in different habitats. However, this has led to the high incidence of snakebite in agriculture and around human settlement (Chippaux, 1998).

Aposematic patterns further increase the chance of encounter with a predator (Higginson & Ruxton, 2010; Mänd *et al.*, 2007). Therefore, a more cryptic colour pattern and disruptive colouration is preferred for lower encounter rate with predators. Species in subgenera *Afronaja* and *Uraeus* generally occupy open habitats and have a relative degree of background matching of pigment to substrate. Although aposematic patterns appear to have been lost in multiple Asian cobra species due to the evolution of spitting, *N. oxiana* has lost both spitting and hood pattern. A hood pattern may be lost in *N. oxiana* due to the transition from a mixed habitat to open habitat where chance of encounter with a predator is higher.

As expected, ventral bands were significantly associated with both a large area and extended ribs, supporting the hypothesis that ventral bands function to attract the focus of the predator to the size of the hood (Nasoori *et al.*, 2016). The black and white colouration and general lack of bright colours mean that ventral bands will attract the attention of mammals with monochromatic vision. However, *N. mossambica* and *Ophiophagus* sometimes have colour such as red or orange as well

as black ventral bands (Lim *et al.*, 2011; Marais, 2005). These colours may be aimed specifically at humans and primates which have evolved colour vision, as well as to increase conspicuousness and contrast from green background vegetation.

Ventral markings are common in most core cobra species which produce a hooding display but are absent in *Walterinnesia*, *Pseudohaje* and *N. multifasciata*. These species also lack extended ribs and either do not produce a hooding display (*Walterinnesia*), or use a very reduced form such as flattening of the neck and slight raising of the forepart of the body. Therefore, the ventral scales are rarely displayed.

This study defined ventral patterns by presence or absence whereas contrast and distinctness of bands may be more informative. Species such as *N. pallida* and *N. nubiae* always have distinct bands (Wüster & Broadley, 2003) whereas bands in *N. siamensis* and *N. sputatrix* are often obscure due to darkening of pigment or mottling (Wüster *et al.*, 1997). Ontogenetic change in ventral bands is also found in many species such as *N. nivea* and *N. annulifera* (Broadley & Wüster, 2004), which may be related to habitat or predator differences. Local adaptation to substrate colour has been found in *N. atra* (Lin *et al.*, 2008) which could obscure or decrease the conspicuousness of bands.

4.4 Further reasons for variation in defensive strategies

In this study, multiple species did not fit into the expected trends. This is potentially due to factors not explored during this thesis such as predator type, frequency of encounter and hunting strategy. An abundance of specialized predators can cause a selection pressure to favour a less conspicuous warning signal. For example, in a study using plasticine models, models with aposematic warning signals were at a disadvantage when specialized snake predators were present (Valkonen *et al.*, 2012). As birds of prey primarily use vision for hunting and many species are known to be predators of cobras, a predator assemblage with a high number of avian predators may lead to selection for less aposematic patterns in cobras. Honey badgers (*Mellivora capensis*) are known predators of cobras, with *N. nivea* making up 10.5% of the biomass in the diet in South Africa (Begg *et al.*, 2003) and have evolved resistance to snake venoms (Drabeck *et al.*, 2015). Honey badgers have poor eyesight and hunt by following scent trails, therefore aposematic markings may have little use in defence. *Naja nivea* lacks a hood pattern and ventral bands fade in colour with age (Broadley & Wüster, 2004).

Another factor not explored in this thesis is behavioural variation and the extent and preference toward which species use different strategies. Some species, such as *N. pallida* and *N. mossambica* spit with little provocation, whereas *N. siamensis* and *Hemachatus* are reluctant to spit

(Rasmussen *et al.*, 1995). The height at which the body is elevated during hooding behaviour is likely to vary between species and also depending on the context of the display (Nasoori *et al.*, 2016). This changes the amount of ventral surface that is displayed and may correlate with the number of ventral bands and proportion of patterned ventral surface. Defensive behaviour of an individual is also influenced by age, experience, temperature and state (Aubret *et al.*, 2011; Mänd *et al.*, 2007; Seigel *et al.*, 1987).

Furthermore, many species included in this study use other defensive behaviours in combination with the three studied such as death feigning, rapid escape, false strikes or hissing. Death feigning behaviour has been observed in *Aspidelaps*, *Hemachatus*, *N. melanoleuca*, *N. annulifera* and *N. mossambica* (Bates & Nuttal, 2013). Rasmussen *et al.* (1995) found that *Hemachatus* performed death feigning at the slightest provocation, whereas spitting was as likely to be used as false strikes. *N. pallida* and *N. mossambica* are very likely to spit, whereas the primary response of *N. nigricollis* is to escape (Rasmussen *et al.*, 1995).

Some species of core cobra use all three strategies. For example, *N. siamensis* uses spitting, sometimes has a hood pattern and has a large hood. The combination of spitting, pattern and hood size may create an additive effect, increasing avoidance by predators (Marples *et al.*, 1994; Rowe, 2002). However, in some cases multiple components do not create an additive effect, but may instead be aimed at different predators with different sensory systems and hunting strategies (Lindstedt *et al.*, 2008; Valkonen *et al.*, 2011). Furthermore, the presence of multiple strategies also allows defences to be deployed on a scale, correlated with an escalating attack from a predator, giving further options if the first strategy does not work (e.g. Ducey *et al.*, 1991; Gibbons & Dorcas, 2002; Schieffelin & De Queiroz, 1991).

However, the primary reason for the presence of such extreme and distinctive defensive strategies in the core cobra group is probably that of innate avoidance. Ignoring an aposematic signal will likely result in the death of a predator. Therefore, natural selection selects for individuals that avoid the aposematic signal (Smith, 1975; 1977). However, specialist snake predators such as mongoose and honey badger (*Mellivora capensis*) have evolved resistance to venom (Drabeck *et al.*, 2015), reducing selection for avoidance of the aposematic signal. Therefore, cobras have evolved other strategies such as hood patterns and spitting or have increased the strength of the aposematic signal such as larger hoods or bolder patterns to increase learning in the predator.

However, most studies involving spitting and hooding behaviour have been in relation to humans and dogs, often in artificial environments. It is largely unknown how cobras use defences and the effectiveness of their defensive displays against other predators such as birds, mammals and other reptiles, requiring extensive further research. Further analysis using a dated phylogeny to identify

the timeframe in which defensive strategies evolved would help to determine triggers for evolution such as expanding grasslands or ranges of potential predators.

4.5 Conclusion

This study has highlighted the high variation in hood size throughout the core cobra group and related Elapid genera. A general trend towards extended ribs is seen throughout all species that display hooding behaviour. Species within the core cobra group that lack extended ribs are generally habitat specialists that may be negatively affected by extended ribs but hooding behaviour is usually still utilized as a defensive strategy. Our study supports the hypothesis of three evolutionary origins of spitting, along with one loss in *N. oxiana*. However, spitting showed no association with any factors included in analysis, suggesting microhabitat or predator complexes may be responsible for spitting evolution. Similarly, no trade-offs between spitting and hood pattern were discovered, primarily due to the presence of hood patterns in Asian spitting *Naja*. No links between hood size and pattern were discovered, potentially due to the conspicuousness of pattern being more important than the size. Some species of Asian *Naja* use all three defensive strategies; they have hood patterns, spit and have a large hood. The use of all three strategies may be due to high selection predators, multiple specialized predators or due to unreliable spitting accuracy.

This study also opens further questions regarding the reasons for spitting evolution, particularly in relation to ecology and the effect of displays on different predators. Overall, this study provides a new insight into a highly complex multicomponent display that has been somewhat neglected despite cultural significance and medical importance.

5.0 Bibliography

- Akani, G. C., Angelici, F. M., & Luiselli, L. (2005). Ecological data on the Goldie's tree cobra, *Pseudohaje goldii* (Elapidae) in southern Nigeria. *Amphibia-Reptilia*, 26, 382–387.
- Alatalo, R. V., & Mappes, J. (1996). Tracking the evolution of warning signals. *Nature*, 382(6593), 709–710.
- Alexander, G. J., & Marshall, C. L. (1998). Diel activity patterns in a captive colony of rinkhals, *Hemachatus haemachatus*. *African Journal of Herpetology*, 47(1), 29–32.
- Allen, W. L., Baddeley, R., Scott-Samuel, N. E., & Cuthill, I. C. (2013). The evolution and function of pattern diversity in snakes. *Behavioral Ecology*, 24(5), 1237–1250.
- Allf, B. C., Durst, P. A. P., & Pfennig, D. W. (2016). Behavioral Plasticity and the Origins of Novelty: The Evolution of the Rattlesnake Rattle. *The American Naturalist*, 188(4), 475–483.
- Amr, Z. S., & Disi, A. M. (2011). Systematics, distribution and ecology of the snakes of Jordan. *Vertebrate Zoology*, 61(2), 179–266.
- Aubret, F., Michniewicz, R. J., & Shine, R. (2011). Correlated geographic variation in predation risk and antipredator behaviour within a wide-ranging snake species (*Notechis scutatus*, Elapidae). *Austral Ecology*, 36(4), 446–452.
- Barbour, T. (1922). Rattlesnakes and Spitting Snakes. *Copeia*, 106, 36–38.
- Bates, M. F., & Nuttal, R. (2013). A case of death-feigning in the striped grass snake *Psammophylax tritaeniatus* (Günther), with a review on the occurrence of this phenomenon in Southern and Eastern African snakes. *African herp news*, 60, 5–9.
- Bauder, J. M., Macey, J. N., Stohlgren, K. M., Day, A., Snow, F., Safer, A., Redmond, R., Waters, J. M., Wallace Sr, M., and Stevenson, D. J. (2015). Factors influencing the display of multiple defensive behaviors in eastern indigo snakes (*Drymarchon couperi*). *Herpetological Conservation and Biology*, 10(2), 559–571.
- Bauwens, D., & Thoen, C. (1981). Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *Journal of Animal Ecology*, 50(3), 733–743.
- Begg, C. M., Begg, K. S., Du Toit, J. T., & Mills, M. G. L. (2003). Sexual and seasonal variation in the diet and foraging behaviour of a sexually dimorphic carnivore, the honey badger (*Mellivora capensis*). *Journal of Zoology*, 260(3), 301–316.
- Bennett, A. F. (1990). Thermal dependence of locomotor capacity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 259(2), 253–258.
- Berthé, R. A. (2011). *Spitting behaviour and fang morphology of spitting cobras*. Doctoral Thesis. Rheinischen Friedrich-Wilhelms-Universität, Bonn, Germany.
- Berthé, R. A., de Pury, S., Bleckmann, H., & Westhoff, G. (2009). Spitting cobras adjust their venom distribution to target distance. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 195(8), 753–757.
- Berthé, R. A., Westhoff, G., & Bleckmann, H. (2013). Potential targets aimed at by spitting cobras when deterring predators from attacking. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 199(5), 335–340.

- Bhaisare, D., Ramanuj, V., Shankar, P. G., Vittala, M., Goode, M., & Whitaker, R. (2010). Observations on a Wild King Cobra (*Ophiophagus hannah*), with Emphasis on Foraging and Diet. *IRCF Reptiles & Amphibians*, 17(2), 95–102.
- Bogert, C. M. (1942). *Pseudohaje* Gunther, a valid genus for two west African arboreal cobras. *American Museum Novitates*, 1174, 1–9.
- Bogert, C. M. (1943). Dentitional phenomena in cobras and other elapids with notes on adaptive modifications of fangs. *Bulletin of the American Museum of Natural History*, 81(3), 260–285.
- Broadley, D. G. (1995). The Snouted Cobra, *Naja annulifera*, a valid species in southern Africa. *The Journal of the Herpetological Association of Africa*, 44(2), 26–32.
- Broadley, D. G., & Baldwin, A. S. (2006). Taxonomy, Natural History, and Zoogeography of the Southern African Shield Cobras, Genus *Aspidelaps* (Serpentes: Elapidae). *Sierra*, 9(2), 163–176.
- Broadley, D. G., & Cock, E. V. (1989). *Snakes of Zimbabwe*. Longman, Zimbabwe.
- Broadley, D. G., & Wüster, W. (2004). A review of the southern African non-spitting cobras (Serpentes: Elapidae: *Naja*). *African Journal of Herpetology*, 53(2), 101–122.
- Brodie III, E. D. (1989). Behavioral modification as a means of reducing the cost of reproduction. *The American Naturalist*, 134(2), 225–238.
- Caro, T., Graham, C., Stoner, C., & Vargas, J. (2004). Adaptive significance of antipredator behaviour in artiodactyls. *Animal Behaviour*, 67(2), 205–228.
- Caro, T., Sherratt, T. N., & Stevens, M. (2016). The ecology of multiple colour defences. *Evolutionary Ecology*, 30(5), 1–13.
- Carpenter, C. C., & Ferguson, G. W. (1977). Variation and evolution of stereotyped behavior in reptiles. In C. Gans & D. W. Tinkle (Eds.), *Biology of the Reptilia: Ecology and Behavior*, vol 7A (pp. 335–554). New York: Academic Press.
- Cascardi, J., Young, B. A., Husic, H. D., & Sherma, J. (1999). Protein variation in the venom spat by the red spitting cobra, *Naja pallida* (Reptilia: Serpentes). *Toxicon*, 37(9), 1271–1279.
- Ceríaco, L. M. P., Marques, M. P., Schmitz, A., & Bauer, A. M. (2017). The “Cobra-preta” of São Tomé Island, Gulf of Guinea, is a new species of *Naja* Laurenti, 1768 (Squamata: Elapidae). *Zootaxa*, 4324(1), 121–141.
- Chippaux, J. P. (1998). Snake-bites: Appraisal of the global situation. *Bulletin of the World Health Organization*, 76(5), 515–524.
- Chippaux, J. P. (2006). *Les serpents d’Afrique occidentale et centrale*. Paris: Institut de Recherche pour le Développement (IRD)
- Chu, E. R., Weinstein, S. A., White, J., & Warrell, D. A. (2010). Venom ophthalmia caused by venoms of spitting elapid and other snakes: report of ten cases with review of epidemiology, clinical features, pathophysiology and management. *Toxicon*, 56(3), 259–272.
- Creer, D. A. (2005). Correlations between Ontogenetic Change in Color Pattern and Antipredator Behavior in the Racer, *Coluber constrictor*. *Ethology*, 111(3), 287–300.
- Dawkins, R., & Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London B*, 205(1161), 489–511.

- Drabeck, D. H., Dean, A. M., & Jansa, S. A. (2015). Why the honey badger don't care: Convergent evolution of venom-targeted nicotinic acetylcholine receptors in mammals that survive venomous snake bites. *Toxicon*, 99, 68–72.
- Ducey, P. K., Anthony, C. D., & Brodie, E. D. (1991). Thresholds and escalation of antipredator responses in the Chinese salamander *Cynops cyanurus*: inter- and intra-individual variation. *Behavioural Processes*, 23(3), 181–191.
- Duchêne, S., & Lanfear, R. (2015). Phylogenetic uncertainty can bias the number of evolutionary transitions estimated from ancestral state reconstruction methods. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 324(6), 517–524.
- Edmunds, M. (1974). *Defence in animals: a survey of anti-predator defences*. Longman Publishing Group.
- Ekstrøm, C. (2017). MESS: Miscellaneous esoteric statistical scripts. R package version 0.2-1.
- Emery, N. J. (2000). The eyes have it: The neuroethology, function and evolution of social gaze. *Neuroscience and Biobehavioral Reviews*, 24(6), 581–604.
- Endler, J. A. (1991). Interactions between predators and prey. In *Behavioural Ecology: An Evolutionary Approach*, 3rd Edition (J. R. Krebs and N. B. Davies, eds.), 169–196. Oxford, UK: Blackwell Scientific Publications.
- Endler, J. A., & Mappes, J. (2004). Predator Mixes and the Conspicuousness of Aposematic Signals. *The American Naturalist*, 163(4), 532–547.
- Etting, S. F., & Isbell, L. A. (2014). Rhesus Macaques (*Macaca mulatta*) use posture to assess level of threat from snakes. *Ethology*, 120(12), 1177–1184.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, 125(1), 1–15.
- Forsman, A., & Merilaita, S. (1999). Fearful symmetry: Pattern size and asymmetry affects aposematic signal efficacy. *Evolutionary Ecology*, 13(2), 131–140.
- Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic Analysis and Comparative Data: A Test and Review of Evidence. *The American Naturalist*, 160(6), 712–726.
- Freyvogel, T. A., & Honegger, C. G. (1965). Der “Speiakt” von “*Naja nigricollis*.” *Acta Tropica*, 22(4), 289–302.
- Gibbons, J. W., & Dorcas, M. E. (2002). Defensive Behavior of Cottonmouths (*Agkistrodon piscivorus*) toward Humans. *Copeia*, 2002(1), 195–198.
- Gittleman, J. L., & Harvey, P. H. (1980). Why are distasteful prey not cryptic? *Nature*, 286(5769), 149–150.
- Gokula, V. (2012). Breeding ecology of the crested serpent eagle *Spilornis cheela* (Latham, 1790)(Aves: Accipitriformes: Accipitridae) in Kolli Hills, Tamil Nadu, India. *TAPROBANICA: The Journal of Asian Biodiversity*, 4(2), 77–82.
- Greene, H. W. (1969). Antipredator Mechanisms in Reptiles. In C. Gans (Ed.), *Biology of the Reptilia* (16th edn., pp. 1–153). Branta Books.
- Greene, H. W. (1979). Behavioral convergence in the defensive displays of snakes. *Experientia*, 35(6), 747–748.

- Hansen, T. F., & Martins, E. P. (1996). Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution*, 50(4), 1404–1417.
- Hasson, O., Hibbard, R., & Ceballos, G. (1989). The pursuit deterrent function of tail-wagging in the zebra-tailed lizard (*Callisaurus draconoides*). *Canadian Journal of Zoology*, 67, 1203–1209.
- Hayes, W. K., Herbert, S. S., Harrison, J. R., & Wiley, K. L. (2008). Spitting versus Biting: Differential Venom Gland Contraction Regulates Venom Expenditure in the Black-Necked Spitting Cobra, *Naja nigricollis nigricollis*. *Journal of Herpetology*, 42(3), 453–460.
- Hayes, W. K., Kaiser, I. I., & Duvall, D. (1992). The mass of venom expended by prairie rattlesnakes when feeding on rodent prey. In J. A. Campbell & E. D. Brodie Jr (Eds.), *Biology of the Pitvipers* (pp. 383–388) Tyler, TX: Selva.
- Helfman, G. S. (1989). Threat-Sensitive Predator Avoidance in Damselfish-Trumpetfish Interactions. *Behavioral Ecology and Sociobiology*, 24(1), 47–58.
- Herrel, A., James, R. S., & Van Damme, R. (2007). Fight versus flight: physiological basis for temperature-dependent behavioral shifts in lizards. *Journal of Experimental Biology*, 210(10), 1762–1767.
- Higginson, A. D., & Ruxton, G. D. (2010). Optimal defensive coloration strategies during the growth period of prey. *Evolution*, 64(1), 53–67.
- Huelsenbeck, J. P., Nielsen, R., & Bollback, J. P. (2003). Stochastic Mapping of Morphological Characters. *Systematic Biology*, 52(2), 131–158.
- Ives, A. R., & Garland, T. (2010). Phylogenetic logistic regression for binary dependent variables. *Systematic Biology*, 59(1), 9–26.
- Jara, M., & Pincheira-Donoso, D. (2015). The neck flattening defensive behaviour in snakes: First record of hooding in the South American colubrid genus *Philodryas*. *Animal Biology*, 65(1), 73–79.
- Jayne, B. C., & Bennett, A. F. (1990). Scaling of speed and endurance in garter snakes: a comparison of cross-sectional and longitudinal allometries. *Journal of Zoology*, 220(2), 257–277.
- Johnson, T. P., Swoap, S. J., Bennett, A. F., & Josephson, R. K. (1993). Body size, muscle power output and limitation on burst locomotor performance in the lizard *Dipsosaurus dorsalis*. *Journal of Experimental Biology*, 174(1), 199–213.
- Kularatne, S. A. M., Budagoda, B. D. S. S., Gawarammana, I. B., & Kularatne, W. K. S. (2009). Epidemiology, clinical profile and management issues of cobra (*Naja naja*) bites in Sri Lanka: first authenticated case series. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 103(9), 924–930.
- Landová, E., Jančúchová-Lásková, J., Musilová, V., Kadochová, Š., & Frynta, D. (2013). Ontogenetic switch between alternative antipredatory strategies in the leopard gecko (*Eublepharis macularius*): Defensive threat versus escape. *Behavioral Ecology and Sociobiology*, 67(7), 1113–1122.
- Langerholm, J. (1991). Facial mimicry in the animal kingdom. *Italian Journal of Zoology*, 58(3), 185–204.

- Langkilde, T., Shine, R., & Mason, R. T. (2004). Predatory attacks to the head vs. body modify behavioral responses of garter snakes. *Ethology*, 110(12), 937–947.
- Lee, M. S. Y., Sanders, K. L., King, B., & Palci, A. (2016). Diversification rates and phenotypic evolution in venomous snakes (Elapidae). *Royal Society Open Science*, 3(1), 150277.
- Lillywhite, H. B. (1987). Circulatory adaptations of snakes to gravity. *Integrative and Comparative Biology*, 27(1), 81–95.
- Lillywhite, H. B., & Gallagher, K. P. (1985). Hemodynamic adjustments to head-up posture in the partly arboreal snake, *Elaphe obsoleta*. *Journal of Experimental Zoology*, 235(3), 325–334.
- Lim, K. K. P., Leong, T. M., & Lim, F. L. K. (2011). The King Cobra, *Ophiophagus hannah* (Cantor) in Singapore (Reptilia: Squamata: Elapidae). *Nature In Singapore*, 4, 143–156.
- Lin, H. C., Li, S. H., Fong, J., & Lin, S. M. (2008). Ventral coloration differentiation and mitochondrial sequences of the Chinese Cobra (*Naja atra*) in Taiwan. *Conservation Genetics*, 9(5), 1089–1097.
- Lindstedt, C., Lindström, L., & Mappes, J. (2008). Hairiness and warning colours as components of antipredator defence: additive or interactive benefits? *Animal Behaviour*, 75(5), 1703–1713.
- Mänd, T., Tammaru, T., & Mappes, J. (2007). Size dependent predation risk in cryptic and conspicuous insects. *Evolutionary Ecology*, 21(4), 485–498.
- Mappes, J., Marples, N., & Endler, J. A. (2005). The complex business of survival by aposematism. *TRENDS in Ecology and Evolution*, 20(11), 598–603.
- Marais, J. (2005). *A Complete Guide to the Snakes of Southern Africa* (2nd ed.). Cape Town, South Africa: Struik Publishers.
- Marples, N. M., van Veelen, W., & Brakefield, P. M. (1994). The relative importance of colour, taste and smell in the protection of an aposematic insect *Coccinella septempunctata*. *Animal Behaviour*, 48(4), 967–974.
- Masataka, N., Hayakawa, S., & Kawai, N. (2010). Human young children as well as adults demonstrate “superior” rapid snake detection when typical striking posture is displayed by the snake. *PLoS ONE*, 5(11), 2–6.
- McCue, M. D. (2006). Cost of Producing Venom in Three North American Pitviper Species. *Copeia*, (4), 818–825.
- Mukherjee, A. K., & Maity, C. R. (1998). The composition of *Naja naja* venom samples from three districts of West Bengal, India. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 119(2), 621–627.
- Nasoori, A., Shahbazzadeh, D., Tsubota, T., & Young, B. A. (2016). The defensive behaviour of *Naja oxiana*, with comments on the visual displays of cobras. *Herpetological Bulletin*, 138, 13–17.
- Nasoori, A., Taghipour, A., Shahbazzadeh, D., Aminirissehei, A., & Moghaddam, S. (2014). Heart place and tail length evaluation in *Naja oxiana*, *Macrovipera lebetina*, and *Montivipera latifii*. *Asian Pacific Journal of Tropical Medicine*, 7(S1), S137–S142.
- Neill, W. T. (1964). Viviparity in Snakes: Some Ecological and Zoogeographical Considerations. *The American Naturalist*, 98(898), 35–55.

- Nilson, G., & Rastegar-Pouyani, N. (2007). *Walterinnesia aegyptia* Lataste, 1887 (Ophidia: Elapidae) and the status of *Naja morgani* Mocquard 1905. *Russian Journal of Herpetology*, 14(1), 7–14.
- O'Shea, M. (2008). *Venomous snakes of the world*. New Holland Publishers.
- Orme, D. (2013). The caper package: comparative analysis of phylogenetics and evolution in R. R Package Version 0.5- 2.
- Panagides, N., Jackson, T.N., Ikononopoulou, M.P., Arbuckle, K., Pretzler, R., Yang, D.C., Ali, S.A., Koludarov, I., Dobson, J., Sanker, B., Asselin, A., Santana, R., Hendriks, I., van der Ploeg, H., Tai-A-Pin, J., van den Bergh, R., Kerckamp, H., Vonk, F., Naude, A., Strydom, M., Jacobsz, L., Dunstan, N., Jaeger, M., Hodgson, W., Miles, J., & Fry, B. (2017). How the Cobra Got Its Flesh-Eating Venom: Cytotoxicity as a Defensive Innovation and Its Co-Evolution with Hooding, Aposematic Marking, and Spitting. *Toxins*, 9(3), 103.
- Paradis, E. (2012). *Analysis of Phylogenetics and Evolution with R* (2nd ed.). New York: Springer-Verlag.
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20(2), 289–290.
- Pintor, A. F. V., Krockenberger, A. K., & Seymour, J. E. (2010). Costs of venom production in the common death adder (*Acanthophis antarcticus*). *Toxicon*, 56(6), 1035–1042.
- Pintor, A. F. V., Winter, K. L., Krockenberger, A. K., & Seymour, J. E. (2011). Venom physiology and composition in a litter of Common Death Adders (*Acanthophis antarcticus*) and their parents. *Toxicon*, 57(1), 68–75.
- Ponder, W. F., Carter, G. A., Flemons, P., & Chapman, R. R. (2001). Evaluation of museum collection data for use in biodiversity assessment. *Conservation Biology*, 15(3), 648–657.
- Popescu, A. A., Huber, K. T., & Paradis, E. (2012). Ape 3.0: New tools for distance-based phylogenetics and evolutionary analysis in R. *Bioinformatics*, 28(11), 1536–1537.
- Radcliffe, C. W., Estep, K., Boyer, T., & Chiszar, D. (1986). Stimulus control of predatory behaviour in red spitting cobras (*Naja mossambica pallida*) and prairie rattlesnakes (*Crotalus v. viridis*). *Animal Behaviour*, 34(3), 804–814.
- Ramakrishnan, U., Coss, R. G., Schank, J., Dharawat, A., & Kim, S. (2005). Snake species discrimination by wild bonnet macaques (*Macaca radiata*). *Ethology*, 111(4), 337–356.
- Rasmussen, S., Young, B., & Krimm, H. (1995). On the “spitting” behaviour in cobras (Serpentes: Elapidae). *Journal of Zoology (London)*, 237(1), 27–35.
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3(2), 217–223.
- Revell, L. J. (2013). Two new graphical methods for mapping trait evolution on phylogenies. *Methods in Ecology and Evolution*, 4(8), 754–759.
- Roper, T. J. (1990). Responses of domestic chicks to artificially coloured insect prey: effects of previous experience and background colour. *Animal Behaviour*, 39(3), 466–473.
- Roth, E. D., & Johnson, J. A. (2004). Size-based variation in antipredator behavior within a snake (*Agkistrodon piscivorus*) population. *Behavioral Ecology*, 15(2), 365–370.

- Rowe, C. (2002). Sound improves visual discrimination learning in avian predators. *Proceedings of the Royal Society B: Biological Sciences*, 269(1498), 1353–1357.
- Ruxton, G. D., Sherratt, T. N., & Speed, M. P. (2004). *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. Oxford University Press.
- Ruxton, G. D., Speed, A. M. P., Broom, A. M., Speed, M. P., & Broom, M. (2009). Identifying the ecological conditions that select for intermediate levels of aposematic signalling. *Evolutionary Ecology*, 23(4), 491–501.
- Santra, V., & Wüster, W. (2017). *Naja kaouthia* (Monocled Cobra). Behavior / spitting. *Herpetological Review*, 48(2), 455–456.
- Scharf, I., Feldman, A., Novosolov, M., Pincheira-Donoso, D., Das, I., Böhm, M., Uetz, P., Torres-Carvajal, O., Bauer, A., Roll, U., & Meiri, S. (2015). Late bloomers and baby boomers: ecological drivers of longevity in squamates and the tuatara. *Global Ecology and Biogeography*, 24(4), 396–405.
- Schieffelin, C. D., & De Queiroz, A. (1991). Temperature and defense in the common garter snake: warm snakes are more aggressive than cold snakes. *Herpetologica*, 47(2), 230–237.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682.
- Schindelin, J., Rueden, C. T., Hiner, M. C., & Eliceiri, K. W. (2015). The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular Reproduction and Development*, 82(7–8), 518–529.
- Seigel, R. A., Huggins, M. M., & Ford, N. B. (1987). Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia*, 73(4), 481–485.
- Shine, R. (1994). Allometric Patterns in the Ecology of Australian Snakes. *Copeia*, 1994(4), 851–867.
- Shine, R., Branch, W. R., Webb, J. K., Harlow, P. S., Shine, T., Keogh, J. S., & Shine, C. R. (2007). Ecology of cobras from southern Africa. *Journal of Zoology*, 272(2), 183–193.
- Shine, R., Sun, L. X., Fitzgerald, M., & Kearney, M. (2002). Antipredator responses of free-ranging pit vipers (*Gloydius shedaoensis*, Viperidae). *Copeia*, 2002(3), 843–850.
- Skelhorn, J., Halpin, C. G., & Rowe, C. (2016). Learning about aposematic prey. *Behavioral Ecology*, 27(4), 955–964.
- Slowinski, J. B., & Wüster, W. (2000). A new cobra (Elapidae : *Naja*) from Myanmar (Burma). *Herpetologica*, 56(2), 257–270.
- Smith, M. T., Ortega, J., & Beaupre, S. J. (2014). Metabolic cost of venom replenishment by Prairie Rattlesnakes (*Crotalus viridis viridis*). *Toxicon*, 86, 1–7.
- Smith, S. M. (1975). Innate recognition of coral snake pattern by a possible avian predator. *Science*, 187(4178), 759–760.
- Smith, S. M. (1977). Coral-snake pattern recognition and stimulus generalisation by naive great kiskadees (Aves: Tyrannidae). *Nature*, 265(5594), 535–536.

- Speed, M. P., Brockhurst, M. A., & Ruxton, G. D. (2010). The dual benefits of aposematism: Predator avoidance and enhanced resource collection. *Evolution*, 64(6), 1622–1633.
- Speed, M. P., & Ruxton, G. D. (2007). How bright and how nasty: explaining diversity in warning signal strength. *Evolution*, 61(3), 623–635.
- Spinner, M., Kovalev, A., Gorb, S. N., & Westhoff, G. (2013). Snake velvet black: Hierarchical micro- and nanostructure enhances dark colouration in *Bitis rhinoceros*. *Scientific Reports*, 3(1), 1846.
- Stevens, M. (2005). The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biological Reviews*, 80(4), 573–588.
- Stevens, M., Hardman, C. J., & Stubbins, C. L. (2008). Conspicuousness, not eye mimicry, makes “eyesspots” effective antipredator signals. *Behavioral Ecology*, 19(3), 525–531.
- Stevens, M., & Ruxton, G. D. (2012). Linking the evolution and form of warning coloration in nature. *Proceedings of the Royal Society B: Biological Sciences*, 279(1728), 417–426.
- Symonds, M. R., & Blomberg, S. P. (2014). A primer on phylogenetic generalised least squares. In *Modern phylogenetic comparative methods and their application in evolutionary biology* (pp. 105–130). Heidelberg, Berlin: Springer.
- Theakston, R. D. G., Phillips, R. E., Warrell, D. A., Galagedera, Y., Abeysekera, D. T. D. J., Dissanayaka, P., de Silva, A., & Aloysius, D. J. (1990). Envenoming by the common krait (*Bungarus caeruleus*) and Sri lankan cobra (*Naja naja naja*): Efficacy and complications of therapy with haffkine antivenom. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 84(2), 301–308.
- Trape, J. F., Chirio, L., Broadley, D. G., & Wüster, W. (2009). Phylogeography and systematic revision of the Egyptian cobra (Serpentes: Elapidae: *Naja haje*) species complex, with the description of a new species from West Africa. *Zootaxa*, 2236, 1–25.
- Tung Ho, L. S., & Ané, C. (2014). A linear-time algorithm for gaussian and non-gaussian trait evolution models. *Systematic Biology*, 63(3), 397–408.
- Umbers, K. D. L., & Mappes, J. (2016). Towards a tractable working hypothesis for deimatic displays. *Animal Behaviour*, 113, e5–e7.
- Valkonen, J. K., Nokelainen, O., & Mappes, J. (2011). Antipredatory function of head shape for vipers and their mimics. *PLoS ONE*, 6(7).
- Valkonen, J. K., Nokelainen, O., Niskanen, M., Kilpimaa, J., Björklund, M., & Mappes, J. (2012). Variation in predator species abundance can cause variable selection pressure on warning signaling prey. *Ecology and Evolution*, 2(8), 1971–1976.
- von Plettenberg Laing, A. (2017). *A multilocus phylogeny of the cobra clade elapids*. Masters of Science by Research thesis, Bangor University, UK.
- Wallach, V., Wüster, W., & Broadley, D. G. (2009). In praise of subgenera: Taxonomic status of cobras of the genus *Naja* Laurenti (Serpentes: Elapidae). *Zootaxa*, 36(2236), 26–36.
- Wang, C. H., Liu, J. H., Lee, S. C., Hsiao, C. D., & Wu, W. G. (2006). Glycosphingolipid-facilitated membrane insertion and internalization of cobra cardiotoxin. The sulfatide-cardiotoxin complex structure in a membrane-like environment suggests a lipid-dependent cell-penetrating mechanism for membrane binding polypeptides. *Journal of Biological Chemistry*, 281(1), 656–667.

- Watt, G., Padre, L., Tuazon, M. L., & Hayes, C. G. (1987). Bites by the Philippine cobra (*Naja naja philippinensis*): an important cause of death among rice farmers. *The American Society of Tropical Medicine and Hygiene*, 37(3), 636–639.
- Wcislo, W. T. (1989). Behavioral environments and evolutionary change. *Annual Review of Ecology and Systematics*, 20, 137–169.
- Westhoff, G., Tzschätzsch, K., & Bleckmann, H. (2005). The spitting behavior of two species of spitting cobras. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 191(10), 873–881.
- Whitaker, P. B., Ellis, K., & Shine, R. (2000). The defensive strike of the Eastern Brownsnake, *Pseudonaja textilis* (Elapidae). *Functional Ecology*, 14(1), 25–31.
- Whitaker, R., & Captain, A. (2004). *Snakes of India*. Chengalpattu, India: Draco Books.
- Wüster, W. (1990). *Population Evolution of the Asiatic Cobra (Naja naja) Species Complex*. Doctoral Thesis. University of Aberdeen, UK.
- Wüster, W. (1996). Taxonomic changes and toxicology: systematic revisions of the Asiatic cobras (*Naja naja* species complex). *Toxicon*, 34(4), 399–406.
- Wüster, W., & Broadley, D. G. (2003). A new species of spitting cobra (*Naja*) from north-eastern Africa (Serpentes: Elapidae). *Journal of Zoology*, 259(4), 345–359.
- Wüster, W., Crookes, S., Ineich, I., Mane, Y., Pook, C. E., Trape, J. F., & Broadley, D. G. (2007). The phylogeny of cobras inferred from mitochondrial DNA sequences: Evolution of venom spitting and the phylogeography of the African spitting cobras (Serpentes: Elapidae: *Naja nigricollis* complex). *Molecular Phylogenetics and Evolution*, 45(2), 437–453.
- Wüster, W., & Thorpe, R. S. (1992a). Asiatic Cobras: Population Systematics of the *Naja naja* Species Complex (Serpentes: Elapidae) in India and Central Asia. *Herpetologica*, 48(1), 117–128.
- Wüster, W., & Thorpe, R. S. (1992b). Dentitional Phenomena In Cobras Revisited: Spitting and Fang Structure In The Asiatic Species of *Naja* (Serpentes: Elapidae). *Herpetologica*, 48(4), 424–434.
- Wüster, W., & Thorpe, R. S. (1994). *Naja siamensis*, a cryptic species of venomous snake revealed by mtDNA sequencing. *Experientia*, 50, 75–79.
- Wüster, W., Warrell, D. A., Cox, M. J., Jintakune, P., & Nabhitabhata, J. (1997). Redescription of *Naja siamensis* (Serpentes: Elapidae), a widely overlooked spitting cobra from SE Asia: geographic variation, medical importance and designation of a neotype. *Journal of Zoology (London)*, 243, 771–788.
- Young, B. A., Dunlap, K., Koenig, Kristen., & Singer, M. (2004). The buccal buckle: the functional morphology of venom spitting in cobras. *The Journal of Experimental Biology*, 207(20), 3483–3493.
- Young, B. A., Boetig, M., & Westhoff, G. (2009). Functional bases of the spatial dispersal of venom during cobra “spitting”. *Physiological and Biochemical Zoology*, 82(1), 80–9.
- Young, B. A., & Kardong, K. V. (2010). The functional morphology of hooding in cobras. *The Journal of Experimental Biology*, 213(9), 1521–1528.
- Zinner, H. (1971). On ecology and the significance of semantic coloration in the nocturnal desert-elapid *Walterinnesia aegyptia* Lataste (Reptiles, Ophidia). *Oecologia*, 7(3), 267–275.

6.0 Supplementary Information

Supplementary Table 1. All specimens used for x-rays and x-ray conditions. Catalogue numbers refer to the British Museum of Natural History (BMNH). Hgr- half-grown.

Species	Subgenus	Catalogue number	Sex	Age	X-ray conditions
<i>Aspidelaps lubricus</i>		1863.2.21.89	Female	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps lubricus</i>		1878.10.12.26	Male	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps lubricus</i>		1867.5.20.1	Male	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps lubricus</i>		1903.4.27.59	Male	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps lubricus</i>		46.6.18.41	Female	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps lubricus</i>		46.6.18.42	Male	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps cowlesi</i>		1937.12.3.148	Male	hgr	25kv, 12ma, 2mins
<i>Aspidelaps scutatus</i>		1936.8.1.730	Female	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps scutatus</i>		1936.8.1.732	Female	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps scutatus</i>		1936.6.13.8	Female	Adult	25 kv 12 ma 1.5mins
<i>Aspidelaps scutatus</i>		1899.3.20.15	Female	Adult	25 kv 12 ma 1.5mins
<i>Bungarus caeruleus</i>		1869.8.28.82	Male	hgr	25kv, 12ma, 2mins
<i>Bungarus caeruleus</i>		1869.8.28.83	Male	hgr	25kv, 12ma, 2mins
<i>Bungarus caeruleus</i>		1891.9.1.6	Female	hgr	25kv, 12ma, 2mins
<i>Bungarus caeruleus</i>		1907.2.14.46	Female	hgr	25kv, 12ma, 2mins
<i>Bungarus caeruleus</i>		1907.2.14.47	Female	hgr	25kv, 12ma, 2mins
<i>Bungarus fasciatus</i>		1930.12.2.14	Male	hgr	25 kv 12 ma 2mins
<i>Bungarus fasciatus</i>		1969.1923	Male	hgr	25 kv 12 ma 2mins
<i>Bungarus fasciatus</i>		1865.4.28.4	Male	hgr	30 kv 12 ma 1.5mins
<i>Bungarus fasciatus</i>		1878.2.14.3	Female	Adult	30 kv 12 ma 1.5mins
<i>Dendroaspis angusticeps</i>		1959.1.8.56	Male	hgr	25kv, 12ma, 2mins
<i>Dendroaspis angusticeps</i>		1959.1.2.24	Female	hgr	25kv, 12ma, 2mins
<i>Dendroaspis angusticeps</i>		1940.2.22.88	Female	hgr	25kv, 12ma, 2mins
<i>Dendroaspis angusticeps</i>		1964.1625	-	Adult	25kv, 12ma, 2mins
<i>Dendroaspis jamesoni</i>		1936.8.1.729	Female	hgr	25kv, 12ma, 2mins
<i>Dendroaspis jamesoni</i>		1865.5.3.1	Female	hgr	25kv, 12ma, 2mins
<i>Dendroaspis jamesoni</i>		1900.2.17.30	Male	hgr	25kv, 12ma, 2mins
<i>Dendroaspis jamesoni</i>		1899.6.26.13	Female	hgr	25kv, 12ma, 2mins
<i>Dendroaspis jamesoni</i>		1886.12.31.3	Male	hgr	25kv, 12ma, 2mins

<i>Dendroaspis jamesoni</i>		1933.9.8.39	Male	hgr	25kv, 12ma, 2mins
<i>Dendroaspis polylepis</i>		1959.1.3.64	Male	hgr	25 kv 12 ma 1.5mins
<i>Dendroaspis polylepis</i>		1905.11.7.57	Male	Adult	25 kv 12 ma 1.5mins
<i>Dendroaspis polylepis</i>		1893.11.21.68	Male	hgr	25 kv 12 ma 1.5mins
<i>Dendroaspis viridis</i>		1960.1.5.46	Male	hgr	25kv, 12ma, 2mins
<i>Dendroaspis viridis</i>		1960.1.5.45	Male	hgr	25kv, 12ma, 2mins
<i>Dendroaspis viridis</i>		1960.1.5.44	Female	hgr	25kv, 12ma, 2mins
<i>Dendroaspis viridis</i>		1845.10.6.3	Male	hgr	25kv, 12ma, 2mins
<i>Dendroaspis viridis</i>		1849.10.99	Female	hgr	25kv, 12ma, 2mins
<i>Elapsoidea boulengeri</i>		1978.589	Female	hgr	25 kv 12 ma 1.5mins
<i>Elapsoidea boulengeri</i>		1978.59	Male	hgr	25 kv 12 ma 1.5mins
<i>Elapsoidea boulengeri</i>		1978.591	Female	hgr	25 kv 12 ma 1.5mins
<i>Elapsoidea boulengeri</i>		1984.86	Female	hgr	25 kv 12 ma 1.5mins
<i>Elapsoidea boulengeri</i>		1950.1.3.52	Female	hgr	25 kv 12 ma 1.5mins
<i>Elapsoidea sundevalli</i>		1907.4.17.79	Male	Adult	25kv, 12ma, 2mins
<i>Elapsoidea sundevalli</i>		1897.9.2.7	Male	Adult	25kv, 12ma, 2mins
<i>Elapsoidea sundevalli</i>		1896.10.6.6	Female	Adult	25kv, 12ma, 2mins
<i>Hemachatus haemachatus</i>		1964.162	Male	Adult	30 kv 12 ma 1.5mins
<i>Hemachatus haemachatus</i>		1964.1621	f? no hemipenes	Adult	25 kv 12 ma 1.5mins
<i>Hemachatus haemachatus</i>		1920.1.20.270	Female	hgr	25 kv 12 ma 1.5mins
<i>Hemachatus haemachatus</i>		1891.5.6.1	Female	hgr	25 kv 12 ma 1.5mins
<i>Hemibungarus calligaster</i>		1872.10.11.13	Male	hgr	25 kv 12 ma 1.5mins
<i>Hemibungarus calligaster</i>		1872.10.11.14	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja ashei</i>	<i>Afronaja</i>	1916.6.24.14	Female	hgr	30 kv 12 ma 2mins
<i>Naja ashei</i>	<i>Afronaja</i>	1954.1.12.46	Female	hgr	30 kv 12 ma 2mins
<i>Naja ashei</i>	<i>Afronaja</i>	1973.3274	Female	hgr	30 kv 12 ma 2mins
<i>Naja katiensis</i>	<i>Afronaja</i>	1962.598	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja katiensis</i>	<i>Afronaja</i>	1975.1146	-	Adult	25 kv 12 ma 1.5mins
<i>Naja katiensis</i>	<i>Afronaja</i>	1962.1829	Male	Adult	25 kv 12 ma 2mins
<i>Naja katiensis</i>	<i>Afronaja</i>	1962.183	Female	Adult	25 kv 12 ma 2mins
<i>Naja katiensis</i>	<i>Afronaja</i>	1962.1831	-	hgr	25 kv 12 ma 2mins
<i>Naja katiensis</i>	<i>Afronaja</i>	1962.146	Juvenile	yg	25 kv 12 ma 2mins
<i>Naja mossambica</i>	<i>Afronaja</i>	1947.1.2.343-5	Female	hgr	25 kv 12 ma 2mins

<i>Naja mossambica</i>	<i>Afronaja</i>	1940.1.18.82	Male	Adult	25 kv 12 ma 1.5mins
<i>Naja mossambica</i>	<i>Afronaja</i>	95.12.12.6	Male	Adult	25 kv 12 ma 1.5mins
<i>Naja mossambica</i>	<i>Afronaja</i>	64.10.29.11	Female	Adult	25 kv 12 ma 2mins
<i>Naja mossambica</i>	<i>Afronaja</i>	1906.11.22.19	Female	Adult	25 kv 12 ma 2mins
<i>Naja mossambica</i>	<i>Afronaja</i>	1940.1.18.81	Male	Adult	25 kv 12 ma 2mins
<i>Naja nigricollis</i>	<i>Afronaja</i>	1975.668	-	hgr	25 kv 12 ma 1.5mins
<i>Naja nigricollis</i>	<i>Afronaja</i>	1975.1	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja nigricollis</i>	<i>Afronaja</i>	1926.5.8.52	Male	Adult	30 kv 12 ma 1.5mins
<i>Naja nigricollis</i>	<i>Afronaja</i>	99.6.9.142	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja nigricollis</i>	<i>Afronaja</i>	1905.10.27.18	Female	yg/hgr	25 kv 12 ma 1.5mins
<i>Naja nubiae</i>	<i>Afronaja</i>	1911.7.15.11	Female	Adult	30 kv 12 ma 2mins
<i>Naja nubiae</i>	<i>Afronaja</i>	97.10.28.615	Female	Adult	30 kv 12 ma 2mins
<i>Naja nubiae</i>	<i>Afronaja</i>	1959.1.5.28	Female	hgr	25 kv 12 ma 2mins
<i>Naja pallida</i>	<i>Afronaja</i>	1932.5.2.105	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja pallida</i>	<i>Afronaja</i>	1900.11.29.4	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja pallida</i>	<i>Afronaja</i>	1905.11.7.55	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja pallida</i>	<i>Afronaja</i>	1954.1.12.90	Male	Adult	25 kv 12 ma 1.5mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1900.2.17.27	Juvenile	Juvenile	25 kv 12 ma 1.5mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1908.5.25.25	Female	Female	30 kv 12 ma 2mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1975.1583	-	hgr	30 kv 12 ma 2mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1908.5.25.25	Female	Female	30 kv 12 ma 2mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1906.12.31.5	Male	hgr	30 kv 12 ma 2mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1953.1.10.90	Male	Adult	30 kv 12 ma 2mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1940.3.8.45	Male	Juvenile	25 kv 12 ma 1.5mins
<i>Naja annulata</i>	<i>Boulengerina</i>	1959.1.2.12	-	Adult	30 kv 12 ma 1.5mins
<i>Naja christyi</i>	<i>Boulengerina</i>	1975.1584	Male	Adult	25 kv 12 ma 1.5mins
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1960.1.3.72	Male	Adult	30 kv 12 ma 2mins
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1958.1.1.51	Male	Juvenile	30 kv 12 ma 2mins
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1960.1.5.43	Male	Juvenile	25 kv 12 ma 1.5mins
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1968.49	Male	Adult	25 kv 12 ma 2mins
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1968.5	Male	Adult	25 kv 12 ma 2mins
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1919.8.16.97	Male	hgr	25 kv 12 ma 2mins
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1959.1.7	Male	Adult	25 kv 12 ma 2mins
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1968.51	Female	Adult	30 kv 12 ma 2mins

<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1967.153	Female	hgr/a	28 kv 12 ma 1.5mins
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1967.154	Female	hgr/a	28 kv 12 ma 1.5mins
<i>Naja multifasciata</i>	<i>Boulengerina</i>	1907.5.22.61	Female	yg	25 kv 12 ma 1.5mins
<i>Naja melanoleuca (West African banded form)</i>	<i>Boulengerina</i>	1968.606	Female	Adult	25 kv 12 ma 1.5mins
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.69	Male	Adult	25 kv 12 ma 2mins
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.54	Male	Adult	25 kv 12 ma 2mins
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.57	Male	Adult	25 kv 12 ma 2mins
<i>Naja subfulva</i>	<i>Boulengerina</i>	1934.12.15.602	Male	Adult	28 kv 12 ma 2mins
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.55	Male	Adult	25 kv 12 ma 1.5mins
<i>Naja subfulva</i>	<i>Boulengerina</i>	1954.1.12	Female	Adult	25 kv 12 ma 1.5mins
<i>Naja subfulva</i>	<i>Boulengerina</i>	1930.6.11.55	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja peroescobari</i>	<i>Boulengerina</i>	1906.3.30.80	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja atra</i>	<i>Naja</i>	1953.1.2.82	Female	Adult	30 kv 12 ma 2mins
<i>Naja atra</i>	<i>Naja</i>	1953.1.2.83	Male	Adult	30 kv 12 ma 2mins
<i>Naja atra</i>	<i>Naja</i>	1956.1.13.11	Female	Adult	30 kv 12 ma 2mins
<i>Naja atra</i>	<i>Naja</i>	1956.1.13.12	Male	Adult	30 kv 12 ma 2mins
<i>Naja atra</i>	<i>Naja</i>	1983.271	Female	Adult	25 kv 12 ma 2mins
<i>Naja atra</i>	<i>Naja</i>	1983.272	Female	Adult	25 kv 12 ma 2mins
<i>Naja kaouthia</i>	<i>Naja</i>	1940.6.5.64	Female	Adult	30kv, 12ma, 1.5mins
<i>Naja kaouthia</i>	<i>Naja</i>	1940.6.5.65	Male	hgr	25kv, 12ma, 2mins
<i>Naja kaouthia</i>	<i>Naja</i>	1987.654	Male	Adult	25kv, 12ma, 2mins
<i>Naja kaouthia</i>	<i>Naja</i>	unknown- India	Male	Adult	25kv, 12ma, 1.5mins
<i>Naja kaouthia</i>	<i>Naja</i>	1987.694	Female	Adult	30 kv 12 ma 2mins
<i>Naja kaouthia</i>	<i>Naja</i>	1987.656	Male	Adult	30 kv 12 ma 2mins
<i>Naja kaouthia</i>	<i>Naja</i>	1905.2.7.9	Female	hgr	25 kv 12 ma 2mins
<i>Naja kaouthia</i>	<i>Naja</i>	1900.9.20.17	Female	Adult	25 kv 12 ma 2mins
<i>Naja kaouthia</i>	<i>Naja</i>	1987.2219	Male	Adult	30kv, 12ma, 1.5mins
<i>Naja kaouthia</i>	<i>Naja</i>	1987.652	Female	Adult	25kv, 12ma, 2mins
<i>Naja miolepis</i>	<i>Naja</i>	1965.265	Female	hgr	25 kv 12 ma 2mins
<i>Naja miolepis</i>	<i>Naja</i>	1965.262	Male	hgr	25 kv 12 ma 2mins
<i>Naja miolepis</i>	<i>Naja</i>	1965.263	Male	hgr	25 kv 12 ma 2mins
<i>Naja miolepis</i>	<i>Naja</i>	1912.6.26.18	Male	hgr	25 kv 12 ma 2mins
<i>Naja miolepis</i>	<i>Naja</i>	1912.6.26.19	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja miolepis</i>	<i>Naja</i>	1933.6.20.31	Female	hgr	25 kv 12 ma 1.5mins

<i>Naja naja</i>	<i>Naja</i>	1860.3.19.1442	Male	Adult	25kv, 12ma, 2mins
<i>Naja naja</i>	<i>Naja</i>	1883.8.2.28	Male	Adult	25kv, 12ma, 2mins
<i>Naja naja</i>	<i>Naja</i>	1860.3.19.1105	Female	Adult	25kv, 12ma, 1.5mins
<i>Naja naja</i>	<i>Naja</i>	1860.3.19.1105	Female	hgr	25kv, 12ma, 1.5mins
<i>Naja naja</i>	<i>Naja</i>	1987.706	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja naja</i>	<i>Naja</i>	1987.707	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja naja</i>	<i>Naja</i>	1913.2.7.3	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja naja</i>	<i>Naja</i>	1913.2.7.4	Female	Juvenile	25 kv 12 ma 1.5mins
<i>Naja naja</i>	<i>Naja</i>	1924.10.13.23	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja naja</i>	<i>Naja</i>	1972.2198 (2197?)	Female	hgr	30 kv 12 ma 2mins
<i>Naja naja</i>	<i>Naja</i>	1901.1.30.106	Female	hgr	25kv, 12ma, 2mins
<i>Naja oxiana</i>	<i>Naja</i>	80.3.15.1	Male	Adult	25 kv 12 ma 2mins
<i>Naja oxiana</i>	<i>Naja</i>	1910.1.4.7	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja oxiana</i>	<i>Naja</i>	1910.1.4.8	Male	Juvenile	25 kv 12 ma 1.5mins
<i>Naja oxiana</i>	<i>Naja</i>	1888.5.25.30	Female	hgr	25kv, 12ma, 2mins
<i>Naja oxiana</i>	<i>Naja</i>	1886.9.21.11.8	Female	Adult	25kv, 12ma, 2mins
<i>Naja sagittifera</i>	<i>Naja</i>	1940.3.9.12	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja samarensis</i>	<i>Naja</i>	77.10.9.65	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja siamensis</i>	<i>Naja</i>	1987.636	Male	Adult	25 kv 12 ma 2mins
<i>Naja siamensis</i>	<i>Naja</i>	1987.635	Male	Adult	25 kv 12 ma 2mins
<i>Naja siamensis</i>	<i>Naja</i>	1987.672	Male	hgr	25 kv 12 ma 2mins
<i>Naja siamensis</i>	<i>Naja</i>	1987.634	-	Adult	25 kv 12 ma 2mins
<i>Naja sputatrix</i>	<i>Naja</i>	97.12.30.23	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja sputatrix</i>	<i>Naja</i>	97.6.21.60	Male	yg	25 kv 12 ma 1.5mins
<i>Naja sumatrana</i>	<i>Naja</i>	1920.1.16.23	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja sumatrana</i>	<i>Naja</i>	1912.2.22.23	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja sumatrana</i>	<i>Naja</i>	1912.2.22.22	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja sumatrana</i>	<i>Naja</i>	89.12.26.17	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja sumatrana</i>	<i>Naja</i>	80.9.10.8	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja sumatrana</i>	<i>Naja</i>	89.12.26.16	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja annulifera</i>	<i>Uraeus</i>	1960.1.6.75	Juvenile	Juvenile	25 kv 12 ma 2mins
<i>Naja annulifera</i>	<i>Uraeus</i>	1908.5.20.14	Juvenile	Juvenile	25 kv 12 ma 2mins
<i>Naja annulifera</i>	<i>Uraeus</i>	1907.4.17.81	Female	Adult	30 kv 12 ma 2mins
<i>Naja arabica</i>	<i>Uraeus</i>	1976.1487	Female	Adult	25 kv 12 ma 2mins

<i>Naja arabica</i>	<i>Uraeus</i>	1931.7.16.73	Female	Adult	25 kv 12 ma 2mins
<i>Naja arabica</i>	<i>Uraeus</i>	1988.313	Juvenile	Juvenile	25 kv 12 ma 2mins
<i>Naja arabica</i>	<i>Uraeus</i>	1979.721	Female	hgr	25 kv 12 ma 2mins
<i>Naja arabica</i>	<i>Uraeus</i>	1903.6.26.46	Male	hgr	25 kv 12 ma 2mins
<i>Naja arabica</i>	<i>Uraeus</i>	1903.6.26.47	Male	hgr	25 kv 12 ma 2mins
<i>Naja haje</i>	<i>Uraeus</i>	97.10.28.618	yg	yg	25 kv 12 ma 1.5mins
<i>Naja haje</i>	<i>Uraeus</i>	1975.654	Female	hgr	25 kv 12 ma 1.5mins
<i>Naja haje</i>	<i>Uraeus</i>	1975.1145	-	hgr	30 kv 12 ma 1.5mins
<i>Naja haje</i>	<i>Uraeus</i>	97.10.28.622	Male	hgr	30 kv 12 ma 1.5mins
<i>Naja haje</i>	<i>Uraeus</i>	1975.652	Female	Adult	30 kv 12 ma 2mins
<i>Naja haje</i>	<i>Uraeus</i>	1962.1833	Male	Adult	30 kv 12 ma 2mins
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.26	Male	hgr	25 kv 12 ma 1.5mins
<i>Naja nivea</i>	<i>Uraeus</i>	1902.1.25.4	Male	Adult	25 kv 12 ma 1.5mins
<i>Naja nivea</i>	<i>Uraeus</i>	1902.1.25.5	Male	Adult	25 kv 12 ma 1.5mins
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.23	Male	Adult	30 kv 12 ma 1.5mins
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.24	Male	hgr	25 kv 12 ma 1.5mins
<i>Ophiophagus hannah</i>		1983.274	Juvenile	Juvenile	25kv, 12ma, 1.5mins
<i>Ophiophagus hannah</i>		1938.8.7.60	Male	Juvenile	25kv, 12ma, 1.5mins
<i>Ophiophagus hannah</i>		1980.935	Female	Adult	30kv, 12ma, 1.5mins
<i>Ophiophagus hannah</i>		1907.12.16.21	Female	Adult	30kv, 12ma, 2mins
<i>Ophiophagus hannah</i>		1968.836	Juvenile	Juvenile	25kv, 12ma, 1.5mins
<i>Ophiophagus hannah</i>		1883.5.26.1	Male	Adult	30kv, 12ma, 1.5 mins
<i>Ophiophagus hannah</i>		1928.2.8.43	Female	Adult	30 kv 12 ma 1.5mins
<i>Ophiophagus hannah</i>		1864.4.7.12	Male	Adult	30 kv 12 ma 1.5mins
<i>Ophiophagus hannah</i>		1900.9.20.19	Female	Adult	30 kv 12 ma 1.5mins
<i>Ophiophagus hannah</i>		1925.6.26.21	Female	Adult	30 kv 12 ma 1.5mins
<i>Ophiophagus hannah</i>		1868.4.3.31	Female	Adult	30 kv 12 ma 1.5mins
<i>Ophiophagus hannah</i>		1995.452	Female	Adult	25 kv 12 ma 1.5mins
<i>Pseudohaje goldii</i>		1936.8.1.723	Female	Adult	30 kv 12 ma 1.5mins
<i>Pseudohaje goldii</i>		1930.6.11.52	Male	Adult	30 kv 12 ma 1.5mins
<i>Pseudohaje goldii</i>		1936.8.1.724	Female	Adult	30 kv 12 ma 1.5mins
<i>Pseudohaje goldii</i>		1950.1.2.10	Male	hgr	25 kv 12 ma 1.5mins
<i>Pseudohaje nigra</i>		1959.1.2.52	-	hgr	25 kv 12 ma 1.5mins
<i>Pseudohaje nigra</i>		1955.1.4.17	Male	Adult	30 kv 12 ma 1.5mins

<i>Walterinnesia aegyptia</i>	1892.6.28.1	Male	Adult	25kv, 12ma, 1.5mins
<i>Walterinnesia aegyptia</i>	1946.1.21.42	Female	Adult	25kv, 12ma, 1.5mins
<i>Walterinnesia aegyptia</i>	1930.12.1.7	Female	hgr	25 kv 12 ma 1.5mins
<i>Walterinnesia morgani</i>	1951.1.1.29	Male	hgr	25 kv 12 ma 1.5mins
<i>Walterinnesia morgani</i>	1951.1.1.30	Female	hgr	25 kv 12 ma 1.5mins
<i>Walterinnesia morgani</i>	1931.12.1.1	Female	hgr	25 kv 12 ma 1.5mins
<i>Walterinnesia morgani</i>	1935.2.1.33	Male	hgr	25 kv 12 ma 1.5mins

Supplementary Table 2. Specimen list of all specimens examined for pattern analysis in subgenera *Uraeus* and *Boulengerina*. Catalogue numbers refer to the British Museum of Natural History (BMNH). J- juvenile, A- adult, HGR- half-grown

Species	Subgenus	Catalogue number	Sex	Lifestage	Country	Locality
<i>Naja anchietae</i>	<i>Uraeus</i>	1906.8.24.77	M	J	Angola	Caconda, Benguela
<i>Naja anchietae</i>	<i>Uraeus</i>	1907.6.29.50-51	-	HGR	Angola	Ponang Kuma, Mossamedes
<i>Naja anchietae</i>	<i>Uraeus</i>	1931.2.3.4	F	A	Botswana	Mongalatsola, Ghanzi, Bechuanaland
<i>Naja annulifera</i>	<i>Uraeus</i>	1902.2.12.103	J	J	Rhodesia	Mazoe
<i>Naja annulifera</i>	<i>Uraeus</i>	1907.4.17.81	F	A	South Africa	Moelini, Zululand
<i>Naja annulifera</i>	<i>Uraeus</i>	1908.5.20.14	J	J	South Africa	Leydenburg district, Transvaal
<i>Naja annulifera</i>	<i>Uraeus</i>	1934.4.6.30	F	HGR	Mozambique	Charrq, Quelimane
<i>Naja annulifera</i>	<i>Uraeus</i>	1954.1.3.37	M	HGR	Rhodesia	Umtali, Southern Rhodesia
<i>Naja annulifera</i>	<i>Uraeus</i>	1954.1.3.38	J	J	Rhodesia	Umtali, Southern Rhodesia
<i>Naja annulifera</i>	<i>Uraeus</i>	1957.1.8.98	-	A	Rhodesia	Umtali, Southern Rhodesia
<i>Naja annulifera</i>	<i>Uraeus</i>	1960.1.6.74	M	HGR/A	Rhodesia	Bembesi, Southern Rhodesia
<i>Naja annulifera</i>	<i>Uraeus</i>	1960.1.6.75	J	J	Rhodesia	Bulawayo, Southern Rhodesia
<i>Naja annulifera</i>	<i>Uraeus</i>	1960.1.6.76	-	A	Rhodesia	Irisvale, Southern Rhodesia
<i>Naja arabica</i>	<i>Uraeus</i>	1962.995	M	HGR/A	Yemen	Kherba, Mukalla
<i>Naja arabica</i>	<i>Uraeus</i>	1962.996	-	J	Yemen	Mukeiras
<i>Naja arabica</i>	<i>Uraeus</i>	1962.997	-	J	Yemen	
<i>Naja arabica</i>	<i>Uraeus</i>	1976.1487	F	A	Oman	Wadi Darbat, Jabal Qara, Dhofar, 560ft
<i>Naja arabica</i>	<i>Uraeus</i>	1977.1198	M	J	Oman	Qadrafi, Jebel Qamar, Dhofar
<i>Naja arabica</i>	<i>Uraeus</i>	1979.721	F	HGR	Saudi Arabia	RRI Camp 5, Wadi Mahra, 1900m

<i>Naja arabica</i>	<i>Uraeus</i>	1982.1166	-	HGR	Yemen	Wadi Ahger, W. of Sanaa, N. Yemen
<i>Naja arabica</i>	<i>Uraeus</i>	1985.744	F	J	Saudi Arabia	An Numos, 2000m
<i>Naja arabica</i>	<i>Uraeus</i>	1985.745	-	A	Saudi Arabia	Dalaghan, 2000m
<i>Naja arabica</i>	<i>Uraeus</i>	1985.911	F	J	Saudi Arabia	Hyla (Km.17 Alka-Khamis Moshayt Road)
<i>Naja arabica</i>	<i>Uraeus</i>	1988.313	-	J	Yemen	Wadi Warazan, Taiz Province, N. Yemen
<i>Naja arabica</i>	<i>Uraeus</i>	1996.435	-	J	Oman	Ayn Razat, Dhofar
<i>Naja arabica</i>	<i>Uraeus</i>	1931.7.16.73	F	HGR	Oman	Khiyunt, 1750ft, Zara Mts, Dhufar
<i>Naja arabica</i>	<i>Uraeus</i>	1931.7.16.74	J	J	Saudi Arabia	Al Qatan, 2000ft, near Bu Matahan, Dhufar, S. Arabia
<i>Naja arabica</i>	<i>Uraeus</i>	1931.7.16.75	J	J	Oman	Ahayrkot, 450ft, Zara Mts, Dhufar
<i>Naja arabica</i>	<i>Uraeus</i>	1931.7.16.76	M	A	Oman	Zara Mts, 500ft, Dhufar
<i>Naja arabica</i>	<i>Uraeus</i>	1938.8.1.33-34	F	A	Yemen	Jebel Jehaf, Aden Protectorate
<i>Naja arabica</i>	<i>Uraeus</i>	1951.1.1.62	M	HGR	Saudi Arabia	Abha
<i>Naja haje</i>	<i>Uraeus</i>	1962.1833	M	A	Nigeria	Galtimore village, 3 miles south of Maiduguri, Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1964.1922	-	A	Kenya	Athi River
<i>Naja haje</i>	<i>Uraeus</i>	1966.161	-	J	Kenya	Athi River, near Nairobi
<i>Naja haje</i>	<i>Uraeus</i>	1975.1145	-	J	Nigeria	Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1975.651	F	A	Nigeria	Near Katsina, Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1975.652	F	A	Nigeria	Katsina area, Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1975.653	M	A	Nigeria	Near Katsina, Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1975.654	F	HGR	Nigeria	Near Katsina, Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1975.655	-	HGR	Nigeria	Katsina area, Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1975.656	-	A	Nigeria	Near Katsina, Northern Nigeria
<i>Naja haje</i>	<i>Uraeus</i>	1860.12.14.1	F	A	Morocco	
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.617	HGR	HGR	Egypt	Beltim, between Rosetta and Damietta
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.618	J	J	Egypt	Giza (below pyramids)
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.619	M	HGR	Egypt	Fayoum
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.620	F	HGR	Egypt	Fayoum
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.621	J	J	Egypt	Maryut
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.622	M	HGR	Egypt	Beni Hassan, Upper Egypt
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.623	F	A	Egypt	Beni Hassan, Lower Egypt
<i>Naja haje</i>	<i>Uraeus</i>	1897.10.28.624	M	HGR/A	Egypt	Tel-el-Amarna, Upper Egypt

<i>Naja haje</i>	<i>Uraeus</i>	1898.4.29.12	J	J	Somalia	Jifa-Uri, inland of Zeila, northeastern Somaliland
<i>Naja haje</i>	<i>Uraeus</i>	1913.2.24.21	M	A	Ethiopia	
<i>Naja haje</i>	<i>Uraeus</i>	1915.10.28.1	J	J	Algeria	Near Biskra
<i>Naja haje</i>	<i>Uraeus</i>	1920.1.20.1857	-	A	Tunisia	Raz-el-Aisum, between Gafsa and Tamaghze
<i>Naja haje</i>	<i>Uraeus</i>	1920.1.20.3056	-	A	Tunisia	Zarzis
<i>Naja haje</i>	<i>Uraeus</i>	1929.11.14.21	-	A	Uganda	Kaiso, E. shore of Lake Albert-Nyanza, Entebbe
<i>Naja haje</i>	<i>Uraeus</i>	1938.3.1.159	J	J	Nigeria	Gadua, N. Prov.
<i>Naja haje</i>	<i>Uraeus</i>	1938.3.1.160	J	J	Nigeria	Gadua, N. Prov.
<i>Naja haje</i>	<i>Uraeus</i>	1940.2.1.20	J	J	Nigeria	
<i>Naja haje</i>	<i>Uraeus</i>	1940.4.25.5	-	HGR	Sudan	Torit
<i>Naja haje</i>	<i>Uraeus</i>	1949.2.2.91	-	A	Somalia	Burao, British Somaliland, 3800ft
<i>Naja haje</i>	<i>Uraeus</i>	1949.2.2.92	F	HGR/A	Somalia	Borama district, British Somaliland, 4500ft
<i>Naja haje</i>	<i>Uraeus</i>	1951.1.5.38	J	J	Uganda	Lira Langa
<i>Naja haje</i>	<i>Uraeus</i>	1951.1.7.68	-	A	Somalia	Errigavo Town, British Somaliland
<i>Naja haje</i>	<i>Uraeus</i>	1956.1.6.57	-	HGR/A	Somalia	Errigavo, Somaliland
<i>Naja haje</i>	<i>Uraeus</i>	1960.1.3.25	-	J	Somalia	Gan-Libah, Somaliland
<i>Naja nivea</i>	<i>Uraeus</i>	1855.10.16.288	-	HGR		
<i>Naja nivea</i>	<i>Uraeus</i>	1888.4.19.3	M	A	South Africa	Port Elizabeth
<i>Naja nivea</i>	<i>Uraeus</i>	1890.2.26.14	M	A	South Africa	Simon's Bay
<i>Naja nivea</i>	<i>Uraeus</i>	1890.9.25.12	F	HGR	South Africa	Port Elizabeth
<i>Naja nivea</i>	<i>Uraeus</i>	1902.1.25.4	F	A	South Africa	Simons Town
<i>Naja nivea</i>	<i>Uraeus</i>	1902.1.25.5	M	A	South Africa	Simons Town
<i>Naja nivea</i>	<i>Uraeus</i>	1908.12.28.78	-	A	South Africa	
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.21	M	HGR	South Africa	
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.23	M	A	South Africa	
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.24	M	HGR	South Africa	
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.25	M	J	South Africa	
<i>Naja nivea</i>	<i>Uraeus</i>	1930.12.3.26	M	HGR	South Africa	
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1946.3.20.18	F	J	Ghana	Bunso
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1958.1.1.50	-	head	Sierra Leone	Bo

<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1958.1.1.51	M	J	Sierra Leone	Bo
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1960.1.3.72	M	A	Sierra Leone	Njala, Kori
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1960.1.5.42	M	J	Ghana	Kumasi
<i>Naja melanoleuca (West African black form)</i>	<i>Boulengerina</i>	1960.1.5.43	F	J	Ghana	Kumasi
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1901.3.12.103	M	J	D.R.C.	Umangi, Congo R.
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1906.5.28.20	M	J	Cameroon	Efulen
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1910.1.11.13	F	J	Nigeria	Oban, Calabar
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1919.8.16.97	M	HGR	D.R.C.	Bafwasende
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1930.6.11.54	F	HGR	D.R.C.	Ituri Forest
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1937.12.1.101	F	J	Cameroon	Lomie District
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1948.1.2.87	M	J	Nigeria	Umuahia
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1957.1.13.94	M	J	Cameroon	Kumba
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1962.267	M	A	Cameroon	Victoria
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1967.152	M	J	Cameroon	Bota
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1967.153	F	HGR	Cameroon	Bota
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1967.154	F	HGR	Cameroon	Bota
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1968.49	M	HGR	Cameroon	Bota
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1968.5	M	HGR	Cameroon	Bota
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1968.51a	M	A	Cameroon	Bota
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1969.525	F	-	Cameroon	Bota
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1971.409	F	-	Cameroon	Mamfe
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1971.41	F	J	Nigeria	Nko, Obubra
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1979.213	F	J	D.R.C.	Kinsuka
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	1979.214	M	J	D.R.C.	Kinsuka
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	88.8.29.19	M	-	Nigeria	Oil River
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	96.5.27.2	M	A	Cameroon	
<i>Naja melanoleuca (West African banded form)</i>	<i>Boulengerina</i>	1911.5.29.12	F	J	Ghana	"Gold Coast"
<i>Naja melanoleuca (West African banded form)</i>	<i>Boulengerina</i>	1968.606	F	HGR	Senegal	Casamance, Boughari
<i>Naja melanoleuca (West African banded form)</i>	<i>Boulengerina</i>	1975.657	M	J	Nigeria	Amadou Bello Uni Hospital, Zaria
<i>Naja subfulva</i>	<i>Boulengerina</i>	88.8.29.20	M	-	Nigeria	Oil River

<i>Naja subfulva</i>	<i>Boulengerina</i>	1908.10.20.21	M	HGR	Uganda	Mabira Forest
<i>Naja subfulva</i>	<i>Boulengerina</i>	1929.8.5.25	M	J	Uganda	Entebbe
<i>Naja subfulva</i>	<i>Boulengerina</i>	1930.6.11.55	F	HGR	Uganda	Wasa R., Semliki valley
<i>Naja subfulva</i>	<i>Boulengerina</i>	1934.12.15.602	M	HGR	Uganda	Mubango, Mabira Forest
<i>Naja subfulva</i>	<i>Boulengerina</i>	1951.1.5.35	M	J	Uganda	Lira, Lango
<i>Naja subfulva</i>	<i>Boulengerina</i>	1951.1.5.36	M	J	Uganda	Kome Island
<i>Naja subfulva</i>	<i>Boulengerina</i>	1951.1.5.37	M	J	Uganda	Katunguru, Kazinga Ch.
<i>Naja subfulva</i>	<i>Boulengerina</i>	1954.1.12.49	M	HGR	Uganda	Jinja
<i>Naja subfulva</i>	<i>Boulengerina</i>	1954.1.12.50	F	J	Uganda	Jinja
<i>Naja subfulva</i>	<i>Boulengerina</i>	1954.1.12.51a	M	J	Uganda	Jinja
<i>Naja subfulva</i>	<i>Boulengerina</i>	1954.1.12.51b	F	HGR	Uganda	Jinja
<i>Naja subfulva</i>	<i>Boulengerina</i>	1954.1.12.51c	M	J	Uganda	Jinja
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.52	F	J	Uganda	Budongo Forest, Bunyoro
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.54	M	HGR	Uganda	Kome Island, Lake Victoria
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.55	M	HGR	Uganda	Koianja, Lake Edward
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.57	M	HGR	Uganda	Katwe, Lake Edward
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.58	M	HGR	Uganda	Mubango, Mabira Forest
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.59	M	J	Uganda	Entebbe
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.60	F	J	Uganda	Mubango, Mabira Forest
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.62	F	J	Uganda	Namantama, Mbira Forest
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.63	M	-	Uganda	Kome Island, Lake Victoria
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.64	F	-	Uganda	Kome Island, Lake Victoria
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.66	F	J	Uganda	Busingiro, Budongo Forest
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.67	M	A	Uganda	Bakalasa, Mengo
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.69	M	HGR	Uganda	Namanyama, Mabira Forest
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.73	M	-	Uganda	Katwe, Lake Edward
<i>Naja subfulva</i>	<i>Boulengerina</i>	1959.1.7.74	M	HGR	Uganda	Bugala Island, Sese
<i>Naja subfulva</i>	<i>Boulengerina</i>	1976.1669	F	-	Ethiopia	Godare
<i>Naja subfulva</i>	<i>Boulengerina</i>	1976.227	F	-	Uganda	Kilembe
<i>Naja peroescobari</i>	<i>Boulengerina</i>	1906.3.30.80	F	HGR	Sao Tome	Agua Ize

Supplementary Table 3. Species used in datasets for hood size, hood size t-tests, PGLS, Phyloglm and ancestral state reconstruction

Species	Subgenus	Hood size data	Hood size more than 4 specimens (for t-tests)	In phylogeny	PGLS analysis	Phyloglm analysis	Ancestral state reconstruction
<i>Naja ashei</i>	<i>Afronaja</i>	y	N	y	y	y	y
<i>Naja katiensis</i>	<i>Afronaja</i>	y	y	y	y	y	y
<i>Naja mossambica</i>	<i>Afronaja</i>	y	y	y	y	y	y
<i>Naja nigricollis</i>	<i>Afronaja</i>	y	y	y	y	y	y
<i>Naja nigricincta</i>	<i>Afronaja</i>	n	n	n	n	y	n
<i>Naja woodi</i>	<i>Afronaja</i>	n	n	n	n	y	n
<i>Naja nubiae</i>	<i>Afronaja</i>	y	y	y	y	y	y
<i>Naja pallida</i>	<i>Afronaja</i>	y	y	y	y	y	y
<i>Naja annulata</i>	<i>Boulengerina</i>	y	y	y	y	y	y
<i>Naja christyi</i>	<i>Boulengerina</i>	y	n	n	n	n	n
<i>Naja melanoleuca</i>	<i>Boulengerina</i>	y	y	y	y	y	y
<i>Naja melanoleuca</i> (W. Africa banded)	<i>Boulengerina</i>	y	n	y	y	y	y
<i>Naja melanoleuca</i> (W. Africa black)	<i>Boulengerina</i>	y	n	y	y	y	y
<i>Naja multifasciata</i>	<i>Boulengerina</i>	y	n	y	y	y	y
<i>Naja peroescobari</i>	<i>Boulengerina</i>	y	n	y	y	y	y
<i>Naja subfulva</i>	<i>Boulengerina</i>	y	n	y	y	y	y
<i>Naja annulifera</i>	<i>Uraeus</i>	y	y	y	y	y	y
<i>Naja arabica</i>	<i>Uraeus</i>	y	y	y	y	y	y
<i>Naja haje</i>	<i>Uraeus</i>	y	y	y	y	y	y
<i>Naja nivea</i>	<i>Uraeus</i>	y	y	y	y	y	y
<i>Naja anchietae</i>	<i>Uraeus</i>	n	n	n	n	y	n
<i>Naja senegalensis</i>	<i>Uraeus</i>	n	n	n	n	y	n
<i>Naja atra</i>	<i>Naja</i>	y	y	y	y	y	y
<i>Naja kaouthia</i>	<i>Naja</i>	y	y	y	y	y	y
<i>Naja miolepis</i>	<i>Naja</i>	y	y	y	y	y	y
<i>Naja naja</i>	<i>Naja</i>	y	y	y	y	y	y
<i>Naja oxiana</i>	<i>Naja</i>	y	y	y	y	y	y

<i>Naja sagittifera</i>	<i>Naja</i>	y	n	y	y	y	y
<i>Naja samarensis</i>	<i>Naja</i>	y	n	y	y	y	y
<i>Naja siamensis</i>	<i>Naja</i>	y	y	y	y	y	y
<i>Naja sputatrix</i>	<i>Naja</i>	y	n	n	n	n	n
<i>Naja sumatrana</i>	<i>Naja</i>	y	y	y	y	y	y
<i>Naja mandalayensis</i>	<i>Naja</i>	n	n	n	n	y	n
<i>Naja philippinensis</i>	<i>Naja</i>	n	n	n	n	y	n
<i>Aspidelaps cowlesi</i>	Core Cobra	y	n	n	n	n	n
<i>Aspidelaps lubricus</i>	Core Cobra	y	y	y	y	y	y
<i>Aspidelaps scutatus</i>	Core Cobra	y	y	y	y	y	y
<i>Hemachatus haemachatus</i>	Core Cobra	y	y	y	y	y	y
<i>Pseudohaje goldii</i>	Core Cobra	y	y	y	y	y	y
<i>Pseudohaje nigra</i>	Core Cobra	y	n	n	n	n	n
<i>Walterinnesia aegyptia</i>	Core Cobra	y	n	y	y	y	y
<i>Walterinnesia morgani</i>	Core Cobra	y	y	n	n	n	y
<i>Bungarus caeruleus</i>	Other Elapid	y	y	y	n	n	y
<i>Bungarus fasciatus</i>	Other Elapid	y	y	y	n	n	y
<i>Dendroaspis angusticeps</i>	Other Elapid	y	y	y	n	n	y
<i>Dendroaspis jamesoni</i>	Other Elapid	y	y	n	n	n	n
<i>Dendroaspis polylepis</i>	Other Elapid	y	n	n	n	n	n
<i>Dendroaspis viridis</i>	Other Elapid	y	y	n	n	n	n
<i>Elapsoidea boulengeri</i>	Other Elapid	y	y	n	n	n	n
<i>Elapsoidea sundevalli</i>	Other Elapid	y	n	n	n	n	n
<i>Hemibungarus calligaster</i>	Other Elapid	y	n	y	n	n	y
<i>Ophiophagus hannah</i>	Other Elapid	y	y	y	n	n	y