

# Sleep in amphibians and reptiles: a review and a preliminary analysis of evolutionary patterns

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## ABSTRACT

Despite the ubiquitous nature of sleep, its functions remain a mystery. In an attempt to address this, many researchers have studied behavioural and electrophysiological phenomena associated with sleep in a diversity of animals. The great majority of vertebrates and invertebrates display a phase of immobility that could be considered as a sort of sleep. Terrestrial mammals and birds, both homeotherms, show two sleep states with distinct behavioural and electrophysiological features. However, whether these features have evolved independently in each clade or were inherited from a common ancestor remains unknown. Unfortunately, amphibians and reptiles, key taxa in understanding the evolution of sleep given their position at the base of the tetrapod and amniote tree, respectively, remain poorly studied in the context of sleep. This review presents an overview of what is known about sleep in amphibians and reptiles and uses the existing data to provide a preliminary analysis of the evolution of behavioural and electrophysiological features of sleep in amphibians and reptiles. We also discuss the problems associated with analysing existing data, as well as the difficulty in inferring homologies of sleep stages based on limited data in the context of an essentially mammalian-centric definition of sleep. Finally, we highlight the importance of developing comparative approaches to sleep research that may benefit from the great diversity of species with different ecologies and morphologies in order to understand the evolution and functions of sleep.

*Key words:* sleep, amphibian, reptile, REM sleep, paradoxical sleep, slow-wave sleep, active sleep, quiet sleep, evolution.

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## I. INTRODUCTION

In the following review, we first provide a brief overview of how sleep is defined, where it is observed in the animal kingdom, and what its possible functions may be. Excellent reviews on these topics are available (Kovalzon, 1976; Hartse, 1994, 2011; Tobler, 1995; Siegel, 2005, 2008, 2009; Rattenborg *et al.*, 2007; Cirelli & Tononi, 2008; Mignot, 2008; Lesku *et al.*, 2008*b*) and, thus, we only briefly touch upon these matters here. A detailed review of the literature on sleep in amphibians and non-avian reptiles is then provided, including a discussion of the behavioural and electrophysiological indicators of sleep in these taxa. We then provide a preliminary quantitative analysis of these data in a phylogenetic framework, and discuss the evolution of sleep in reptiles and amphibians in the context of the evolution of sleep in vertebrates more generally. In doing so, we place specific emphasis on the difficulties encountered in defining sleep or sleep stages in reptiles and amphibians based on either behavioural or electrophysiological data. Finally, we discuss the need to develop comparative and developmental analyses to understand sleep in its evolutionary

context and to identify its function(s). We also emphasize the need for additional studies on taxa such as reptiles and amphibians, and raise the question of the presence of a paradoxical sleep-like state in reptiles and amphibians.

### (1) What is sleep?

Together with reproduction and feeding, sleep appears to be one of the fundamental requirements of all vertebrates. Most of the vertebrates studied to date display a daily period of prolonged immobility that can be considered as a sleep-like state. A behavioural definition of sleep was proposed just over a century ago and can be used to study the presence or absence of sleep in different organisms (Piéron, 1913). The behavioural features of sleep include: (i) the spontaneous use of a stereotypic or species-specific posture during periods of immobility, (ii) the maintenance of behavioural immobility, (iii) an elevated behavioural response threshold to arousal stimuli, and (iv) a rapid behavioural state reversibility upon stimulation. After the discovery of variation in brain waves related to vigilance in animals (Caton, 1877) and in man (Berger, 1929),

electrophysiological criteria were added to the definition of sleep (Loomis, Harvey & Hobart, 1938).

A sleep state associated with rapid eye movements (REM sleep) was identified first in man and later in the cat (Aserinsky & Kleitman, 1953; Dement, 1958). Jouvet, Michel & Courjon (1959) showed that a muscle atonia appears during this state in the cat. They referred to this state as 'paradoxical sleep' because the electroencephalogram (EEG) pattern resembles the EEG of an awake animal. After these discoveries, electroencephalography, electromyography (EMG), and electro-oculography (EOG) were accepted as valid methods to identify sleep in mammals. These techniques subsequently allowed the identification of two distinct sleep states in birds as well (Klein, Michel & Jouvet, 1964; Ookawa & Gotoh, 1964). Consequently, two main states are now recognized in both birds and mammals: slow-wave sleep, also called non-REM sleep, or quiet sleep, and paradoxical sleep, also known as REM sleep, or active sleep. The specific physiological and behavioural correlates associated with these two sleep states are detailed below. In the following review we use the terms 'quiet sleep' and 'active sleep' to avoid the confusion induced by naming sleep states based on only a single feature. This terminology is often used in ontogenetic studies when talking about sleep in newborn animals. The active sleep of newborns and embryos contains many twitches and motor automatisms and is defined as such without the electrophysiological criteria typically used for adults (Corner, 1977; Blumberg & Lucas, 1996). We use the terms 'rest' or 'quiescence' when we do not postulate a true homology with mammalian sleep. This also pertains to the term 'quiet wakefulness'.

Adult mammalian quiet sleep is characterized by a relatively low-frequency, large-amplitude EEG signal. However, this state is composed of many specific electrophysiological patterns including hippocampal sharp waves, sleep spindles, K-complexes, and delta waves (Sirota & Buzsáki, 2005). As a consequence, human quiet sleep has been separated into as many as three different states. Hippocampal sharp waves are an important feature of quiet wakefulness and quiet sleep, and have been recorded in the CA1 region of the hippocampus. Hartse *et al.* (1979) refer to these waves in the cat as ventral hippocampal spikes. Sharp waves begin during quiet wakefulness and appear in bursts when an animal falls asleep. These hippocampal sharp waves are also associated with high-frequency (200 Hz) fusiform waves called ripples in rats and mice (Ylinen *et al.*, 1995). During quiet sleep heart rate, respiratory rate, and muscle tone are reduced compared to the waking state. Metabolism is maintained at a lower level, but thermoregulatory mechanisms such as shivering, sweating, piloerection, and vasomotion are maintained (Parmeggiani, 2003). Brain temperature, on the other hand, decreases.

Mammalian active sleep is characterized by an EEG signal with lower amplitude than during quiet sleep. A hippocampal regular oscillation at 4–9 Hz also exists in rodents and cats. EEG patterns called ponto-geniculate occipital (PGO) waves can be recorded in the pons, lateral geniculate nuclei, and in the occipital cortex of cats (Jouvet *et al.*, 1959; McCarley, Nelson & Hobson, 1978). During

active sleep the animal displays atonia of the postural muscles (Jouvet *et al.*, 1959) and eye movements (Aserinsky & Kleitman, 1953). Twitches of the limbs or the tail are often present as well, and remain the main component of active sleep in newborns (Blumberg & Lucas, 1996). Penile and clitoral erections are also characteristic of this state (Schmidt *et al.*, 1994). Finally, thermoregulation mechanisms such as vasomotion, piloerection, shivering, and sweating are not maintained, and an increase of the brain temperature has been reported in rabbits, cats, rats and mice (Parmeggiani, 2003).

One characteristic that the two sleep states have in common is their homeostatic regulation (Tobler, 2011). After a quiet sleep or active sleep deprivation, a recovery of the deprived state is observed. An increase of the power of the slow waves during quiet sleep after deprivation has also been reported and is referred to as an increase in slow-wave activity (SWA) in mammals (Franken *et al.*, 1991) and birds (Rattenborg, Martinez-Gonzalez & Lesku, 2009).

However, the electrophysiological criteria and physiological correlates of sleep are not universal, with different patterns being present in some adult mammals (Siegel, 2009), neonate mammals (Blumberg & Lucas, 1996), and some birds (Rattenborg *et al.*, 2011*b*). An alternative EEG manifestation, associated with unihemispheric quiet sleep, has been observed in some species of birds, cetaceans, manatees, and otarid seals. This sleep state is characterized by a unilateral slow-wave activity EEG signal while the animal maintains a waking EEG pattern in the contralateral cortex. The occurrence of this type of sleep has been suggested to be related to the need to remain vigilant in areas with high predation pressure in birds (Rattenborg, Lima & Amlaner, 1999), and to the need to resurface for breathing in marine mammals (Lyamin *et al.*, 2008). Additionally, cetaceans do not exhibit active sleep (Lyamin *et al.*, 2008). The arousal threshold is also not uniform across mammals, being lowest during active sleep in humans, but highest during this state in rats and most other mammals (Siegel, 2009). Brain temperature changes are also not uniform across mammals, increasing in rat, cat, sheep, rabbit, and dog during active sleep, whereas in monkeys and humans a decrease in brain temperature has been reported (Denoyer *et al.*, 1991). Interestingly, in some basal mammals and birds (i.e. the platypus; *Ornithorhynchus anatinus*, the echidna; *Tachyglossus aculeatus*, and the ostrich; *Struthio camelus*), eye movements and a reduced muscle tone appear to be associated with 'cortical' slow waves characteristic of quiet sleep (Siegel *et al.*, 1996; Lesku *et al.*, 2011) and sometimes also with a 'typical' active sleep EEG (Nicol *et al.*, 2000; Lesku *et al.*, 2011). The amount of active and quiet sleep decrease continuously across the life of nearly all species studied (Roffwarg, Muzio & Dement, 1966) and neonate mammals do not present the EEG features typically associated with quiet and active adult mammalian sleep (Seelke & Blumberg, 2008).

Other states of prolonged immobility exist across vertebrates. They are referred to as dormancy states, often called hibernation, torpor, or aestivation. These particular states present the same behavioural characteristics as sleep, but in mammals and birds they are mainly associated with a

reduction in body temperature and metabolic rate (Geiser, 1988, 2004). At an electrophysiological level, mammals in torpor cease active sleep below 25°C, yet the EEG still displays slow-wave oscillations like those observed during typical quiet sleep, even if these waves tend to decrease with temperature until a hibernation state with little EEG activity is observed (Walker *et al.*, 1977). Another interesting feature of these states is the rebound of slow-wave sleep after daily torpor (Deboer & Tobler, 2000, 2003), which suggest a distinct difference between dormancy and sleep, even if a continuum between sleep and torpor likely exists (Walker *et al.*, 1983; Berger, 1984).

## (2) Who sleeps?

Even if some authors reported that some fish, frogs, turtles, and crocodiles never sleep (Hobson, 1967; Susic, 1972; Van Twyver, 1973; Kavanau, 1998), the current consensus is that virtually all animals, including insects, nematodes, scorpions, spiders, and vertebrates, show some form of sleep, or at least sleep-like states (Campbell & Tobler, 1984; Hartse, 1994; Siegel, 2008). Both quiet sleep and active sleep have been clearly identified in terrestrial mammals, seals, manatees, and birds. The presence of these two states in amphibians and non-avian reptiles remains debated and is discussed later in this review (Fig. 1). Cetaceans, by contrast, do not display electrophysiological features of active sleep, but short periods of muscle jerks and eyelid movements have been reported during the resting period in some species (Lyamin *et al.*, 2008). It has also been demonstrated recently that migratory birds can fly continuously for over 6 months (Liechti *et al.*, 2013), raising the question of whether they sleep during this period, or not (Rattenborg, 2006a). Unihemispheric sleep could be a solution to long-term migration, even if it has never been demonstrated using EEG recordings during migrant flight. However, some laboratory studies have been performed during the migration period and show a clear reduction in the quantity of sleep and brief naps of uni- or bihemispheric sleep have been reported (Rattenborg *et al.*, 2004; Fuchs *et al.*, 2009). When considering the diversity of species studied, it becomes clear that the types of sleep observed and the characteristics of sleep at a behavioural and electrophysiological level may differ dramatically even among closely related species. Moreover it is important to consider sleep also in an ontogenetic context as the quantity of sleep and active sleep are greater at the beginning of life in all species studied in this context. Yet, studies examining the evolution of sleep typically focus on sleep in mature animals only.

## (3) Why sleep?

Whereas the functions of the main waking behaviours, like reproduction, locomotion, feeding, and foraging are obvious and needed to maximize the lifetime reproductive success (fitness) of all living animals, the role of being inactive, unconscious of the environment and by consequence exposed to predation, remains unclear. If sleep is not necessary for an animal, this state would likely have been

eliminated by natural selection, at least in some species (Rechtschaffen, 1998; Cirelli & Tononi, 2008; Siegel, 2008). However, most animals spend a significant proportion of their life asleep. As a consequence, sleep must provide real benefits for the organism, but its functions currently remains unclear and highly debated (Rechtschaffen, 1998; Siegel, 2005; Mignot, 2008). Some authors assert that sleep is simply a way to pass time that cannot be spent performing waking-related functions (Meddis, 1975; Rial *et al.*, 2007), however, it is generally accepted that sleep has vital and important functions. Indeed, many physiological changes occur during sleep, and sleep is also associated with changes in gene expression. Moreover, cognitive changes are observed as the result of sleep deprivation. Consequently, many theories have been proposed to explain why animals sleep. These theories can be classified based on the three main suggested functions: restorative, cognitive, and developmental.

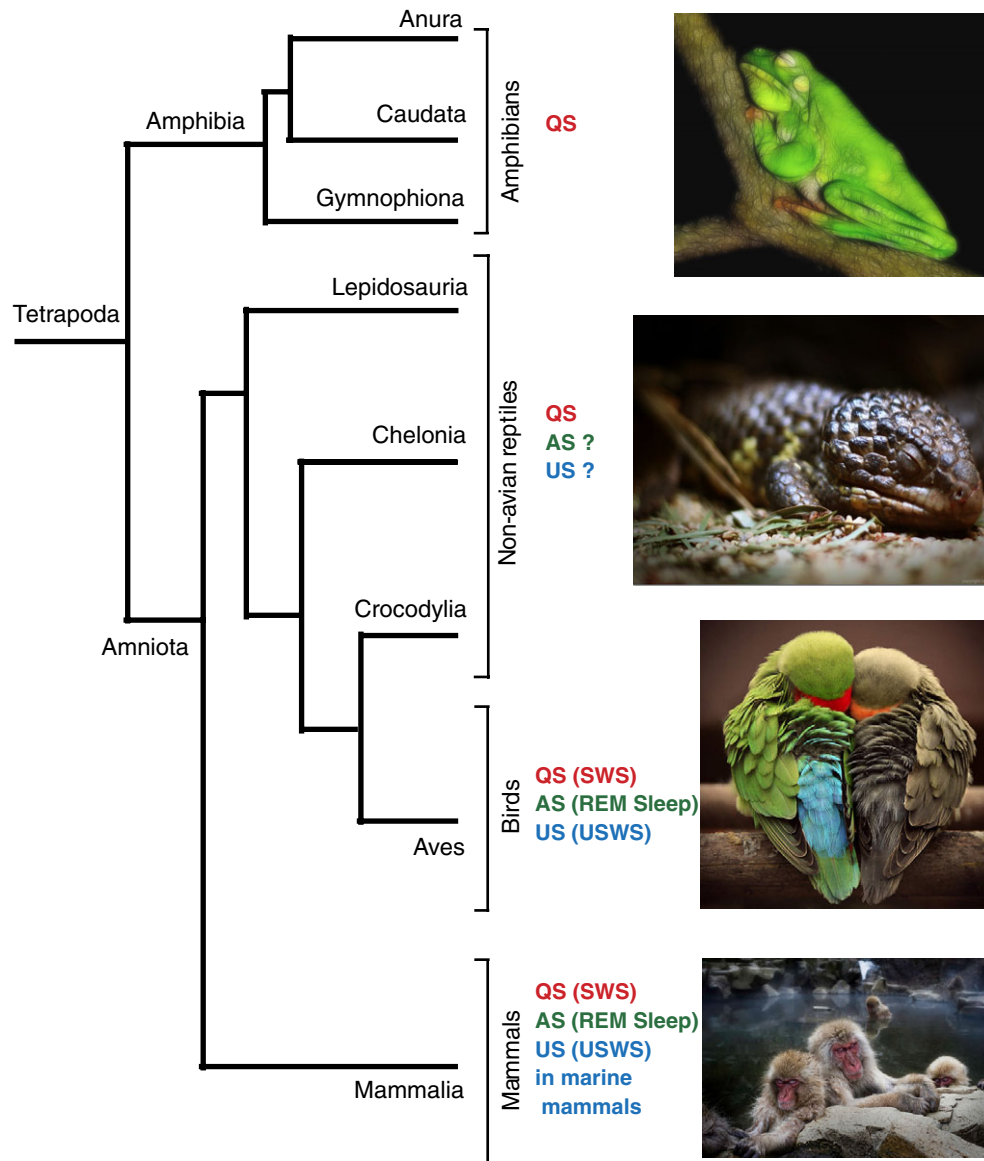
Firstly, some sleep researchers have proposed that sleep may be beneficial for energy conservation or restoration. These hypotheses are mainly based on the fact that temperature decreases during sleep in endotherms. Berger & Phillips (1995) proposed that sleep could serve to conserve energy. Benington & Heller (1995) proposed a function for quiet sleep in the replenishment of cerebral glycogen depleted during the waking state. Still others have proposed that sleep serves a role in molecular biosynthesis or gene expression (Mignot, 2008).

Secondly, molecular, anatomical, and behavioural data suggest a role for sleep in brain function and memory. It has been proposed that during sleep, and particularly during quiet sleep, a synaptic downscaling occurs in order to reorganize synaptic connectivity (Tononi & Cirelli, 2006). Others have suggested a role of sleep in memory consolidation (Stickgold, 2005; Stickgold & Walker, 2005), and a specific role of quiet and active sleep in contextual and emotional memory, respectively (Walker, 2010).

Lastly, based the fact that the amount of sleep, particularly active sleep, is greater during the juvenile stages of many animals (Roffwarg *et al.*, 1966; Jouvet-Mounier & Astic, 1968), and that juveniles display a greater quantity of this state during the first few days of their lives (Jouvet-Mounier, Astic & Lacote, 1970), some authors proposed a role of active sleep in brain maturation by promoting brain and neuromuscular development (Roffwarg *et al.*, 1966; Blumberg, 2010). This hypothesis is also supported by the fact that behavioural patterns of active sleep, like spontaneous motility are also present *in ovo* and *in utero* in all species studied, even if those species do not display an active sleep-like state as adults (Corner, 1977; Blumberg & Lucas, 1996).

To test some of these hypotheses, correlation analyses relating sleep duration and sleep fragmentation to body mass, metabolism, diet, brain size, etc., across different species of mammals have been performed (Zepelin & Rechtschaffen, 1974; Zepelin, 2000; Lo *et al.*, 2004; Lesku *et al.*, 2006, 2008b; Capellini *et al.*, 2008). However, these correlative approaches reveal some contradictions due to the different methods employed, and no clear consensus has emerged. Recently, Schmidt (2014) proposed a





**Fig. 1.** Simplified cladogram illustrating the relationships among extant vertebrate groups and the sleep states currently accepted to be present. Question marks indicate that the presence of some sleep states in this group remains controversial. Sleep states AS (active sleep), QS (quiet sleep) and US (unihemispheric sleep) are based on behavioural criteria only. States in parentheses including rapid eye movement (REM) sleep, slow-wave sleep (SWS), and unihemispheric slow-wave sleep (USWS) are defined based on electrophysiological criteria. Cladogram based on Pyron & Wiens (2011) and Chiari *et al.* (2012). Picture credits: tree frog (*Litoria sp.*), Helmut Hess; sleepy lizard (*Tiliqua rugosa*), Lachlan Sear; love birds (*Agapornis roseicollis*), Ansgar Trimborn; Japanese macaque (*Macaca fuscata*), Trey Ratcliff.

unifying theory of the function of sleep based on results from comparative, ecological, metabolic, cellular, phylogenetic, and ontogenetic data. This ‘energy allocation model’ proposes that the wake/sleep cycle constitutes a trade-off of temporal utilization of energy to maximize fitness. This model proposed a specific role of mammalian active sleep to enhance energy allocation to somatic and central nervous system processes. Surprisingly, despite its ubiquitous nature and our understanding of the various processes and physiological changes associated with sleep across phylogeny and ontogeny, we still do not know exactly why we sleep.

#### (4) The evolution of sleep

Although many papers have discussed the evolution of sleep, relatively few have done so in a quantitative and phylogenetically informed way (Lesku *et al.*, 2006, 2008*b*; Roth *et al.*, 2006; Capellini *et al.*, 2008). In general, it is considered that all vertebrates sleep or show sleep-like states. Quiet sleep with slow waves is thought to be present only in mammals and birds, however. As mammalian active sleep has not been demonstrated unequivocally in amphibians or reptiles, it is often considered to have evolved independently in mammals and birds (Rattenborg, Lesku &

Martinez-Gonzalez, 2011). Moreover, it is thought that this convergent evolution is accompanied by the appearance of homeothermy (Kavanau, 2002). Berger (1984) raised the possibility that mammalian active sleep could be a 'vestige of a reptilian ectothermic state of inactivity' based on the loss of thermoregulation in mammals during this state (Berger, 1984, p. 320). Karmanova (1982) developed comparative studies to understand the evolution of sleep in vertebrates and postulated that amphibians and fish display three forms of rest or sleep-like states (SLS), named 'protosleep' or 'primary sleep': cataleptic (plastic muscle tone) or protosleep 1 (P1), catatonic (rigid muscle tone) or protosleep 2 (P2), and cataleptic sleep (atonia) or protosleep 3 (P3). The P1 state appears mainly during the day when the eyes are open, whereas P2 and P3 are observed during nighttime rest periods. The arousal threshold increases relative to the type of protosleep, and the heart rate decreases accordingly. Karmanova's research group refers to the resting periods of reptiles as intermediate sleep, this state containing mainly P1 and P3. They further suggest that P3 sleep is the evolutionary precursor of mammalian and bird quiet sleep, and that P1 and P2 are precursors of quiet wakefulness. They described periods of motor automatisms in amphibians and reptiles, sometimes associated with high-voltage sharp waves and with an EEG similar to that observed during the awake state. They proposed that these activation phases are an ancient form of mammalian active sleep (Karmanova & Lazarev, 1979; Karmanova, 1982). Others have proposed, based on the presence of slow waves during the awake state in some reptiles, that the awake state of reptiles evolved into the slow-wave sleep observed in mammals (Rial *et al.*, 2010).

##### **(5) Amphibians and non-avian reptiles, key but challenging taxa in understanding the functions and evolution of sleep**

Amphibians evolved during the Devonian some 370 million years ago. Extant amphibians are composed of three major groups (Fig. 1); Anura (frogs and toads), Caudata (salamanders and newts), and Gymnophiona (caecilians). Of these, the Anura make up 88% of all species. Amphibians live in a wide variety of habitats and range from fossorial, terrestrial, and arboreal, to entirely aquatic. Due to their pivotal placement at the transition from water to land at the base of the tetrapod tree, these animals may provide important clues to the changes that occurred during the transition to a terrestrial habitat. Non-avian reptiles are a heterogeneous group of amniotes including crocodiles, turtles, and lepidosaurians (lizards, snakes, and rhynchocephalians) (Fig. 1). Among these groups, lepidosaurians are the most diverse with 9413 species ([www.reptile-database.org](http://www.reptile-database.org)). Turtles are much less numerous with 328 identified species, and extant crocodylians contain only 25 species, despite their evolutionary abundance in the fossil record. Like amphibians, reptiles are extremely diverse and have invaded all possible niches ranging from the marine environment to species that are exclusively fossorial.

Sleep is clearly related to thermoregulation, metabolism, cognition, and development, and its description is mainly based on work using mammals as a model system. In consequence, the phylogenetic position of amphibians and non-avian reptiles, their ectothermy, and their precocial lifestyle at birth make them important taxa in understanding the function and origin of sleep. With respect to the special case of active sleep, their position becomes even more crucial as only terrestrial mammals and birds appear to display this sleep state. Moreover, if active sleep is related to maturation (Roffwarg *et al.*, 1966; Blumberg, 2010) then this state is likely only present during the developmental phase of these species and may no longer be present in the adult. This raises the importance of studying sleep throughout ontogeny when trying to infer homologies between sleep states. However, most work on amphibians and reptiles involves adults despite the fact that phasic twitches are present *in ovo* in many amphibians and reptiles (Corner, 1977). Studying sleep in amphibians and reptiles remains challenging, however. Many have relatively small brains, are often aquatic or semi-aquatic, and the literature on their neuroanatomy is sparse. Moreover, the currently accepted mammalian-centric definition of sleep which is mostly based on cortical electrophysiological patterns may impose difficulties when applied to other taxa. For example, brain waves are related to neuronal networks and are influenced by temperature. As a consequence, the poikilothermic nature and the absence of a neocortex in amphibians and non-avian reptiles constitute an important hurdle when attempting to draw parallels with sleep in mammals. These difficulties may also explain why the behavioural and electrophysiological data available for amphibians and non-avian reptiles are rather sparse.

In the context of the present review we first provide an overview of what is known about sleep in amphibians and non-avian reptiles. We present for each clade the experimental conditions in which animals have been recorded including the light cycle, temperature, and the environment in which experiments were conducted. Next, we review the behaviour patterns related to sleep including continuous immobility, arousal threshold, and the presence of eye movements, and motor automatism during sleep. We then review the electrophysiological patterns associated with sleep including what is known about EEG frequency and amplitude variations relative to the EEG during the awake state, the muscle tone, the heart and respiratory rates, and the presence of high-voltage sharp waves (HShW). Finally, we present data on sleep deprivation, where investigated, and the possible mammalian sleep-state homologies discussed in the literature. We discuss the limitations and problems associated with these data and subsequently provide a preliminary analysis of the evolution of associated sleep traits in reptiles and amphibians and highlight the difficulties of proposing hypotheses pertaining to the evolution of sleep without simultaneously integrating behavioural, neuroanatomical, molecular, and electrophysiological aspects of sleep.

## II. AMPHIBIANS

With over 7235 described species (www.amphibiaweb.org), amphibians are probably the most poorly studied taxa among tetrapods with sleep–wake data existing for only a handful of species (0.14% of all known species). Whereas sleep data exist for only 0.15% of all species of salamanders, fewer data are available for frogs (0.14%) and caecilians (0%). Moreover, existing data for frogs are highly skewed towards derived taxa such as bufonids, hylids, and ranids, effectively leaving most of the ecological and phylogenetic diversity unexplored. Below we describe the behavioural and electrophysiological information available for the different taxa studied. Data for all amphibians are summarized in Tables 1–4.

### (1) Caudata

#### (a) Experimental conditions

One species of salamander has been studied in the context of sleep, the tiger salamander (*Ambystoma tigrinum*; Lucas & Sterman, 1969). Neither the light cycle, nor the temperature and recording duration were reported.

#### (b) Behavioural evidence

The only investigation in salamanders was performed on 27 *Ambystoma tigrinum* (Lucas & Sterman, 1969) and showed that they display rhythmic behavioural activity with a 4 h rest–activity cycle.

#### (c) Electrophysiological evidence

The EEG analysis in *Ambystoma tigrinum* revealed a decrease in frequency and amplitude during prolonged resting periods. The absence of clear neck atonia and distinct eye movements obviated the identification of any state analogous to mammalian active sleep.

#### (d) Summary

In the only species of salamander studied to date, arousal threshold and sleep deprivation were not evaluated. Based on this experiment we can conclude, however, that at least one species of salamander displays a rest–activity cycle during which the rest phase could potentially represent sleep. *A. tigrinum* displays slower brain activity during prolonged resting versus the awake state.

### (2) Anura

#### (a) Experimental conditions

Nine different species of anuran have been studied in the context of sleep: two species of bufonids, the western toad [*Anaxyrus (Bufo) boreas*; Huntley, Donnelly & Cohen, 1978] and the common African toad [*Amietophrynus (Bufo) regularis*; Laming, 1982], however, this last species was studied only relative to its behavioural activity. Three species of hylids were also studied: the tree frogs *Osteopilus (Hyla*

*septentrionalis*, *Hyla squirella*, and *Hyla cinerea* (Hobson, Goin & Goin, 1968); and four species of ranids: the European common frog (*Rana temporaria*; Lazarev, 1978a; Karmanova & Lazarev, 1979; Karmanova, 1982; Laming, 1982; Belich, 1984; Aristakesyan & Karmanova, 1998, 2007), the American bullfrog [*Lithobates (Rana) catesbeianus*; Hobson, 1967], the marsh frog [*Pelophylax (Rana) ridibunda*; Karmanova, 1982] and the Emei music frog (*Babina daunchina*; Fang *et al.*, 2012). One-third of these experiments did not report recording duration and only half reported a recording duration greater than or equal to 24 h. The light cycle was specified as a natural cycle or as a 12:12 h light:dark cycle for half of these experiments. Ambient temperature was described as constant in most of these studies but was not reported in four cases. Hobson *et al.* (1968) were the only authors to make behavioural observations and an arousal threshold evaluation in the field as well as under laboratory conditions.

#### (b) Behavioural evidence

All anuran species studied to date, in which frequency rate and heart rate has been recorded, display a specific posture of immobility with a decrease in those parameters (Hobson, 1967; Hobson *et al.*, 1968; Lazarev, 1978a; Karmanova, 1982; Belich, 1984). Karmanova (1982) reported that *Pelophylax (Rana) ridibunda* spends most of its time in a resting state (80–90% of a 24 h period) and was also able to identify three kinds of resting states (sleep-like states or protosleep). A P3 with eyes closed was observed in some animals during less than 10% of the 24 h period. This state was characterized by a slowing of the heart and respiratory rate. Arousal threshold was examined in only three publications. Although *Anaxyrus boreas* showed an increased arousal threshold to gentle handling during rest (Huntley *et al.*, 1978), there was no such change in *Lithobates catesbeianus* in response to electrical stimulation between the awake and resting states (Hobson, 1967). Three species of hylid studied by Hobson *et al.* (1968) showed a higher arousal threshold to gentle handling in laboratory compared to field conditions. In laboratory conditions no differences in arousal threshold were observed between awake and resting states, indeed they stated that: ‘frogs are inert and non reactive unless heavily stimulated’ (p. 386). Studies by Karmanova (1982) and Karmanova & Lazarev (1979) associated the three SLS categories with an increased arousal threshold. However, we could not find any specific details of the experiments performed. Rhythmic movements of the feet during sleep-like states were reported only in *Rana temporaria* (Lazarev, 1978a,b; Belich, 1984).

#### (c) Electrophysiological evidence

Huntley *et al.* (1978) and Lazarev (1978a) reported a decrease in EEG frequency and amplitude for *Anaxyrus boreas* and *Rana temporaria* in a sleep-like state compared to when awake. Hobson *et al.* (1968) and Fang *et al.* (2012) report decreased EEG amplitude in the three tree frogs and *Babina daunchina*, respectively. The factorial analysis

Table 1. Amphibian sleep: experimental parameters

Order	Family	Genus	Species	Author	No. of animals	Recording duration	Light cycle	Ambient temperature	EOG recorded	EMG recorded	Arousal threshold evaluated	SLS deprivation evaluated
Caudata	Ambystomatidae	<i>Ambystoma</i>	<i>tigrinum</i>	Lucas & Sterman (1969)	27	NR	NR	NR	Yes	Yes	NR	NR
Anura	Bufonidae	<i>Amietophrynus</i>	<i>regularis</i>	Laming (1982)	7	<24 h	12:12 h	24 ± 3°C	NR	NR	NR	NR
Anura	Bufonidae	<i>Anaxyrus</i>	<i>boreas</i>	Huntley <i>et al.</i> (1978)	NR	≥24 h	Natural cycle	20°C	NR	Yes	Yes	NR
Anura	Hylidae	<i>Osteopilus</i>	<i>septentrionalis</i>	Hobson <i>et al.</i> (1968)	6	≥24 h	Natural cycle	20–24°C	NR	NR	Yes	NR
Anura	Hylidae	<i>Hyla</i>	<i>squirella</i>	Hobson <i>et al.</i> (1968)	NR	≥24 h	Natural cycle	20–24°C	NR	NR	Yes	NR
Anura	Hylidae	<i>Hyla</i>	<i>cinerea</i>	Hobson <i>et al.</i> (1968)	NR	≥24 h	Natural cycle	20–24°C	NR	NR	Yes	NR
Anura	Ranidae	<i>Babina</i>	<i>daunchina</i>	Fang <i>et al.</i> (2012)	10	NR	NR	NR	NR	NR	NR	NR
Anura	Ranidae	<i>Lithobates</i>	<i>atesbeianus</i>	Hobson (1967)	20	≥24 h	NR	22–24°C	Yes	Yes	Yes	NR
Anura	Ranidae	<i>Pelophylax</i>	<i>ridibundus</i>	Karmanova (1982)	6	≥24 h	NR	NR	NR	NR	NR	NR
Anura	Ranidae	<i>Rana</i>	<i>temporaria</i>	Laming (1982)	6	<24 h	12:12 h	5 ± 1°C	NR	NR	NR	NR
Anura	Ranidae	<i>Rana</i>	<i>temporaria</i>	Aristakesyan & Karmanova (1998)	10	NR	NR	NR	NR	Yes	NR	Yes
Anura	Ranidae	<i>Rana</i>	<i>temporaria</i>	Belich (1984)	NR	≥24 h	NR	15–16°C	NR	NR	NR	NR
Anura	Ranidae	<i>Rana</i>	<i>temporaria</i>	Lazarev (1978a)	8	NR	NR	20 ± 2°C	NR	Yes	NR	NR
Anura	Ranidae	<i>Rana</i>	<i>temporaria</i>	Reviews: Karmanova (1982) and Karmanova & Lazarev (1979)	NR	NR	NR	NR	NR	Yes	Yes	NR

Wake duration includes quiet wake and active wake. Wake EEG characteristics are based on quiet-wake EEGs. Quiet sleep duration in *L. atesbeianus* is based on the duration in a reclined position.



Table 2. Amphibian sleep: behavioural parameters

Genus	Species	Author	Heart rate, wakefulness versus SLS	Respiratory rate, wakefulness versus SLS	Eye movements during SLS	Period of full atonia recorded during SLS	Twitches or motor automatism during SLS	Arousal threshold during quiescence compared to the awake state	SLS recovery after deprivation
<i>Ambystoma</i>	<i>tigrinum</i>	Lucas & Sterman (1969)	NR	NR	No	NR	NR	—	—
<i>Amietophrynus</i>	<i>regularis</i>	Laming (1982)	NR	NR	NR	NR	NR	—	—
<i>Anaxyrus</i>	<i>boreas</i>	Huntley <i>et al.</i> (1978)	Decrease	Decrease	NR	NR	NR	Higher	—
<i>Osteopilus</i>	<i>septentrionalis</i>	Hobson <i>et al.</i> (1968)	Decrease	NR	NR	NR	NR	Higher	—
<i>Hyla</i>	<i>squirella</i>	Hobson <i>et al.</i> (1968)	Decrease	NR	NR	NR	NR	Higher	—
<i>Hyla</i>	<i>cinerea</i>	Hobson <i>et al.</i> (1968)	Decrease	NR	NR	NR	NR	Higher	—
<i>Babina</i>	<i>daunchina</i>	Fang <i>et al.</i> (2012)	NR	NR	NR	NR	NR	—	—
<i>Lithobates</i>	<i>catesbeianus</i>	Hobson (1967)	Decrease	Decrease	No	NR	NR	No change	—
<i>Pelophylax</i>	<i>ridibunda</i>	Karmanova (1982)	NR	Decrease	NR	NR	NR	—	—
<i>Rana</i>	<i>temporaria</i>	Laming (1982)	NR	NR	NR	NR	NR	—	—
<i>Rana</i>	<i>temporaria</i>	Aristakesyan & Karmanova (1998)	NR	NR	NR	NR	NR	—	Yes
<i>Rana</i>	<i>temporaria</i>	Belich (1984)	Decrease	NR	NR	NR	Yes	—	—
<i>Rana</i>	<i>temporaria</i>	Lazarev (1978a)	Decrease	NR	NR	NR	NR	—	—
<i>Rana</i>	<i>temporaria</i>	Reviews: Karmanova (1982) and Karmanova & Lazarev (1979)	Decrease	NR	NR	NR	Yes	Higher	—

Only protosleep 3 is considered as a sleep-like state in the studies by Karmanova (1982), Lazarev (1978a), Belich (1984) and Aristakesyan & Karmanova (1998). In a review Karmanova (1982) mentions that the arousal threshold is higher in P3. However, we could not find any details about the actual experiments. We do not consider the P3 of Karmanova (1982) and Lazarev, 1978a as showing period of full atonia as no phasic decrease in muscle tone was reported during this state. NR, not reported; SLS, sleep-like state.

Table 3. Amphibian sleep: electrophysiological parameters

Genus	Species	Author	EEG correlated with respiratory rate	Presence of HShW	EEG wake amplitude (µV)	EEG wake frequency (Hz)	EEG SLS1 amplitude (µV)	EEG SLS1 frequency (Hz)	EEG SLS2 amplitude (µV)	EEG SLS2 frequency (Hz)	Main SLS EEG amplitude relative to the awake state	Main SLS EEG frequency relative to the awake state
<i>Ambystoma</i>	<i>tigrinum</i>	Lucas & Sterman (1969)	Yes	NR	—	20–40	—	1–20	—	—	Decrease	Decrease
<i>Amietophrynus</i>	<i>regularis</i>	Laming (1982)	Yes	NR	—	—	—	—	—	—	—	—
<i>Anaxyrus</i>	<i>boreas</i>	Huntley <i>et al.</i> (1978)	Yes	NR	5–10	10–14	3–7	5–7	—	—	Decrease	Decrease
<i>Osteopilus</i>	<i>septentrionalis</i>	Hobson <i>et al.</i> (1968)	Yes	NR	50–100	5–8	<50	8–30	—	—	Decrease	—
<i>Hyla</i>	<i>squirella</i>	Hobson <i>et al.</i> (1968)	Yes	NR	50–100	5–8	<50	8–30	—	—	Decrease	—
<i>Hyla</i>	<i>cinerea</i>	Hobson <i>et al.</i> (1968)	Yes	NR	50–100	5–8	<50	8–30	—	—	Decrease	—
<i>Babina</i>	<i>daurichina</i>	Fang <i>et al.</i> (2012)	NR	NR	—	—	—	—	—	—	—	—
<i>Lithobates</i>	<i>catesbeianus</i>	Hobson (1967)	Yes	NR	20–30	6–15	—	—	—	—	—	—
<i>Pelophylax</i>	<i>ridibundus</i>	Karmanova (1982)	NR	NR	—	—	—	—	—	—	—	—
<i>Rana</i>	<i>temporaria</i>	Laming (1982)	Yes	NR	—	—	—	—	—	—	—	—
<i>Rana</i>	<i>temporaria</i>	Aristakesyan & Karmanova (1998)	NR	NR	—	—	—	—	—	—	—	—
<i>Rana</i>	<i>temporaria</i>	Karmanova (1998)	NR	NR	—	—	—	—	—	—	—	—
<i>Rana</i>	<i>temporaria</i>	Belich (1984)	NR	During quiescence	—	0.5–1.5; 3–4	10–20	0.5–2.0	—	—	Decrease	Decrease
<i>Rana</i>	<i>temporaria</i>	Lazarev (1978a)	NR	During quiescence	10–40	0.5–1.5; 3–4	—	0.5–2.0	—	—	Decrease	Decrease
<i>Rana</i>	<i>temporaria</i>	Reviews: Karmanova (1982) and Karmanova & Lazarev (1979)	NR	During quiescence	10–40	0.5–1.5; 3–4	—	0.5–2.0	—	—	Decrease	Decrease

Only protosleep 3 is considered as a sleep-like state in the studies by Karmanova (1982), Lazarev (1978a), Belich (1984) and Aristakesyan & Karmanova (1998). EEG, electroencephalography; HShW, high-voltage sharp waves; NR, not reported; SLS, sleep-like state.

Table 4. Amphibian sleep: vigilance state parameters

Genus	Species	Author	SLS duration (%) (AS + QS)	AS duration (%) (if described)	AS episode mean duration (s) (if described)	No. of SLS	Mammalian AS homology	Mammalian AS QS homology
<i>Ambystoma</i>	<i>tigrinum</i>	Lucas & Sterman (1969)	—	—	—	1	—	—
<i>Amietophrynus</i>	<i>regularis</i>	Laming (1982)	—	—	—	NR	—	—
<i>Anaxyrus</i>	<i>boreas</i>	Huntley <i>et al.</i> (1978)	53	—	—	1	—	—
<i>Osteopilus</i>	<i>septentrionalis</i>	Hobson <i>et al.</i> (1968)	—	—	—	1	—	—
<i>Hyla</i>	<i>squirella</i>	Hobson <i>et al.</i> (1968)	—	—	—	1	—	—
<i>Hyla</i>	<i>cinerea</i>	Hobson <i>et al.</i> (1968)	—	—	—	1	—	—
<i>Babina</i>	<i>daunchina</i>	Fang <i>et al.</i> (2012)	—	—	—	1	—	—
<i>Lithobates</i>	<i>calesbeianus</i>	Hobson (1967)	71	—	—	0	—	—
<i>Pelophylax</i>	<i>ridibunda</i>	Karmanova (1982)	10	—	—	NR	—	—
<i>Rana</i>	<i>temporaria</i>	Laming (1982)	—	—	—	NR	—	—
<i>Rana</i>	<i>temporaria</i>	Aristakesyan & Karmanova (1998)	11.1	—	—	1	—	—
<i>Rana</i>	<i>temporaria</i>	Belich (1984)	—	—	—	1	—	—
<i>Rana</i>	<i>temporaria</i>	Lazarev (1978a)	—	—	—	1	—	—
<i>Rana</i>	<i>temporaria</i>	Reviews: Karmanova (1982) and Karmanova & Lazarev (1979)	—	—	—	1	—	—

Only protosleep 3 is considered as a sleep-like state in the studies by Karmanova (1982), Lazarev, (1978a), Belich (1984) and Aristakesyan & Karmanova (1998). AS, active sleep; NR, not reported; QS, quiet sleep; SLS, sleep-like state.

study of Fang *et al.* (2012) found a statistical link between EEG band, vigilance state, and electrode placement. A fusiform oscillation at 12–14 Hz during the resting state was also observed; this was not present in the alert state. Karmanova & Lazarev (1979) reported a polymorphic and irregular EEG with a low amplitude in passive wakefulness similar to the waking state in *Rana temporaria*, whereas during the cataplexy state (P3), slow-wave activity was present on the EEG (0.5–2 Hz) (Lazarev, 1978*a*; Karmanova & Lazarev, 1979).

#### (d) Sleep deprivation

The only sleep deprivation study on an amphibian (*Rana temporaria*) reported a decrease of wakefulness during the recovery phase (20% awake in baseline to 5% during recovery) after 6 h of sleep deprivation using a gentle handling method (Aristakesyan & Karmanova, 1998). Similar to mammals and birds, for the first 2 h after rest deprivation the EEG of the frog during the deepest sleep state showed a statistically significant increase in the low-frequency power and an inhibition of the faster frequency component as compared to the baseline signal.

#### (e) Summary

For anurans, two main trends can be inferred from the EEG recordings: (i) high-voltage slow waves are more prominent during the awake state than during rest, and (ii) a slight enhancement in slow-wave activity may occur during the deepest sleep state (Karmanova, Belekova & Churnosov, 1971; Aristakesyan & Karmanova, 1998). Another feature of the EEG during the resting state in amphibians is the presence of EEG fusiform activity correlated with respiration, a finding reported in all studies to date that have recorded respiratory rate. Despite conflicting results from some studies, we conclude that the amphibian species studied to date display behavioural characteristics of sleep. Moreover, high-voltage sharp waves (HShW; often referred to as sharp waves or spike waves in the reptilian sleep literature) were only reported by Lazarev (1978*a,b*) for *Rana temporaria*. These HShW were 30–50  $\mu$ V in amplitude with a duration of 80–110 ms and were recorded mostly in the optic tectum and more rarely in the medial cortex (generally accepted to be the homologue of the mammalian hippocampus). Lazarev (1978*a,b*) noted that these patterns sometimes coincided with a brief rise in electromyogram (EMG) amplitude and that the spectral EEG during these waves was almost identical to that of wakefulness. The majority of studies did not report complete atonia or eye movements during the resting state suggesting the absence of an active sleep state in amphibians. However, Karmanova & Lazarev (1979) did report an ‘activation phase’ with an EEG similar to that observed during the awake state, motor automatisms, and a phasic transitory heart rate increase associated with HShW in *R. temporaria*. It should be noted, however, that only one study recorded electro-oculographic activity and only four reported electromyographic data.

### III. NON-AVIAN REPTILES

Data on sleep are available for only 0.24% of all reptiles. Whereas 12% of all crocodylian species have been examined, data are available for only 2.43% of all turtles, and a mere 0.12% of all lizards. Taxa such as crocodylians are key to our understanding of the evolution of avian sleep, however, given that extant species of birds are most closely related to crocodyles. Although the position of turtles in the amniote tree remains debated, turtles are generally considered to be the sister taxon to both crocodylians and birds based on large-scale molecular analyses (Crawford *et al.*, 2012). The most important, yet least studied, group of reptiles – given their position at the base of the reptilian tree – is clearly the Lepidosauria. Data on non-avian reptiles are critical to the evaluation of hypotheses on the origin of sleep and its potential convergence in mammals and birds (Rattenborg *et al.*, 2011*b*). Unfortunately, data for reptilian species are scarce, but the available information is summarized in Tables 5–8.

#### (1) Turtles

##### (a) Experimental conditions

Nine species of turtles and tortoise were studied in the context of sleep, the yellow-footed tortoise (*Chelonoidis denticulata*; Walker & Berger, 1973), the red-footed tortoise (*Chelonoidis carbonaria*; Flanigan, 1974), *Kinosternon* sp. (Ayala-Guerrero, 1987), the box turtle (*Terrapene carolina*; Flanigan *et al.*, 1974; Eiland, Lyamin & Siegel, 2001), the European pond turtle (*Emys orbicularis*; Vasilescu, 1970; Karmanova *et al.*, 1971; Belich, 1984), the bolson tortoise (*Gopherus flavomarginatus*; Ayala-Guerrero, Calderon & Perez, 1988), the marginated tortoise (*Testudo marginata*; Hermann, Jouvett & Klein, 1964), the Russian tortoise (*Testudo horsfieldi*; Aristakesyan, 2009), and the aquatic loggerhead sea turtle (*Caretta caretta*; Susic, 1972). Half of the studies recorded animals during 24 h or more in a chamber at constant temperature. A third of the papers report constant illumination during the experiment.

##### (b) Behavioural evidence

All chelonians studied to date display a state of prolonged immobility, different from basking, with the eyes closed, the plastron resting on the ground, and the head fully relaxed. All studies that recorded heart rate and/or respiratory rate noted a diminution of these parameters during sleep. Of interest is that half of the studies report eye movements and/or twitches during sleep-like states. Four publications report arousal thresholds in turtles (Walker & Berger, 1973; Flanigan, 1974; Flanigan *et al.*, 1974; Ayala-Guerrero, 1987). Except for the study of Walker & Berger (1973) where no differences in arousal threshold were detected for *Chelonoides denticulata*, longer response latencies were found. Ayala-Guerrero (1987) evaluated arousal threshold by gentle handling; the three other studies used electric shocks. Walker & Berger (1973) administered electric shocks at different intensities every



Table 5. Reptilian sleep: experimental parameters

Order	Family	Genus	Species	Author	No. of animals	Recording duration	Light cycle	Ambient temperature	EOG recorded	EMG recorded	Arousal threshold evaluated	SLS deprivation evaluated
Chelonia	Emydidae	<i>Emys</i>	<i>orbicularis</i>	Vasilescu (1970)	33	<24h	NR	NR	Yes	Yes	NR	NR
Chelonia	Emydidae	<i>Emys</i>	<i>orbicularis</i>	Karmanova <i>et al.</i> (1971)	10	≥24h	Natural cycle	NR	Yes	Yes	NR	NR
Chelonia	Emydidae	<i>Emys</i>	<i>orbicularis</i>	Belich (1984)	NR	≥24h	NR	Water at 15–16°C	No	NR	NR	NR
Chelonia	Emydidae	<i>Terrapene</i>	<i>carolina</i>	Flanigan <i>et al.</i> (1974)	10	>48h	Constant light	26–29°C	Yes	Yes	Yes	Yes
Chelonia	Emydidae	<i>Terrapene</i>	<i>carolina</i>	Eiland <i>et al.</i> (2001)	4	>48h	12:12h	23–29°C	Yes	Yes	NR	NR
Chelonia	Kinosternidae	<i>Kinosternon</i>	sp.	Ayala-Guerrero (1987)	4	<24h	Constant Light	23°C	Yes	Yes	Yes	NR
Chelonia	Testudinidae	<i>Chelonoidis</i>	<i>carbonaria</i>	Flanigan (1974)	6	≥48h	Constant light	27–29°C	Yes	Yes	Yes	Yes
Chelonia	Testudinidae	<i>Chelonoidis</i>	<i>denticulata</i>	Walker & Berger (1973)	7	≥24h	12:12h	25°C day, 23°C night	Yes	Yes	Yes	NR
Chelonia	Testudinidae	<i>Gopherus</i>	<i>flavomarginatus</i>	Ayala-Guerrero <i>et al.</i> (1988)	4	≥24h	Constant light	25–28°C	Yes	Yes	NR	NR
Chelonia	Testudinidae	<i>Testudo</i>	<i>horsfieldi</i>	Aristakesyan (2009)	NR	NR	NR	NR	NR	NR	NR	NR
Chelonia	Testudinidae	<i>Testudo</i>	<i>marginala</i>	Hermann <i>et al.</i> (1964)	3	<24h	NR	NR	Yes	Yes	NR	NR
Chelonia	Testudinidae	<i>Caretta</i>	<i>caretta</i>	Susic (1972)	3	NR	Natural cycle	Water 22–24°C	Yes	Yes	NR	Yes
Crocodylia	Alligatoridae	<i>Alligator</i>	<i>mississippiensis</i>	Van Twyver (1973)	7	NR	NR	Different temperatures 4–32°C	Yes	Yes	NR	NR
Crocodylia	Alligatoridae	<i>Caiman</i>	<i>latirostris</i>	Peyrethron & Dusan-Peyrethron (1969)	1	≥48h	12:12h	26°C	Yes	Yes	NR	NR
Crocodylia	Alligatoridae	<i>Caiman</i>	<i>sclerops</i>	Meglason & Huggins (1979)	5	≥24h	12:12h	37 ± 1°C	No	Yes	Yes	NR
Crocodylia	Alligatoridae	<i>Caiman</i>	<i>sclerops</i>	Rechtschaffen <i>et al.</i> , 1968	4	NR	NR	NR	Yes	Yes	NR	NR
Crocodylia	Alligatoridae	<i>Caiman</i>	<i>sclerops</i>	Flanigan <i>et al.</i> (1973)	10	≥48h	Constant light	Water 25–28°C	Yes	Yes	Yes	Yes
Crocodylia	Alligatoridae	<i>Caiman</i>	<i>sclerops</i>	Warner & Huggins (1978)	7	<24h	12:12h	Constant warm water at 27–30°C	No	Yes	NR	NR
Squamata	Anguillidae	<i>Ophisaurus</i>	<i>apodus</i>	Aristakesyan (2009)	NR	NR	NR	NR	NR	NR	NR	NR
Squamata	Chamaeleonidae	<i>Trioceros</i>	<i>jacksonii</i>	Tauber <i>et al.</i> (1966)	2	NR	NR	NR	NR	NR	NR	NR
Squamata	Chamaeleonidae	<i>Trioceros</i>	<i>malleni</i>	Tauber <i>et al.</i> (1966)	2	<24h	NR	NR	No	Yes	NR	NR
Squamata	Iguanidae	<i>Ctenosaura</i>	<i>pectinata</i>	Ayala-Guerrero & Huitron-Resendiz (1991)	4	24h	Constant light	Constant + hot spot 25–29°C	Yes	Yes	Yes	NR
Squamata	Iguanidae	<i>Ctenosaura</i>	<i>pectinata</i>	Tauber <i>et al.</i> (1968)	36	<24h	12:12h	23–26°C	Yes	Yes	Yes	NR
Squamata	Iguanidae	<i>Ctenosaura</i>	<i>pectinata</i>	Flanigan (1973)	3	≥48h	12:12h	Thermal gradient + hot spot day: 34–50°C + hot spot 4h night 27–32°C	Yes	Yes	Yes	Yes
Squamata	Iguanidae	<i>Ctenosaura</i>	<i>similis</i>	Ayala-Guerrero & Vargas Reyna (1987)	7	≥48h	Constant light	29–32°C	No	Yes	Yes	NR
Squamata	Iguanidae	<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley <i>et al.</i> (1977)	8	<24h	12:12h	21°C	No	Yes	NR	NR
Squamata	Iguanidae	<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley & Cohen (1980)	10	NR	NR	10°C, 20°C, 30°C	No	Yes	NR	NR
Squamata	Iguanidae	<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley (1987)	37	NR	Natural cycle	10°C, 20°C, 30°C	No	Yes	Yes	NR
Squamata	Iguanidae	<i>Iguana</i>	<i>iguana</i>	Peyrethron & Dusan-Peyrethron (1969)	1	≥48h	NR	NR	Yes	Yes	Yes	NR
Squamata	Iguanidae	<i>Iguana</i>	<i>iguana</i>	Ayala-Guerrero & Mexicano (2008a)	10	≥24h	Constant Light	25–30°C	Yes	Yes	Yes	Yes

Table 5. Continued

Order	Family	Genus	Species	Author	No. of animals	Recording duration	Light cycle	Ambient temperature	EOG recorded	EMG recorded	Arousal threshold evaluated	SLS deprivation evaluated
Squamata	Iguanidae	<i>Iguana</i>	<i>iguana</i>	Flanigan (1973)	3	≥48 h	12:12 h	Thermal gradient + hot spot day: 34–50°C + hotspot 4 h night 27–32°C	Yes	Yes	Yes	Yes
Squamata	Iguanidae	<i>Saurornathus</i>	<i>obsesus</i>	Stropes (1975)	8	NR	12:12 h	29–34°C	Yes	Yes	Yes	NR
Squamata	Phrynosomatidae	<i>Phrynosoma</i>	<i>solare</i>	Romo <i>et al.</i> (1978)	13	≥24 h	12:12 h	30 ± 0.5°C	No	Yes	NR	NR
Squamata	Phrynosomatidae	<i>Uma</i>	<i>notata</i>	Stropes (1971)	4	≥24 h	Constant light	36–38°C	Yes	Yes	NR	NR
Squamata	Pythonidae	<i>Python</i>	<i>sebae</i>	Peyrethron & Dusan-Peyrethron (1969)	1	≥48 h	NR	NR	Yes	Yes	NR	NR
Squamata	Varanidae	<i>Varanus</i>	<i>griseus</i>	Karmanova <i>et al.</i> (1971)	1	≥24 h	Natural cycle	NR	Yes	Yes	NR	NR

Wake duration includes quiet wake and active wake. Wake EEG characteristics are based on quiet-wake EEG. We included the PID level 3 of Meglasson & Huggins (1979) as sleep. We considered postures 3 and 4 described by Flanigan (1973, 1974) and Flanigan *et al.* (1973, 1974) as sleep. EMG, electromyography; EOG, electro-oculography; NR, not reported; SLS, sleep-like state.

10 min during a 6–8 h period of sleep for 3–4 days, whereas Flanigan (1974) and Flanigan *et al.* (1974) administered shocks at an intensity which generated arousal in 50% of cases observed, with at least one stimulation every 60 min.

(c) Electrophysiological evidence

Of the 10 experiments reporting EEG activity, 5 reported no changes in EEG frequency and amplitude between wakefulness and resting or sleep-like states. These experiments involved the aquatic *Caretta caretta* (Susic, 1972) and the terrestrial *Chelonoidis denticulata*, *Ch. carbonaria* and *Terrapene carolina* (Walker & Berger, 1973; Flanigan, 1974; Flanigan *et al.*, 1974; Eiland *et al.*, 2001). Susic (1972) did not report HShW, but the four other experiments cited above noted the presence of HShW during the resting period, including quiet wake as well as sleep. Three other experiments also reported HShW during rest (Karmanova *et al.*, 1971; Ayala-Guerrero, 1987; Ayala-Guerrero *et al.*, 1988). Ayala-Guerrero *et al.* (1988); Ayala-Guerrero (1987) and Karmanova *et al.* (1971) noted a diminution in EEG amplitude and a decrease in EEG frequency in *Gopherus flavomarginatus*, *Kinosternon* sp., and *Emys orbicularis* associated with reduced vigilance. By contrast, Vasilescu (1970) reported only a decrease in EEG frequency in *Emys orbicularis*. Hermann *et al.* (1964) was the only experiment to report an increase in EEG amplitude but a decrease in EEG frequency during sleep (in *Testudo marginata*), thus concluding that turtle sleep is similar to mammalian slow-wave sleep.

(d) Sleep deprivation

Only three studies investigated sleep deprivation. Flanigan *et al.* (1974) and Flanigan (1974) report an increase in the quantity of sleep during the recovery phase after 48 h of constant arousal. However, Susic (1972) found no increase in the quantity of rest following 12 h of constant arousal in the fully aquatic *Caretta caretta*. Flanigan *et al.* (1974) and Flanigan (1974) also noted an increase in the quantity of HShW during the recovery period after sleep deprivation.

(e) Sleep state homologies

Three publications describe the presence of two sleep-like states, including the presence of a mammalian active sleep-like state (Vasilescu, 1970; Ayala-Guerrero, 1987; Ayala-Guerrero *et al.*, 1988). They based their conclusions on the presence of eye movements and motor twitches during sleep. Vasilescu (1970) also reported atonia during a state that he called ‘paradoxical sleep’. In contrast to the above, neither Susic (1972) nor Walker & Berger (1973) reported changes in the EEG and Walker & Berger (1973) report no change in the arousal threshold response, concluding that *Caretta caretta* and *Chelonoidis denticulata* do not sleep. They consequently suggested that prolonged resting periods in reptiles should be considered as an inactive behaviour rather than true sleep. Walker & Berger (1973) was the only study included in this review to record oxygen consumption over a 4–6 h period. Oxygen consumption

Table 6. Reptilian sleep: behavioural parameters

Genus	Species	Author	Heart rate, wakefulness <i>versus</i> SLS	Respiratory rate, wakefulness <i>versus</i> SLS	Eye movements during SLS	Period of full atonia recorded during SLS	Twitches or motor automatism during SLS	Arousal threshold during quiescence compared to the awake state	SLS recovery after deprivation
<i>Emys</i>	<i>orbicularis</i>	Vasilescu (1970)	Decrease	NR	Yes	Yes	Yes	—	—
<i>Emys</i>	<i>orbicularis</i>	Karmanova <i>et al.</i> (1971)	NR	NR	No	No	No	—	—
<i>Emys</i>	<i>orbicularis</i>	Belich (1984)	Decrease	NR	NR	NR	Yes	—	—
<i>Terrapene</i>	<i>carolina</i>	Flanigan <i>et al.</i> (1974)	Decrease	Decrease	Yes	No	NR	Higher	Yes
<i>Terrapene</i>	<i>carolina</i>	Eiland <i>et al.</i> (2001)	NR	NR	Yes	No	Yes	—	—
<i>Kinosternon</i>	sp.	Ayala-Guerrero (1987)	Decrease	NR	Yes	No	Yes	Higher	—
<i>Chelonoidis</i>	<i>carbonaria</i>	Flanigan (1974)	Decrease	Decrease	Yes	No	No	Higher	Yes
<i>Chelonoidis</i>	<i>denticulata</i>	Walker & Berger (1973)	Decrease	NR	Yes	No	No	Higher	—
<i>Gopherus</i>	<i>flavomarginatus</i>	Ayala-Guerrero <i>et al.</i> (1988)	Decrease	NR	Yes	No	NR	No change	—
<i>Testudo</i>	<i>horsfieldi</i>	Aristakesyan (2009)	NR	NR	NR	NR	NR	—	—
<i>Testudo</i>	<i>marginata</i>	Hermann <i>et al.</i> (1964)	Decrease	Decrease	No	No	NR	—	—
<i>Caretta</i>	<i>caretta</i>	Susic (1972)	NR	NR	No	No	NR	—	No
<i>Alligator</i>	<i>mississippiensis</i>	Van Tuyver (1973)	NR	NR	NR	No	NR	—	—
<i>Caiman</i>	<i>latirostris</i>	Peyrethron & Dusan-Peyrethron (1969)	Decrease	Decrease	Yes	No	Yes	—	—
<i>Caiman</i>	<i>sclerops</i>	Meglasson & Huggins (1979)	NR	NR	NR	No	NR	Higher	—
<i>Caiman</i>	<i>sclerops</i>	Rechtschaffen <i>et al.</i> (1968)	NR	NR	No	Yes	NR	No change	—
<i>Caiman</i>	<i>sclerops</i>	Flanigan <i>et al.</i> (1973)	Decrease	Decrease	No	No	NR	No change	Yes
<i>Caiman</i>	<i>sclerops</i>	Warner & Huggins (1978)	Decrease	Decrease	NR	No	NR	—	—
<i>Ophisaurus</i>	<i>apodus</i>	Aristakesyan (2009)	NR	NR	NR	NR	NR	—	—
<i>Triceros</i>	<i>jacksonii</i>	Tauber <i>et al.</i> (1966)	NR	NR	NR	NR	NR	—	—
<i>Triceros</i>	<i>melleri</i>	Tauber <i>et al.</i> (1966)	Decrease	NR	Yes	No	NR	—	—
<i>Ctenosaura</i>	<i>pectinata</i>	Ayala-Guerrero & Huitron-Resendiz (1991)	Decrease	NR	Yes	No	Yes	Higher	—
<i>Ctenosaura</i>	<i>pectinata</i>	Tauber <i>et al.</i> (1968)	Decrease	NR	Yes	No	NR	Higher	—
<i>Ctenosaura</i>	<i>pectinata</i>	Flanigan (1973)	Decrease	Decrease	No	No	Yes	Higher	Yes
<i>Ctenosaura</i>	<i>similis</i>	Ayala-Guerrero & Vargas Reyna (1987)	Decrease	NR	No	No	Yes	Higher	—
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley <i>et al.</i> (1977)	Decrease	Decrease	Yes	No	Yes	Higher	—
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley & Cohen (1980)	NR	Decrease	NR	Yes	NR	Higher	—
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley (1987)	Decrease	Decrease	NR	No	NR	Higher	—
<i>Iguana</i>	<i>iguana</i>	Peyrethron & Dusan-Peyrethron (1969)	Decrease	Decrease	No	Yes	NR	Higher	—
<i>Iguana</i>	<i>iguana</i>	Ayala-Guerrero & Mexicano (2008a)	Decrease	NR	Yes	No	No	Higher	Yes
<i>Iguana</i>	<i>iguana</i>	Flanigan (1973)	Decrease	Decrease	No	No	Yes	Higher	Yes
<i>Scaromatus</i>	<i>obsesus</i>	Stropes (1975)	Decrease	NR	No	No	Yes	Higher	—
<i>Phrynosoma</i>	<i>solare</i>	Romo <i>et al.</i> (1978)	NR	NR	NR	No	NR	—	—
<i>Uma</i>	<i>notata</i>	Stropes (1971)	Decrease	NR	Yes	No	NR	—	—
<i>Python</i>	<i>sebae</i>	Peyrethron & Dusan-Peyrethron (1969)	Decrease	Decrease	NR	No	NR	—	—
<i>Vivanus</i>	<i>griseus</i>	Karmanova <i>et al.</i> (1971)	NR	NR	No	No	NR	—	—

NR, not reported; SLS, sleep-like state.

Table 7. Reptilian sleep: electrophysiological parameters

Genus	Species	Author	Presence of HSW	EEG wake amplitude (µV)	EEG wake frequency (Hz)	EEG SLS1 amplitude (µV)	EEG SLS1 frequency (Hz)	EEG SLS2 amplitude (µV)	EEG SLS2 frequency (Hz)	EEG correlated with respiratory rate		Main SLS EEG	
										EEG wake amplitude (µV)	EEG wake frequency (Hz)	amplitude relative to the awake state	frequency relative to the awake state
<i>Emys</i>	<i>orbicularis</i>	Vasilescu (1970)	NR	10–15	8–11	10–15	3–8	35	8–15	No change	No change	Decrease	Decrease
<i>Emys</i>	<i>orbicularis</i>	Karmanova <i>et al.</i> (1971)	During quiescence	—	3–4; 8–12	—	3–7	—	—	Decrease	Decrease	Decrease	Decrease
<i>Emys</i>	<i>orbicularis</i>	Belich (1984)	NR	—	—	—	—	—	—	NR	NR	NR	NR
<i>Terrapene</i>	<i>carolina</i>	Flanigan <i>et al.</i> (1974)	During quiescence	2–23.4	—	2–21.2	—	—	—	No change	No change	No change	No change
<i>Terrapene</i>	<i>carolina</i>	Eiland <i>et al.</i> (2001)	During quiescence	—	—	—	—	—	—	No change	No change	No change	No change
<i>Kinosternon</i>	sp.	Ayala-Guerrero (1987)	During quiescence	20–90	10–25	—	—	—	—	Decrease	Decrease	Decrease	Decrease
<i>Chelonoidis</i>	<i>carbonaria</i>	Flanigan (1974)	During quiescence	2.2–70	—	2–70	—	—	—	No change	No change	No change	No change
<i>Chelonoidis</i>	<i>denticulata</i>	Walker & Berger (1973)	During quiescence	<40	6–10	<40	6–10	—	—	No change	No change	No change	No change
<i>Gopherus</i>	<i>flavomarginatus</i>	Ayala-Guerrero <i>et al.</i> (1988)	During quiescence	—	—	—	—	—	—	Decrease	Decrease	Decrease	Decrease
<i>Testudo</i>	<i>horsfieldi</i>	Aristakesyan (2009)	NR	—	—	—	—	—	—	NR	NR	NR	NR
<i>Testudo</i>	<i>marginata</i>	Hermann <i>et al.</i> (1964)	NR	12–15	11–13	50	6–8	—	—	Increase	Increase	Decrease	Decrease
<i>Caretta</i>	<i>caretta</i>	Susic (1972)	NR	15–25	8–16	—	—	—	—	No change	No change	No change	No change
<i>Alligator</i>	<i>mississippiensis</i>	Van Twyver (1973)	During quiescence	—	—	—	—	—	—	No change	No change	No change	No change
<i>Caiman</i>	<i>latirostris</i>	Peyrethron & Dusan-Peyrethron (1969)	During wake	20	7–8	30	4–5	30	7–8	Increase	Increase	Decrease	Decrease
<i>Caiman</i>	<i>sclerops</i>	Meglasson & Huggins (1979)	During wake and quiescence	20–70	10–16	50–130	0.8–2.2	—	—	Increase	Increase	Decrease	Decrease
<i>Caiman</i>	<i>sclerops</i>	Rechtschaffen <i>et al.</i> (1968)	NR	10–30	1–10	—	—	—	—	Increase	Increase	Decrease	Decrease
<i>Caiman</i>	<i>sclerops</i>	Flanigan <i>et al.</i> (1973)	During quiescence	2–63	4–11; 19–23	2–33	2–5; 7–10; 19–23	—	—	Decrease	Decrease	Decrease	Decrease
<i>Caiman</i>	<i>sclerops</i>	Warner & Huggins (1978)	NR	20–70	10–16	50–130	0.8–2.2	—	—	Increase	Increase	Decrease	Decrease
<i>Ophisaurus</i>	<i>apodus</i>	Aristakesyan (2009)	NR	—	—	—	—	—	—	NR	NR	NR	NR
<i>Trociros</i>	<i>jacksonii</i>	Tauber <i>et al.</i> (1966)	NR	—	—	—	—	—	—	NR	NR	NR	NR



Table 7. Continued

Genus	Species	Author	EEG correlated with respiratory rate	Presence of HShW	EEG wake amplitude ( $\mu$ V)	EEG wake frequency (Hz)	EEG SLS1 amplitude ( $\mu$ V)	EEG SLS1 frequency (Hz)	EEG SLS2 amplitude ( $\mu$ V)	EEG SLS2 frequency (Hz)	Main SLS EEG amplitude relative to the awake state	Main SLS EEG frequency relative to the awake state
<i>Triturus</i>	<i>malleri</i>	Tauber <i>et al.</i> (1966)	NR	During quiescence	35–45	7–9	35–45	6–8	—	—	No change	Decrease
<i>Ctenosaura</i>	<i>pectinata</i>	Ayala-Guerrero & Huitron-Resendiz (1991)	NR	During quiescence	—	9–10.2	—	5.4–6	—	9–10.2	Decrease	Decrease
<i>Ctenosaura</i>	<i>pectinata</i>	Tauber <i>et al.</i> (1968)	NR	During wake and quiescence	15–50	15–18	—	13–15	—	13–15	No change	Decrease
<i>Ctenosaura</i>	<i>pectinata</i>	Flanigan (1973)	Yes	During quiescence	2.9–152.3	3–8; 10–13; 15–18; 20–24	2.6–90	2–6; 8–10; 12–18; 20–23	—	—	No change	Decrease
<i>Ctenosaura</i>	<i>similis</i>	Ayala-Guerrero & Vargas Reyna (1987)	NR	During quiescence	20–160	12–25	—	—	—	—	Decrease	Decrease
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley <i>et al.</i> (1977)	NR	NR	100	13–16	1–3	10–13	—	10–15	Decrease	Decrease
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley & Cohen (1980)	NR	During wake	—	—	—	—	—	—	NR	NR
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley (1987)	NR	During wake	30–50	8.7–10.4	3–5	4.2–7.5	—	7.9–14.6	Decrease	Decrease
<i>Iguana</i>	<i>iguana</i>	Peyrethon & Dusan-Peyrethon (1969)	Yes	During quiescence	50	40–42	30	30–35	—	—	No change	No change
<i>Iguana</i>	<i>iguana</i>	Ayala-Guerrero & Mexicano (2008a)	NR	During quiescence	24.2	9–11.4	9.2	5–6	19	8–9	Decrease	Decrease
<i>Iguana</i>	<i>iguana</i>	Flanigan (1973)	NR	During quiescence	2.9–152.3	3–8; 10–13; 15–18; 20–24	2.6–90	2–6; 8–10; 12–18; 20–23	—	—	No change	Decrease
<i>Sauromalus</i>	<i>obsesus</i>	Stropes (1975)	NR	During wake	35	7–16	30	1–14	10–60	3–11	No change	Decrease
<i>Phrynosoma</i>	<i>solaris</i>	Romo <i>et al.</i> (1978)	NR	NR	5–10	—	—	—	—	—	Increase	Decrease
<i>Uma</i>	<i>notata</i>	Stropes (1971)	NR	NR	10–40	6–9	15–50	0–4	10–30	5–8	No change	Decrease
<i>Python</i>	<i>sebae</i>	Peyrethon & Dusan-Peyrethon (1969)	Yes	During quiescence	30	20–25	30	14–15	—	—	No change	Decrease
<i>Varanus</i>	<i>griseus</i>	Karmanova <i>et al.</i> (1971)	NR	During quiescence	—	3–4; 8–12	—	3–7	—	—	Decrease	Decrease

EEG, electroencephalography; HShW, high-voltage sharp waves; NR, not reported; SLS, sleep-like state.

Table 8. Reptilian sleep: vigilance state parameters

Genus	Species	Author	SLS duration (%) (AS+ QS)	AS duration (%) (if described)	AS episode mean duration (s) (if described)	No. of SLS	Mammalian AS homology	Mammalian QS homology
<i>Emys</i>	<i>orbicularis</i>	Vasilescu (1970)	—	—	<15	2	Yes	—
<i>Emys</i>	<i>orbicularis</i>	Karmanova <i>et al.</i> (1971)	—	—	—	1	—	—
<i>Emys</i>	<i>orbicularis</i>	Belich (1984)	—	—	—	1	—	—
<i>Terrapene</i>	<i>carolina</i>	Flanigan <i>et al.</i> (1974)	85	—	—	1	—	—
<i>Terrapene</i>	<i>carolina</i>	Eiland <i>et al.</i> (2001)	—	—	—	1	—	—
<i>Kinosternon</i>	sp.	Ayala-Guerrero (1987)	—	—	19.2	2	Yes	—
<i>Chelonoidis</i>	<i>carbonaria</i>	Flanigan (1974)	91	—	—	1	—	—
<i>Chelonoidis</i>	<i>denticulata</i>	Walker & Berger (1973)	—	—	—	0	—	—
<i>Gopherus</i>	<i>flavomarginatus</i>	Ayala-Guerrero <i>et al.</i> (1988)	72.5	—	11	2	Yes	—
<i>Testudo</i>	<i>horsfieldi</i>	Aristakesyan (2009)	—	—	—	1	—	—
<i>Testudo</i>	<i>marginata</i>	Hermann <i>et al.</i> (1964)	48	—	—	1	—	Yes
<i>Caretta</i>	<i>caretta</i>	Susic (1972)	—	—	—	0	—	—
<i>Alligator</i>	<i>mississippiensis</i>	Van Twyver (1973)	—	—	—	0	—	—
<i>Caiman</i>	<i>latirostris</i>	Peyrethron & Dusan-Peyrethron (1969)	67	0.35	50	2	Yes	Yes
<i>Caiman</i>	<i>sclerops</i>	Meglsson & Huggins (1979)	12.7	—	—	1	—	Yes
<i>Caiman</i>	<i>sclerops</i>	Rechtschaffen <i>et al.</i> (1968)	—	—	—	1	—	—
<i>Caiman</i>	<i>sclerops</i>	Flanigan <i>et al.</i> (1973)	>50	—	—	1	—	—
<i>Caiman</i>	<i>sclerops</i>	Warner & Huggins (1978)	—	—	—	1	—	—
<i>Ophisaurus</i>	<i>apodus</i>	Aristakesyan (2009)	—	—	—	1	—	—
<i>Troceros</i>	<i>jacksonii</i>	Tauber <i>et al.</i> (1966)	—	—	—	1	—	—
<i>Troceros</i>	<i>melleri</i>	Tauber <i>et al.</i> (1966)	—	—	—	1	—	—
<i>Ctenosaura</i>	<i>pectinata</i>	Ayala-Guerrero & Huitron-Resendiz (1991)	68	0.61	12.9	2	Yes	—
<i>Ctenosaura</i>	<i>pectinata</i>	Tauber <i>et al.</i> (1968)	—	—	—	2	Yes	—
<i>Ctenosaura</i>	<i>pectinata</i>	Flanigan (1973)	34–66.5	—	—	1	—	—
<i>Ctenosaura</i>	<i>similis</i>	Ayala-Guerrero & Vargas Reyna (1987)	—	1	22.4	2	Yes	—
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley <i>et al.</i> (1977)	71	13.5	—	2	Yes	—
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley & Cohen (1980)	—	—	—	2	—	—
<i>Dipsosaurus</i>	<i>dorsalis</i>	Huntley (1987)	50–62	1–5.9	—	NR	—	—
<i>Iguana</i>	<i>iguana</i>	Peyrethron & Dusan-Peyrethron (1969)	67.5	—	—	2	—	—
<i>Iguana</i>	<i>iguana</i>	Ayala-Guerrero & Mexicano (2008a)	51	0.54	22	2	Yes	—
<i>Iguana</i>	<i>iguana</i>	Flanigan (1973)	34–66.5	—	—	1	—	—
<i>Sauromalus</i>	<i>obesus</i>	Stropes (1975)	—	—	—	2	—	—
<i>Phrynosoma</i>	<i>solare</i>	Romo <i>et al.</i> (1978)	50	—	—	2	Yes	—
<i>Uma</i>	<i>notata</i>	Stropes (1971)	61	—	—	2	—	—
<i>Python</i>	<i>sebae</i>	Peyrethron & Dusan-Peyrethron (1969)	65–85	—	—	1	—	—
<i>Varanus</i>	<i>griseus</i>	Karmanova <i>et al.</i> (1971)	—	—	—	1	—	—

AS, active sleep; NR, not reported; QS, quiet sleep; SLS, sleep-like state.

was related to EMG activity but the 'spike rate' (presumably meaning the sharp wave rate) was not correlated to oxygen consumption resulting in the following observation: 'The absence of negative correlations between spiking and O<sub>2</sub> consumption indicates that in this respect spiking is not analogous to SWS' (Walker & Berger, 1973, p. 462).

Eiland *et al.* (2001) made unit recordings in the brainstem to search for evidence of active-sleep-specific activity. Although specific pontine neurons in the mammalian locus coeruleus and dorsal raphe cease discharging during active sleep while other cholinergic neurons increase in activity during this state, Eiland *et al.* (2001) found that most recorded brainstem neurons discharged only when the animal was active and reduced their discharge rates with immobility. Thus, they were unable to identify neurons related to a specific vigilance state that could be homologous to those observed during active sleep in mammals. Of note is the presence of EMG potentials during sleep that resemble mammalian active-sleep-related twitches (Eiland *et al.*, 2001). However, these authors were unable to confirm this finding using simultaneous polygraphic and video records.

#### (f) Summary

Data on sleep in turtles are sparse but a consensus suggests that they show an immobility state associated with a reduction of heart and respiratory rates. This state may represent a sleep state as the arousal threshold was usually higher in this state when investigated. The electrophysiological results are more difficult to interpret. Five studies reported no change in EEG variables related to the diminution of vigilance, three reported a decrease in EEG amplitude and frequency, one a decrease only in frequency, and one an increase in amplitude and decrease in frequency. HShW have been reported to be more prominent during sleep in half of the studies on turtles. Three studies reported the presence of a mammalian active sleep-like state, and one the presence of mammalian quiet sleep-like state. The only study on a fully aquatic turtle species (Susic, 1972) concluded the absence of sleep in that species based on an unchanged EEG during immobility and the absence of a recovery period after continuous arousal.

## (2) Crocodylians

### (a) Experimental conditions

Three species of Crocodylia were studied, the American alligator (*Alligator mississippiensis*; Van Twyver, 1973), the spectacled caiman (*Caimans sclerops*; Parsons & Huggins, 1965*a,b*; Rechtschaffen, Bassan & Ledecy-Janecek, 1968; Flanigan, Wilcox & Rechtschaffen, 1973; Warner & Huggins, 1978; Meglasson & Huggins, 1979), and the broad-snouted caiman (*Caiman latirostris*; Peyrethon & Dusan-Peyrethon, 1969). Of these eight papers, two (Parsons & Huggins, 1965*a,b*) investigated the effect of temperature on EEG variables, but outside of the context of sleep. Of the remaining six, only three reported recordings lasting 24 h or longer, and only three reported the

use of a day/night light cycle. The others gave no information on the light cycle used, or used constant illumination. The ambient temperature was reported in most cases but was too low for normal behaviour in half of the studies where it was reported. Warner & Huggins (1978) were the only authors to record animals in a breeding colony. Flanigan *et al.* (1973) reported that animals took days to fall asleep under laboratory conditions, even when they were isolated in a sound-attenuated chamber. By contrast, Warner & Huggins (1978) found that sleep was easy to identify in the same species under semi-natural conditions at a higher temperature. This suggests that unnatural laboratory conditions may influence the sleep-wake cycle and that provision of an environment more similar to the natural environment may be required in studies of sleep in reptiles and amphibians.

### (b) Behavioural evidence

Three species of crocodiles have been studied behaviourally in relation to vigilance states. For *Caiman sclerops* (Rechtschaffen *et al.*, 1968; Flanigan *et al.*, 1973; Warner & Huggins, 1978; Meglasson & Huggins, 1979) all reports describe a prolonged resting stage involving total immobility with the eyes closed. The three studies (on *C. sclerops* and *C. latirostris*) that quantified respiratory rate and heart rate reported a decrease in both parameters during sleep (Peyrethon & Dusan-Peyrethon, 1969; Flanigan *et al.*, 1973; Warner & Huggins, 1978). Only Peyrethon & Dusan-Peyrethon (1969) reported fast twitches of the anterior limbs and fingers and eye movement during a sleep-like state in *C. latirostris*. Three arousal threshold evaluation tests on juvenile *C. sclerops* gave variable results: whereas Rechtschaffen *et al.* (1968) found that the animal was always easily aroused, Flanigan *et al.* (1973) highlight difficulties in evaluation of the arousal response because the animals adapted rapidly to the stimuli, and Meglasson & Huggins (1979) reported a higher arousal threshold during sleep.

### (c) Electrophysiological evidence

In contrast to other reptilian species, most studies reported a slight increase in EEG amplitude and a decrease in EEG frequency in the sleep-like state. However, Flanigan *et al.* (1973) noted a decrease in both amplitude and frequency and Van Twyver (1973) reported no change in EEG activity. The latter study concluded that *Alligator mississippiensis* do not sleep as the eyes were rarely closed during periods of immobility. Four studies investigated the relationship between HShW and vigilance. Van Twyver (1973) and Flanigan *et al.* (1973) reported a negative correlation between the number of HShW and overall activity level. Meglasson & Huggins (1979) reported that HShW were present during all activity states (quiescence, arousal, and diving). Peyrethon & Dusan-Peyrethon (1969), by contrast, reported these features only during active wakefulness and suggested a positive relationship between HShW and attention level in *C. latirostris*. Peyrethon & Dusan-Peyrethon (1969) provide the only report of a

mammalian active sleep-like state in one individual in the form of short phases lasting 50 s on average during which the animal displayed rapid eye movements with the eyelids closed. However, the nuchal muscle tone did not change compared to the other sleep stages, and the EEG was similar to that of the awake state. Fast twitches of the anterior limbs and toes were also reported.

(d) *Sleep deprivation*

Flanigan *et al.* (1973) performed a gentle-handling sleep deprivation study on four animals over periods varying between 24 and 48 h. They reported an increase in the duration of immobility periods after sleep deprivation, and a substantial increase in the number of HShW which they referred to as a 'spike rebound'.

(e) *Summary*

Despite the low number of publications concerning sleep in crocodiles we can conclude that all studies report behavioural sleep except for one study on *A. mississippiensis*. The authors of this study concluded that this species does not sleep based on the fact that the animal rarely closed its eyes and that the EEG did not change throughout the day. In contrast to other reptilian species, the EEG amplitude and frequency during sleep-like states in crocodiles appears to change, similar to what is observed during mammalian quiet sleep. Only one study reported eye movements and motor automatisms during behavioural sleep not associated with muscle atonia. The presence of HShW during quiescence was reported in only two studies. Sleep-deprivation studies were performed only once but showed an increase in the immobility period and the number of HShW of normal sleep during the recovery period.

### (3) Squamates

(a) *Experimental conditions*

Most studies on squamates (lizards and snakes) report both behavioural and electrophysiological data, and most involved iguanians. Only two publications deal with more actively foraging species and include a varanid, the desert monitor (*Varanus griseus*; Karmanova *et al.*, 1971), and a glass lizard (*Ophisaurus apodus*; Aristakesyan, 2009). Forty-five percent of experiments report recordings lasting less than 24 h, and 55% did not report the light cycle or used constant illumination. Constant temperatures were used in most experiments, often selected to approximate natural conditions, including the provision of a higher-temperature basking spot in three studies.

(b) *Behavioural evidence*

All studies reported behavioural immobility with the eyes closed; there were no reports concluding that these animals do not sleep. All studies which measured heart and respiratory rates reported a decrease in these parameters related to quiescence. Ten experiments evaluated

the arousal threshold during sleep in squamates, albeit including either gentle handling or electric shocks. All report a higher arousal threshold or an increase in latency of response to stimulation during sleep (Tauber, Rojas-Ramirez & Hernandez Peon, 1968; Peyrethon & Dusan-Peyrethon, 1969; Flanigan, 1973; Stropes, 1975; Huntley & Cohen, 1980; Huntley, 1987; Ayala-Guerrero & Vargas Reyna, 1987; Ayala-Guerrero & Huitron-Resendiz, 1991; Ayala-Guerrero & Mexicano, 2008a).

(c) *Electrophysiological evidence*

Comparing the basic sleep-like state with the awake state, six experiments reported a decrease in both EEG amplitude and frequency, and seven studies reported a decrease in EEG frequency alone. Romo, Cepeda & Velasco (1978) found an increase in EEG amplitude but a decrease in EEG frequency for the regal horned lizard (*Phrynosoma solare*), concluding that there was a clear parallel with the slow-wave sleep of mammals. Peyrethon & Dusan-Peyrethon (1969) reported no change in EEG variables of the green iguana (*Iguana iguana*) during different behavioural states, and a decrease in EEG frequency in the only snake studied to date (*Python sebae*; Peyrethon & Dusan-Peyrethon, 1969). Tauber *et al.* (1968) found no correlation between the number of HShW and behaviour in the Mexican spiny-tailed iguana (*Ctenosaura pectinata*). Huntley (1987); Huntley & Cohen (1980) and Stropes (1975), however, reported a greater HShW occurrence during wake compared to sleep in the desert iguana (*Dipsosaurus dorsalis*) and the chuckawalla (*Sauromalus obesus*), respectively. Nine publications reported an increase in the number of HShW during quiescence. Peyrethon & Dusan-Peyrethon (1969) and Flanigan (1973) for *Iguana iguana*, *Python sebae* and *Ctenosaura pectinata* reported a correlation between respiratory rate and the presence of EEG fusiform oscillations. An interesting behavioural observation involved the presence of asynchronous eye closure in the western fence lizard (*Sceloporus occidentalis*; Mathews *et al.*, 2006) which the authors suggested was correlated with predation risk. Other studies have reported unilateral eye closure in reptiles (Tauber, Roffwarg & Weitzman, 1966; Tauber *et al.*, 1968; Peyrethon & Dusan-Peyrethon, 1969; Flanigan, 1973, 1974; Flanigan *et al.*, 1974; Warner & Huggins, 1978). For a detailed review on unihemispheric sleep see Rattenborg, Amlaner & Lima (2000). The presence of unihemispheric sleep in reptiles remains, however, unclear.

(d) *Sleep deprivation*

Two studies reported the use of a 48 h sleep-deprivation study to test for a homeostatic response (Flanigan, 1973; Ayala-Guerrero & Mexicano, 2008a). Both noted the presence of a recovery period (in *Iguana iguana* and *Ctenosaura pectinata*). Flanigan (1973) also reported a substantial increase in the number of HShW after deprivation, similarly to his results on sleep-deprived chelonians (Flanigan, 1974; Flanigan *et al.*, 1974) and crocodylians (Flanigan *et al.*, 1973).



*(e) Sleep state homologies*

Nine publications report electrophysiological data suggesting the presence of two sleep states in lizards. Three of these identified a sleep stage with high-amplitude slow waves and a second stage with low-amplitude fast waves (Stropes, 1971, 1975; Romo *et al.*, 1978). Other authors propose a homology of this second sleep-like state with mammalian active sleep based on the presence of nuchal muscle atonia (Huntley, Donnelly & Cohen, 1977; Huntley, 1987), eye movements (Tauber *et al.*, 1966; Ayala-Guerrero & Vargas Reyna, 1987; Ayala-Guerrero & Huitron-Resendiz, 1991; Ayala-Guerrero & Mexicano, 2008*a*), motor automatisms (Ayala-Guerrero & Vargas Reyna, 1987; Ayala-Guerrero & Huitron-Resendiz, 1991; Ayala-Guerrero & Mexicano, 2008*a*), or EEG activity (Romo *et al.*, 1978). Tauber *et al.* (1966) studied two species of chameleon [*Trioceros (Chamaeleo) jacksoni*, *Trioceros (Chamaeleo) melleri*] that demonstrate a high degree of eye mobility and visual acuity during wakefulness. They found that eye movements of chameleons during sleep are disconjugate but not associated with atonia, motor twitches, or changes in EEG pattern. They proposed homology with eye movement during sleep in humans but did not suggest homology with mammalian active sleep (Tauber *et al.*, 1966). Stropes (1971, 1975) also reported eye movements during sleep, in *Sauromalus obesus* and the fringe-toed lizard (*Uma notata*), but these eye movements were present during both of the two electrophysiological sleep-like states identified.

*(f) Summary*

All studies agree on the presence of sleep-like states in squamates, but the diversity of findings prevents clear conclusions regarding the electrophysiological nature of sleep in these animals. Interestingly, however, just under half of the studies on squamates report eye movements during sleep, and three studies describe muscle atonia. Moreover, six studies report motor automatisms during sleep, and half of all studies agree on the presence of two sleep states. Finally, five studies concluded the presence of a sleep state homologous to mammalian active sleep. Unfortunately, none of the studies on squamates performed to date have examined all of the electrophysiological and physiological traits that allow the characterization of active sleep in mammals.

**IV. LIMITATIONS OF THE DATA**

Any review aiming to synthesize the existing literature is limited by the quality and quantity of the available data. With respect to sleep in amphibians and reptiles, these limitations are significant. Below we list the principal limitations encountered in trying to derive a broader understanding on the evolution of sleep from these data.

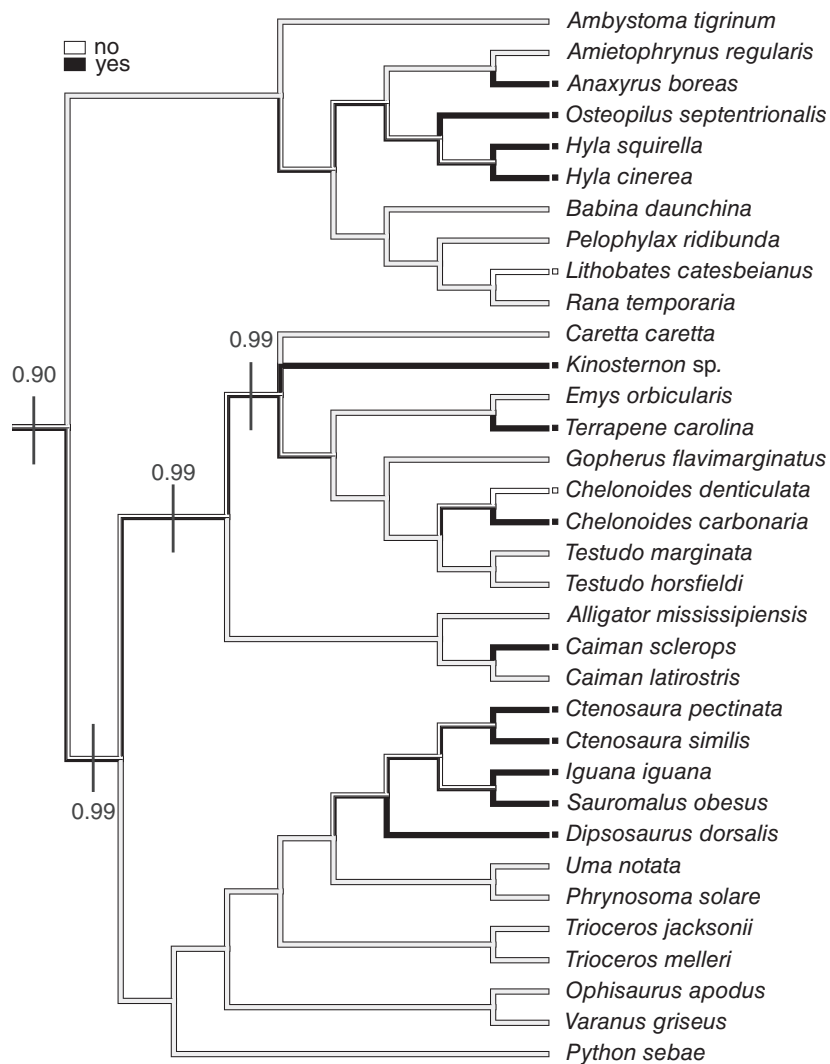
**(1) Methodological limitations**

In mammals it has been shown that sleep is strongly influenced by environmental variables such as lighting

conditions and temperature (Berger & Phillips, 1995). Due to the poikilothermic nature of amphibians and reptiles, these species are strongly influenced by environmental conditions such as light and temperature. Unfortunately, only a few studies have explicitly tested the influence of temperature on the activity–rest cycle and on EEG amplitude or high-amplitude EEG wave distribution in amphibians and reptiles, and not necessarily in the context of sleep. In general, these studies report a reduction of EEG amplitude and a decrease in EEG frequency in relation to ambient temperature (Hunsaker & Lansing, 1962; Parsons & Huggins, 1965*b*; Van Twyver, 1973; Huntley, 1987; De Vera, Gonzalez & Rial, 1994) as has also been shown in mammals (Deboer, 1998). In sleep studies where HShW were recorded, their number appears to decrease with decreasing temperature (Flanigan *et al.*, 1973; Van Twyver, 1973; Huntley & Cohen, 1980; Huntley, 1987). The most complete study on the effect of temperature and seasonal light cycle duration on sleep was performed by Huntley (1987) on *Dipsosaurus dorsalis* who recorded EEG patterns in these lizards at 10°C, 20°C and 30°C during spring, autumn, and winter. He reported that the proportion of sleep decreased slightly with duration of the night. He also found that the occurrence of HShW diminished, and that the EEG amplitude and frequency decreased with temperature suggesting that light cycle and temperature fluctuations have a strong impact on the sleep cycle (Huntley, 1987). However, most studies on amphibian and reptilian sleep report experiments that were performed under constant light and temperature conditions. Moreover, about half of the studies monitored animals for less than 24 h or did not report this parameter, thus imposing strong limitations on the use of these data in a broader comparative context.

Another important factor that may bias sleep patterns is the age of the animal. In mammals and birds, sleep duration, and particularly active sleep duration, is known to decrease with age (Jouvet-Mounier & Astic, 1966; Scriba *et al.*, 2013). To our knowledge, there are no studies exploring the effects of age on sleep patterns in amphibians and reptiles. However, all crocodiles studied to date were juveniles, all turtles were adults, and the lizards included both juveniles and adults. If an age effect is present as in mammals and birds, then this may bias attempts at comparative analyses of sleep using these data. Similarly, although all amphibians studied were non-larval, their exact ages were not reported. If the developmental hypothesis of Roffwarg *et al.* (1966) holds for non-avian reptiles and amphibians, active sleep may be present only *in ovo* during the maturation phase of the animal and may disappear soon after hatching. Corner (1977) reviewed data on motility cycles in early life and during development in the context of sleep and reported that brief movement patterns during periods of relative inactivity were found in all species studied including arthropods, molluscs, fishes, amphibians, birds and mammals. This underlines the importance of investigating the role of development in sleep studies.

The ability of an animal to interact with its environment is also known to influence sleeping patterns. For example, Rattenborg *et al.* (2008) showed that sloths (*Bradypus*

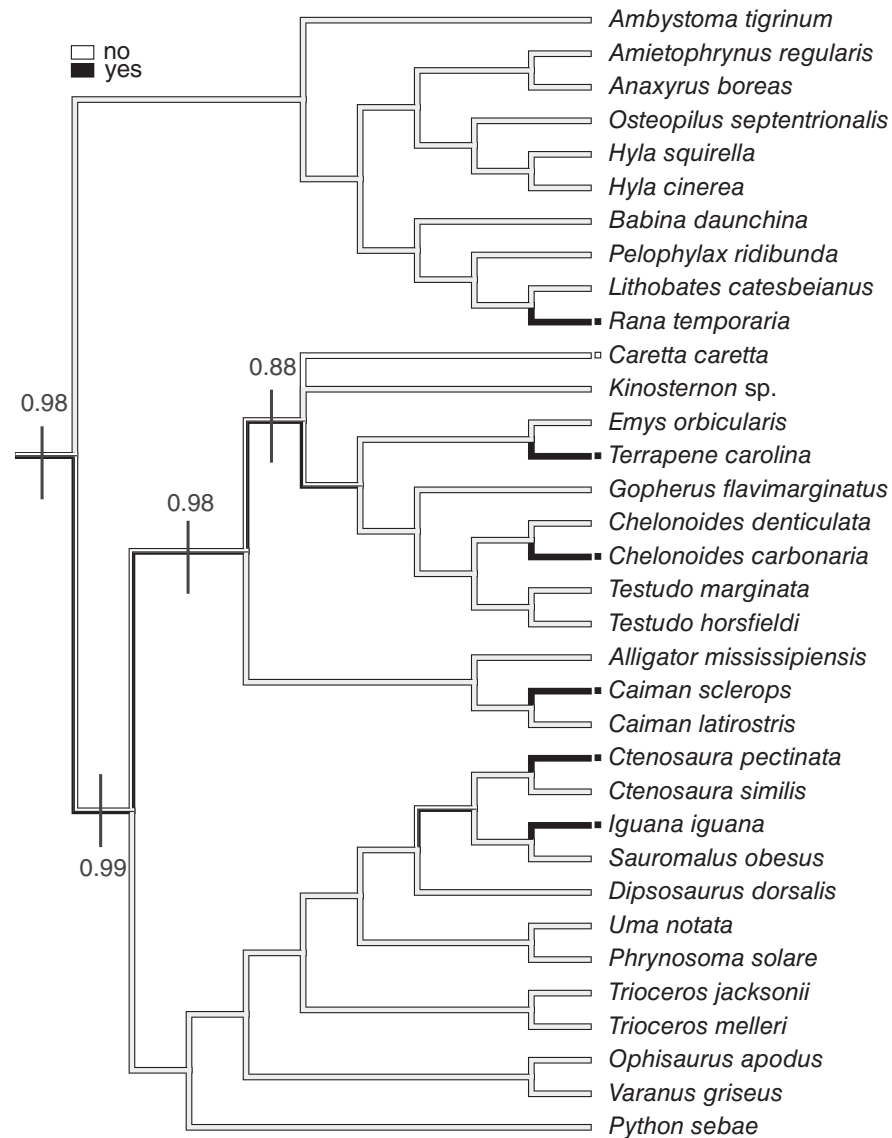


**Fig. 2.** Maximum-likelihood-based ancestral character state reconstruction of the presence of an elevated arousal threshold during quiescence. Black squares indicate the presence of an elevated arousal threshold in terminal taxa. Shading along the branches illustrates the reconstructed ancestral states. Numbers indicate the likelihood of the presence of the character at a given node. Phylogeny based on Pyron & Wiens (2011) for amphibians, Chiari *et al.* (2012) for amniotes, Pyron, Burbrink & Wiens (2013) for squamates and Guillon *et al.* (2012) for turtles. All branches are set to unit lengths as branch lengths were not available for all taxa.

*variegatus*) sleep 40% less when recorded in their natural environment compared to laboratory conditions. Warner & Huggins (1978) noted that animals recorded in a semi-natural environment appear to fall asleep faster than animals recorded in isolation in laboratory conditions. This suggests the importance of factors such as social context when working on gregarious species as has been demonstrated for mice. Febinger *et al.* (2014) reported that mice housed in a group have shorter bouts of active sleep and quiet sleep during the light phase and more active sleep during the dark phase. Moreover, a recent field study focusing on the link between predation and sleep showed that high predation risk may influence the timing of sleep, but not the amount of sleep in sloths (Voinir *et al.*, 2014). It has been shown that simulated predator encounters reduced the amount of sleep in wild rats due to a lower number of

sleep episodes (Lesku *et al.*, 2008a). Further investigations pertaining to the ecological context of sleep are required for non-avian reptiles and amphibians (Lima *et al.*, 2005; Capellini *et al.*, 2008; Revell & Hayes, 2009).

In our overview of the literature, we also observed methodological differences in the evaluation of the arousal threshold response. Flanigan *et al.* (1973) identified the problem of animals habituating to the presentation of cutaneous stimuli. In subsequent studies these workers switched to the use of electric shocks calibrated to a 50% awakening threshold, with sufficient time between shocks to avoid habituation (Flanigan, 1973). However, others did not note a difference in the arousal threshold when using shocks of varying intensity (Walker & Berger, 1973). Unfortunately, shocks in that study were administered at very short intervals (every 10 min on average),

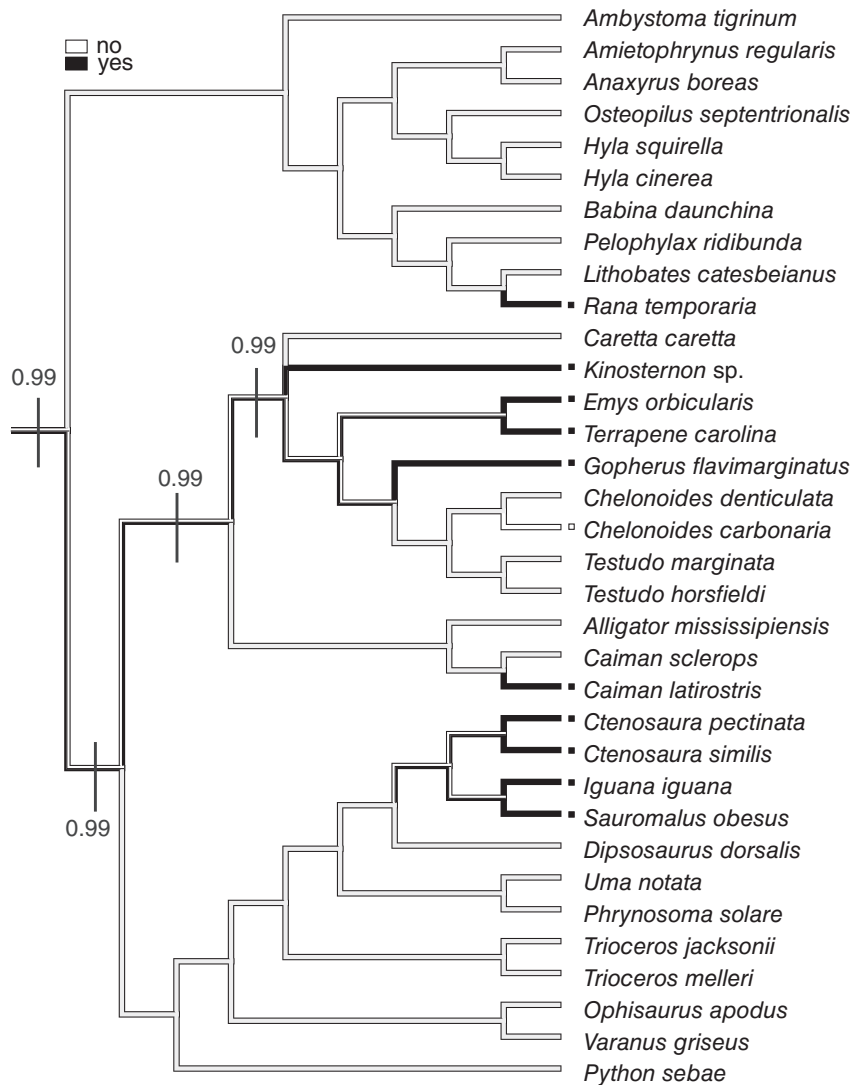


**Fig. 3.** Maximum-likelihood-based ancestral character state reconstruction of the presence of 'sleep homeostasis' (i.e. an increase in sleep-like state duration after sleep deprivation). Black squares indicate the presence of sleep homeostasis in terminal taxa. Other details are as in Fig. 2.

potentially rendering their results on arousal threshold questionable. The same reservations may apply to the study of Hobson (1967) on *Lithobates catesbeianus* where stimuli were administered at very short intervals. Arousal threshold has been evaluated in 22 experiments on amphibians and reptiles, of which 18 report an elevated arousal threshold when animals were in behavioural sleep. Of the four studies that reported no change in arousal threshold during behavioural sleep, two used manual stimulation of which one noted probable habituation (Rechtschaffen *et al.*, 1968; Flanigan *et al.*, 1973), and the two others used electric shocks but with very short latencies (Hobson, 1967; Walker & Berger, 1973).

Another potential confounding factor in comparing data recorded using different protocols is the type of recording device. Two kinds of electrodes are typically

used for recording brain waves: wires and screws. Wire electrodes, because of their smaller contact surface area, record more-local electrical fields in the brain. By contrast, EEGs recorded using screws are averaged over a larger surface area and local activity may be less detectable, resulting in different patterns. About half of the studies in reptiles and amphibians used screw electrodes while the others used wire electrodes, representing a potentially confounding difference. Another important feature of the electrode is the nature of the metal used; the conductivity and the impedance of an electrode are related both to the metal used and to the size of the electrode tip. These properties affect how brain waves are measured especially amplitude measurements, and may thus hinder comparative analyses. Moreover, brain waves are specific to the neuronal organization of the study organism, its



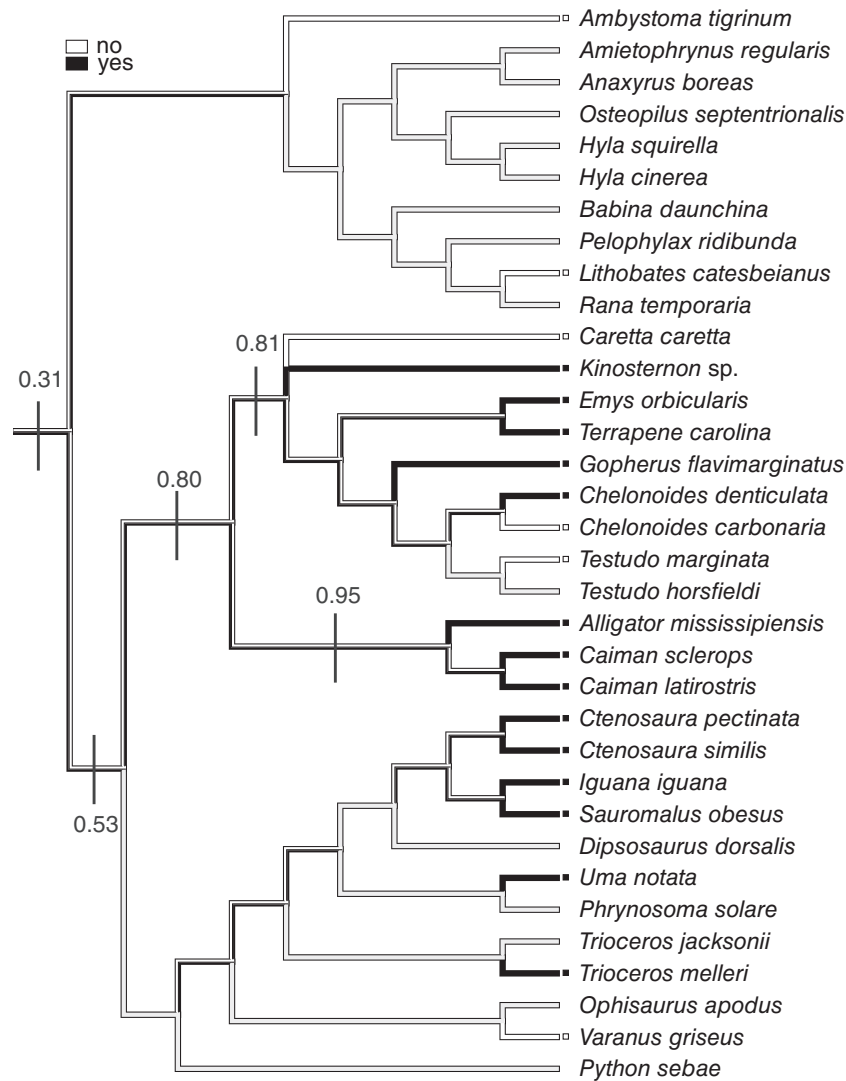
**Fig. 4.** Maximum-likelihood-based ancestral character state reconstruction of the presence of twitches and motor automatisms during sleep-like states. Black squares indicate the presence of twitches or motor automatisms during sleep-like states. Other details are as in Fig. 2.

neuroanatomy, and neuronal connectivity (Bullock & Basar, 1988; Bullock, 1997; Buzsaki, Anastassiou & Koch, 2012). The positioning of the electrodes thus may influence the measurement of brain waves, and as a consequence homology in the nature and origin of the high-amplitude EEG waves reported by different authors is questionable. As no detailed neuro-anatomical atlas exists for reptiles and amphibians, and as electrode placements are often not verified histologically, comparisons among species and studies are rendered difficult. Electromyographic recordings may also be influenced by interspecific differences in muscle fibre-type composition. In contrast to many mammals, the dorsal nuchal musculature in many amphibians and reptiles is not postural in nature and thus may be a very poor indicator of muscle atonia in these animals. In fact, the slow-twitch muscle fibres, which are important in the maintenance of head posture, are present in reptiles, but positioned deep, adjacent to the vertebral

column (Schilling, 2011). The superficial muscles such as the m. spinalis capitis and m. obliquus capitis (Herrel & De Vree, 1999), which are easily recordable, contain mostly fast-twitch fibres and typically are recruited for phasic movements such as cranial elevation during feeding (Gans, Carrier & De Vree, 1985; Herrel, Cleuren & Vree, 1996). It is thus possible that many electromyographic recordings in reptiles may be affected by the positioning of electrodes in non-postural muscles, yet this remains to be verified.

## (2) Limitations of mammalian-centred definitions of sleep

Another important limitation when trying to compare sleep in different groups of vertebrates is the definition of sleep. The criteria used to define sleep and its different stages are principally based on work in mammals. At a behavioural level, parameters are more easily generalized

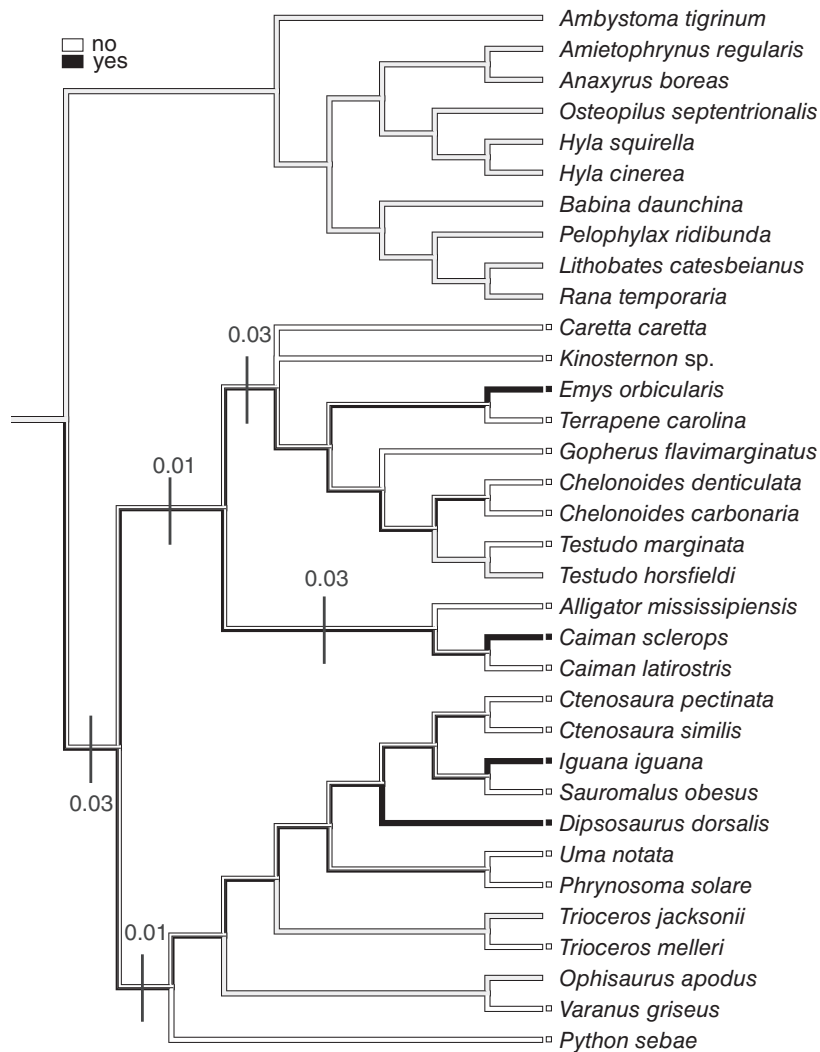


**Fig. 5.** Maximum-likelihood-based ancestral character state reconstruction of the presence of eye movements during sleep-like states. Black squares indicate the presence of eye movements during sleep-like states in terminal taxa. Other details are as in Fig. 2.

as they involve the presence of a stereotypic posture, immobility, rapid state reversibility, an elevated arousal threshold, eye closure, and phasic motor automatisms. However, when working with physiological and electroencephalographic parameters, criteria that are used to identify sleep and its different stages cannot be generalized so easily, particularly since these parameters are linked to the lifestyle, neuroanatomy, and metabolism of a species. In consequence dormancy states have often been considered as independent from sleep states. As ectothermic animals have a body temperature that is dependent on the environment and behaviourally regulated, the classical mammalian sleep definition may be limited when working with non-homeothermic species. As a result, the establishment of homology of sleep based on these classical criteria is tenuous. Moreover, the variability of these phenomena is large in a phylogenetic and ontogenetic context, thus rendering broad-scale interpretations difficult.

Thus, evaluating the presence of mammalian active sleep, or its equivalent, in amphibians and non-avian reptiles is extremely difficult. All studies regarding the presence of an active sleep-like state in reptiles raise the question as to whether their results potentially represent an alternative state such as a short waking event rather than true mammalian active sleep. Yet, reptiles possess pontine structures homologous to those of mammals (Medina *et al.*, 1993; Northcutt, 2002; Ayala-Guerrero & Mexicano, 2008b), and periventricular hypothalamic peptides such as orexin and melanin-concentrating hormone (Cardot, Fellmann & Bugnon, 1994; Dominguez *et al.*, 2010) involved in the regulation of active sleep in mammals (Saper *et al.*, 2010; Luppi, Peyron & Fort, 2013). Basal birds and mammals also have the largest amounts of behavioural active sleep (Siegel *et al.*, 1996, 1999; Lesku *et al.*, 2011) suggesting that this may be an ancestral trait. Phasic motility also appears to exist during the development of reptiles, raising the possibility





**Fig. 6.** Maximum-likelihood-based ancestral character state reconstruction of the presence of muscle atonia during sleep-like states. Black squares indicate the absence of muscle tone during sleep-like states in terminal taxa. Other details are as in Fig. 2.

that this type of sleep may have been present in a common ancestor, and is still present in early ontogenetic stages. This raises questions about the real nature, origin and functions of active sleep across species and its evolutionary and developmental origins.

Ayala-Guerrero & Mexicano (2008a) attempted to test arousal threshold during a supposed active sleep state. They were able to demonstrate an elevated arousal threshold suggesting that these animals were not awake. Whether EEG activity, muscle atonia or the presence of eye movements are better indicators of the presence of an active sleep homologue in amphibians or non-avian reptiles remains unresolved. Because of these limitations, we believe that it is essential to base comparative analyses of sleep, especially in ectothermic animals, on a combination of behavioural, physiological, electroencephalographic, neuro-anatomical, developmental, and ecological variables. Clearly, behavioural features of sleep are more easily interpreted and could be the first to be evaluated in a broad comparative context.

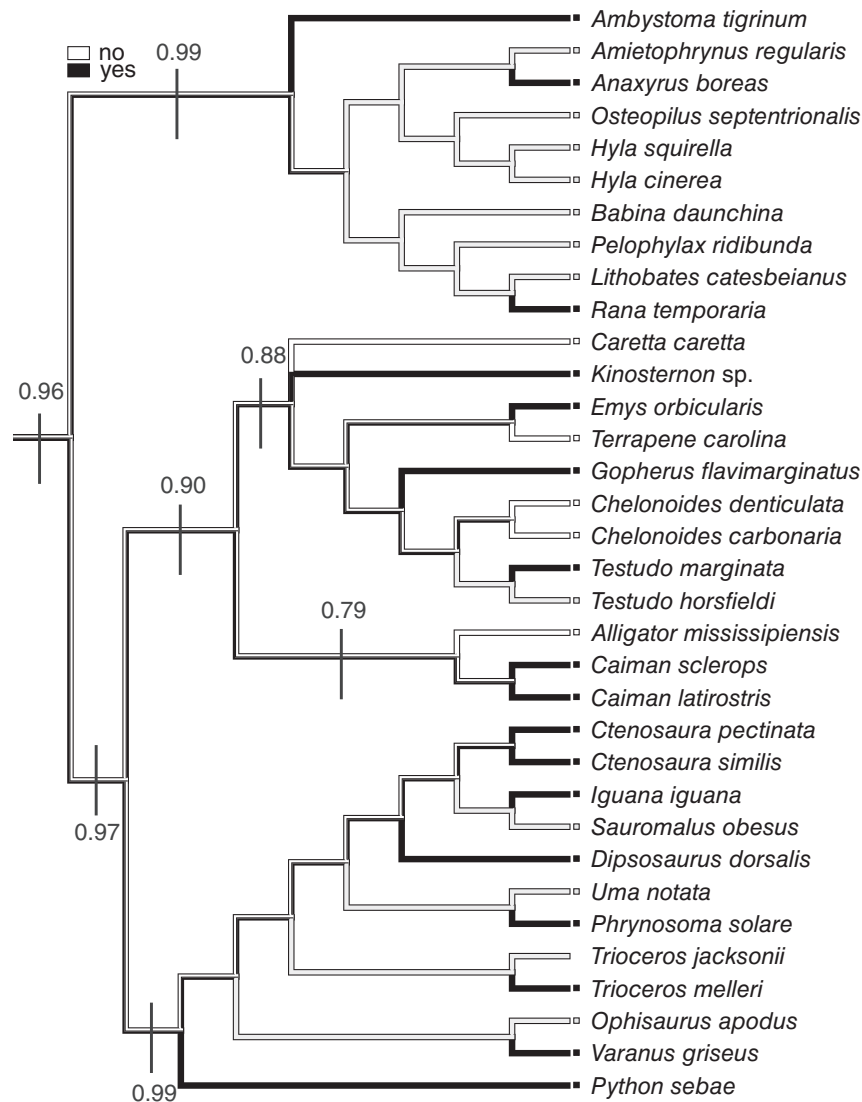
## V. DISCUSSION

### (1) Phylogenetic analysis

In an attempt to infer common sleep features at the origin of tetrapods and amniotes using data on sleep available in the literature, we reconstructed ancestral character states using maximum likelihood methods (Pagel, 1999). In doing so, we attempt to infer patterns rather than processes or function. We focused our analysis on behavioural and electrophysiological parameters given their wider availability in the literature. We could not use sleep and wake quantities as parameters because of the differences in environmental conditions.

#### (a) Behavioural patterns

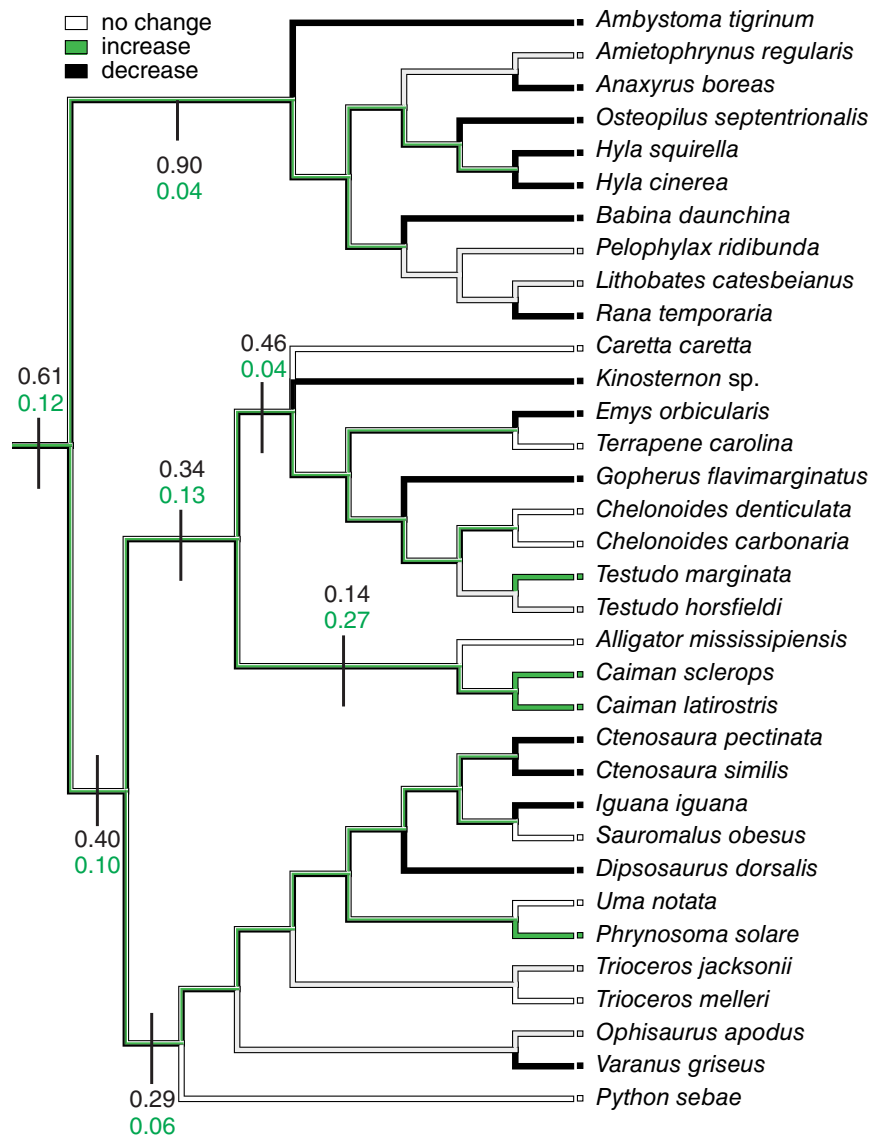
Despite differences in environmental conditions during recording and non-continuous monitoring it appears that all amphibians and non-avian reptiles studied to date



**Fig. 7.** Maximum-likelihood-based ancestral character state reconstruction of the presence of a decrease in EEG frequency during sleep-like states. Black squares indicate a decrease in EEG frequency in terminal taxa. Other details are as in Fig. 2.

display a daily phase of immobility with stereotypic postures that involve eye closure (except in species lacking eyelids). The heart rate and respiratory rate, when measured, show a tendency to decrease when amphibians and reptiles are in a sleep-like state. The evolution of other traits is less clear, but our maximum-likelihood estimates may be informative in inferring evolutionary patterns. The likelihood that an increase in the arousal threshold during quiescence (Fig. 2) is an ancestral feature of tetrapods is high (0.90). Similarly, the likelihood that an increased arousal threshold is an ancestral feature of non-avian reptiles is very high (0.99). A similar analysis of the presence of 'sleep homeostasis', i.e. the presence of a sleep-like recovery state after sleep deprivation (Fig. 3) gave a high likelihood of this being an ancestral feature for tetrapods (0.98) and non-avian reptiles (0.99). The only species that appears to have lost this physiological trait is the only fully aquatic species recorded: the

loggerhead sea turtle (*C. caretta*). This exception suggests constraints imposed by an aquatic environment on the evolution of sleep, as also has been suggested for mammals (Lyamin *et al.*, 2008; Madan & Jha, 2012). Of the behavioural features typically associated with mammalian and avian active sleep, twitches and motor automatisms during sleep (Fig. 4) have a high likelihood (0.99) that they were present in the ancestor of reptiles. The presence of eye movements during periods of behavioural sleep (Fig. 5) is a feature commonly linked to mammalian active sleep. The analysis shows that the likelihood of this being a shared feature of tetrapods is low (0.31) although the likelihood of this feature being ancestral for non-avian reptiles (0.53) and present in the ancestor of archosaurs and chelonians is higher (0.80). Together, these results are consistent with the premise that behavioural sleep is present in amphibians and reptiles, but the behavioural distinction between quiet sleep and active sleep is more



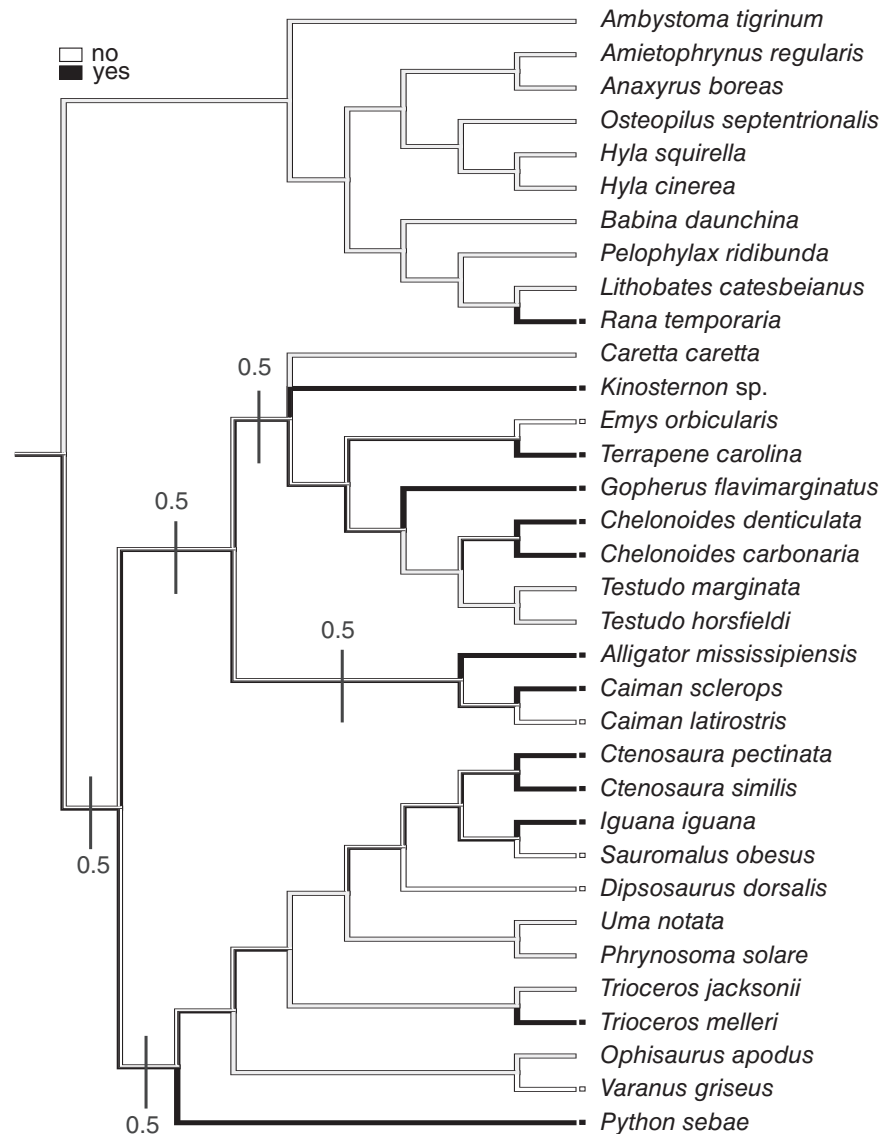
**Fig. 8.** Maximum-likelihood-based ancestral character state reconstruction of EEG amplitude during sleep-like states. Black squares indicate the presence of a decreased EEG amplitude; white squares indicate no change in EEG amplitude, and green squares indicate an increase in EEG amplitude in terminal taxa. The black number at each node is the likelihood of a decrease in EEG amplitude; the green number represents the likelihood of an increase in EEG amplitude. Other details are as in Fig. 2.

difficult despite the probable presence of some features of mammalian active sleep at the base of non-avian reptiles (Fig. 1).

#### (b) Electrophysiological patterns

A feature often thought to characterize active sleep in mammals is the presence of periods of complete muscle atonia. However, the presence of this feature at the base of the reptilian tree (Fig. 6) is unlikely (0.03). This feature has formed the basis of arguments over the presence of active sleep in reptiles. Due to potential bias caused by the different brain regions recorded and by the different methodologies used to record brain waves, we chose to quantify the variation in frequency and amplitude during

sleep relative to the awake state. EEG frequency decreases during sleep-like states in nearly all amphibians and reptiles where it has been measured (Fig. 7). Consequently, the likelihood that this is an ancestral feature for amniotes (0.96) and lissamphibians (0.99) is high. The pattern with respect to EEG amplitude is different, however (Fig. 8). Whereas the likelihood for a decrease in EEG amplitude during sleep-like states to be ancestral for lissamphibians is high (0.90), the likelihood for decreased amplitude to be an ancestral state is much lower at the base of the amniotes (0.40). The condition of no change in EEG amplitude relative to the vigilant state is the more likely at the base of amniotes. Finally, the ancestral character state reconstruction of the presence of HSHW at the base of the reptilian tree is also equivocal (0.5; see Fig. 9).



**Fig. 9.** Maximum-likelihood-based ancestral character state reconstruction of the presence of high-voltage sharp waves during quiescence. Black squares indicate the presence of high-voltage sharp waves during quiescence in terminal taxa. Other details are as in Fig. 2.

## (2) The evolution of sleep

The above review suggests that although some reptilian and amphibian features of sleep are different from sleep characteristics observed in mammals and birds, there are also similarities, suggesting either a common origin or a strong convergence in the evolution of behavioural sleep. However, the origin of the separation of mammalian sleep into two distinct states remains unclear. During active sleep birds display phases of eye movements and occasional twitching (Rattenborg *et al.*, 2011*b*). The EEG patterns of birds during quiet sleep and active sleep are similar to those of mammals. However, some characteristics of mammalian brain activity during sleep, such as thalamocortical spindles, hippocampal sharp wave ripples, and hippocampal theta waves, have not been observed in

birds (Rattenborg *et al.*, 2011*b*) suggesting subtle but potentially important differences. The presence of atonia in postural muscles during active sleep has been reported in birds (Dewasmes *et al.*, 1985), but it is not as clearly defined as in mammals (Amlaner & Ball, 1994; Rattenborg *et al.*, 2011*b*), meaning that conclusions regarding homology cannot be drawn. The same issue concerns changes in sleep during development: in newborn rats an activated EEG, rapid eye movements, muscle atonia, inhibition of the thermoregulatory response, and hippocampal theta waves are not observed during active sleep. Such considerations raise concern regarding the use of only electrophysiological features to identify sleep homologies. Moreover, although the presence of HShW during episodes of behavioural quiescence has been proposed as a marker of quiet sleep in reptiles (Hartse, 2011), HShW have been reported in

amphibians only in the optic tectum of *Rana temporaria* (Lazarev, 1978*a,b*). In reptiles, studies on the presence of HShW are sparse. Some pharmacological experiments in turtles revealed that HShW react in the same way to a pharmacological agent as do ventral hippocampal sharp waves in mammals (Hartse & Rechtschaffen, 1982). The origin of HShW in reptiles, however, remains unknown, despite the fact that some *in vitro* and *in vivo* local field potential recordings in brain areas like the thalamus, medial and dorsal cortex, and the optic tectum have been performed (Servit, Strejckova & Volanschi, 1971; Gaztelu, Garcia-Austt & Bullock, 1991; Lorenzo, Macadar & Velluti, 1999). Thus, additional studies are needed to understand better whether reptilian HShW are generated by structures homologous to those observed in mammals.

The integration of neuroanatomy with brain activity may provide a more comprehensive understanding on sleep-state homologies and evolution. For example, Rattenborg (2006*b*) postulated that the origin of the slow oscillations recorded during sleep in birds could be explained by their higher degree of cortico-cortical (i.e. pallio-pallial) connectivity. He proposed that the origin of these slow waves could be linked to convergent evolution of a higher degree of cortico-cortical connectivity in both mammals and birds. Such observations reveal the challenges of inferring homologies for species that do not possess the neocortical organization necessary for generating the slow waves characteristic of mammalian active sleep.

A high level of variation of active sleep is observed in relation to ambient temperature (Sokoloff & Blumberg, 1998). In addition, the proportion of active sleep is greatest early in development and greatest when animals are at a thermoneutral temperature (Szymusiak & Satinoff, 1981). Consequently, development, the temperature dependence of active sleep, and the mammalian-centric definition mostly based on electrophysiological patterns may prevent its identification in adult reptiles and amphibians. However, our phylogenetic analysis shows that twitches and eye movements were likely present during behavioural sleep at the stem of the reptilian tree. These behavioural patterns of active sleep coupled with the presence of phasic motility *in ovo* in non-avian reptiles, makes the presence of active sleep at the base of the amniotes more than likely even if it might display an electrophysiological phenotype different from that of mammals.

What does this mean for the evolution of sleep? If mammals and birds do not show the same sleep states as their more basal reptilian ancestors, this would suggest convergent evolution of sleep in these two taxa, potentially associated with their homeothermic physiology and higher energy requirements. An alternative hypothesis is that all tetrapods share similar sleep states and neuronal sleep generators, but that the electrophysiological correlates of sleep are induced by differences in neuroanatomy and by consequence result in differences in brain activity. In that case sleep would be ancestral for tetrapods with modification of brain connectivity in mammals and birds driving the differences in brain signatures observed. Thus, all living tetrapods may share a common ancestral type of sleep, but the features common to this ancestral sleep have

not yet been identified. Irrespective of the hypothesis to be tested, data on sleep in amphibians and reptiles are crucial to be able to discriminate among them.

## VI. CONCLUSIONS

(1) Most amphibians and reptiles display behavioural criteria of sleep, including stereotypic postures, maintenance of behavioural immobility, an elevated behavioural response threshold to arousal stimuli, eye closure, and homeostatic regulation of sleep. Twitches or motor automatisms and eye movements during sleep are likely present in reptiles, but their significance and origin remain poorly understood.

(2) The respiratory rate and heart rate appear to decrease in reptiles and amphibians during sleep-like states as is the case in mammals and birds. The decrease in EEG frequency in sleep-like states compared to the awake state is likely an ancestral feature in tetrapods. However, the amplitude of the EEG is more variable across amphibians and non-avian reptiles than in mammals during sleep. High-voltage sharp waves, considered a marker of sleep in mammals, cannot be clearly related to sleep in either amphibians or reptiles, raising the question of whether their presence is really related to sleep.

(3) The combination of all behavioural and electrophysiological patterns typically associated with mammalian active sleep has never been reported in amphibians. However, the behavioural characteristics of mammalian active sleep are likely to have been present at the stem of reptiles. It is not possible to link these features clearly to active sleep in mammals because of the variability in electrophysiological features in reptiles.

(4) Studying sleep in a comparative context is essential, using as many behavioural, physiological, and electrophysiological variables as possible to gain better insights into the nature and evolution of sleep in non-avian reptiles and amphibians. The poikilothermic lifestyle of these groups is associated with important differences in physiology and behaviour when compared to mammals or birds. The parameters typically used to describe sleep in mammals may not apply to animals with such a different physiology, neuroanatomy, and behaviour. The diversity of mechanisms implicated in the control of sleep–wake physiology, as well as the variety of epiphenomena related to sleep observed across vertebrates, may partly explain the lack of consensus regarding the function, evolution, and nature of sleep. Any analysis of the ontogenetic and phylogenetic features of sleep encounters the difficulty of identifying homologous features across species, which may hinder phylogenetic inferences on the evolutionary origins of sleep states (Blumberg, 2013). Despite this, comparative and developmental approaches remain essential to understanding sleep in all of its manifestations. Indeed, the time lost by sleeping rather than being invested in reproduction, parental care, or foraging suggests an essential role for sleep. Finally, the variability in duration, fragmentation, and physiological modifications of sleep across the animal kingdom reveals its



adaptive nature, making it both interesting and crucial to draw parallels among species in an evolutionary context.

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