Salinity influences the distribution of marine snakes: implications for evolutionary transitions to marine life

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Secondary transitions from terrestrial to marine life provide remarkable examples of evolutionary change. Although the maintenance of osmotic balance poses a major challenge to secondarily marine vertebrates, its potential role during evolutionary transitions has not been assessed. In the current study, we investigate the role of oceanic salinity as a proximate physiological challenge for snakes during the phylogenetic transition from the land to the sea. Large-scale biogeographical analyses using the four extant lineages of marine snakes suggest that salinity constrains their current distribution, especially in groups thought to resemble early transitional forms between the land and the sea. Analyses at the species-level suggest that a more efficient salt-secreting gland allows a species to exploit more saline, and hence larger, oceanic areas. Salinity also emerged as the strongest predictor of sea snake richness. Snake species richness was negatively correlated with mean annual salinity, but positively correlated with monthly variation in salinity. We infer that all four independent transitions from terrestrial to marine life in snakes may have occurred in the Indonesian Basin, where salinity is low and seasonally variable. More generally, osmoregulatory challenges may have influenced the evolutionary history and ecological traits of other secondarily marine vertebrates (turtles, birds and mammals) and may affect the impact of climate change on marine vertebrates.

Evolutionary transitions between habitats provide powerful opportunities to understand how selective pressures imposed by the new habitat have shaped the morphology, physiology, and behaviour of organisms. For example, secondary transitions from terrestrial to marine life provide remarkable examples of evolutionary change, driven by contrasting physical and chemical properties of the two environments (Mazin and de Buffrénil 2001). As a result, secondarily marine air-breathing vertebrates display a suite of specializations linked to marine life (Kooyman 1989). Compared to their terrestrial counterparts, these species exhibit a specialized morphology that allows efficient movement through water; their ability to store large amounts of oxygen, and to decrease rates of oxygen use, allow them to remain underwater for long periods; and their reduced susceptibility to high hydrostatic pressures allows them to dive deeply (Kooyman 1989, Boyd 1997, Butler and Jones 1997).

Adaptations of the respiratory system to marine life have attracted extensive research (Seymour and Webster 1975, Boyd 1997, Halsey et al. 2006, Brischoux et al. 2008). For example, a capacity for prolonged apnoea may well have been critical to the evolutionary success of secondarily marine air-breathing vertebrates. However, marine life poses physiological challenges other than respiration – notably, related to the chemical composition of seawater and, in particular, the high concentration of sodium chloride (Schmidt-Nielsen

1983). Because seawater is hyperosmotic relative to the internal milieu of most vertebrates, marine forms will tend to gain salt and lose water across permeable surfaces (Schmidt-Nielsen 1983). Additionally, drinking seawater (inevitable during prey capture) will impose a supplementary salt-load (Costa 2002, Houser et al. 2005). As a consequence, living in seawater entails a significant risk of dehydration, and most marine vertebrates have to regulate their hydro-mineral balance in order to survive (Schmidt-Nielsen 1983).

Secondarily marine vertebrates have evolved a diversity of excretory structures that eliminate excess salt and maintain hydro-mineral balance within a range compatible with life (Schmidt-Nielsen 1983, Houser et al. 2005). The kidneys of marine mammals are lobulated or reniculated, and the countercurrent geometry of elongated nephrons allows them to maintain osmotic balance by excreting large ion loads (Ortiz 2001). Reptilian kidneys lack the loops of Henle that are characteristic of mammals, and they are not able to excrete large ion loads in highly concentrated urine (Peaker and Linzell 1975). However, marine reptiles (sensu lato i.e. including birds) possess specialized extra-renal salt glands capable of excreting concentrated solutions of salt to maintain osmotic balance (Peaker and Linzell 1975).

We have very little fossil evidence of the taxa that are transitional between terrestrial and aquatic habitats – and

even when such fossils are available, they are unlikely to be preserved in enough detail to clarify critical aspects of physiology or behaviour (Mazin and de Buffrénil 2001). Hence, it is difficult to identify the role of physiological challenges (such as those linked to osmoregulation) during phylogenetic transitions to marine life. For example, the presence of salt glands in extinct marine reptiles is still a topic of active debate (Witmer 1997, Modesto 2006, Young et al. 2010, but see Fernández and Gasparini 2008). Additionally, morphological features alone may not provide unequivocal evidence as to function: for example, the specialised salt-excreting features of marine mammals (lobulated kidneys) are also seen in terrestrial ungulates (e.g. ruminants, pigs: Houser et al. 2005). Similarly, salt-excreting glands occur in many terrestrial birds and lizards and some freshwater crocodilians (Peaker and Linzell 1975).

In the current investigation, we examine a study system that facilitates exploration of the hypothesis that oceanic salinity was a major proximate challenge during evolutionary transitions from terrestrial to marine life. We use snakes as our model system because this lineage displays a combination of characteristics that circumvent some of the limitations highlighted above. First, four phylogenetic lineages of snakes independently underwent the transition to marine life; and those four lineages are spread across three Families (Homalopsidae, Acrochordidae and within Elapidae, the subfamilies Laticaudinae and Hydrophiini [Heatwole 1999]). Second, all of these independent transitions exhibit convergent evolution of salt-secreting glands (modified sub-lingual glands in Acrochordidae, Laticaudinae and Hydrophiini [Dunson 1976] and modified pre-maxillary glands in Homalopsidae [Dunson and Dunson 1979]), whereas no extant terrestrial or freshwater snakes are known to possess any such salt-secreting adaptations (Babonis et al. 2011). Third, the high ratio of surface area to volume imposed by the snake body plan (Brischoux and Shine 2011) likely makes maintaining osmotic balance a major physiological challenge for marine snakes, and some species cannot survive without access to fresh or brackish water (Lillywhite and Ellis 1994, Lillywhite et al. 2008). Finally, these lineages of marine snakes vary significantly in their degree of emancipation from the terrestrial environment, covering a continuum of intermediate ecological stages between the land and the ocean (Heatwole 1999). Some marine snakes are among the most fully marine tetrapod taxa, completely independent from land, whereas others depend upon terrestrial habitats for many of their daily activities.

This unique combination of traits within snakes provides a model system for investigating the role of oceanic salinity as a physiological challenge during the colonization of marine environments by terrestrial vertebrates, and suggests the following predictions: 1) salinity should constrain the oceanic distributions of marine snakes, and the extent of this constraint should vary concomitantly with their degree of marine life. 2) Marine snake richness should be negatively correlated with oceanic salinity, and positively correlated with variation in salinity because highly variable salinity should provide frequent access to rehydration with less saline water. 3) Snake species with more effective salt-excreting glands should extend into more saline areas, and hence be distributed over larger areas. 4) The transition from terrestrial

to marine life is most likely to have occurred in areas with low and/or variable salinity.

To test these predictions, we adopted two complementary approaches. First, we used large-scale analyses on broad phylogenetic groups (i.e. the four lineages of marine snakes) to identify abiotic factors affecting sea snake distribution, and snake richness. Second, we used a fine-scale (species-level) analysis to investigate the relationship between salt gland function and the geographic distributions of sea snake species.

Material and methods

Marine snake groups

Snakes underwent four independent transitions to marine life (ancestors of the present-day Homalopsidae, Acrochordidae and [within the Elapidae] the Laticaudinae and Hydrophiini). The potential minimum crown age for the marine adapted homalopsids is 18 My; 16 My for acrochordids; 13 My for laticaudines; and 7 My for hydrophiines (Alfaro et al. 2008, Sanders and Lee 2008, Sanders et al. 2010a). Although the minimum ages for these transitions fall in the Miocene, the transition to marine life could have occurred earlier (e.g. due to possible disparities between stem and crown ages for some of these clades). We did not include one facultatively marine species of Colubridae (the salt-marsh snake *Nerodia clarkii*) in our analyses because the osmoregulatory biology (e.g. presence or absence of a salt gland) remains unknown in this species (Babonis et al. 2011).

The species belonging to these four phylogenetic groups are conservative in their broad life-history traits (except hydrophiines, see below).

1) The Acrochordidae (filesnakes) consists of three extant species, placed within a single genus, *Acrochordus*. The three acrochordid species span the entire range from freshwater (*A. arafurae*) through brackish (*A. javanicus*) to saltwater (*A. granulatus*) (McDowell 1979). We considered only the two latter species in our analysis. These species are widely distributed through marine, estuarine and freshwater habitats, especially mangrove areas, and feed mainly on gobioid fishes which are located by active foraging in small crevices on the sea bottom (Voris and Glodek 1980). The posterior sublingual gland of *A. granulatus* is a salt gland (Dunson and Dunson 1973, see Biogeography and salt gland function). Freshwater drinking is important to water balance of *A. granulatus* (Lillywhite and Ellis 1994).

2) The Homalopsidae (Oriental-Australian rear-fanged water snakes) include 10 genera and 34 species of medium-sized snakes distributed from India across southeast Asia to northern Australia (Gyi 1970, Greer 1997, Voris et al. 2002). Eight species are coastal, living in mangrove forests, tidal mudflats, near-shore coastal waters and estuarine habitats (*Bitia hydroides*, *Cantoria violacea*, *C. annulata*, *Cerberus rynchops*, *Enhydris bennetti*, *Fordonia leucobalia*, *Gerarda prevostiana*, *Myron richardsonii*, Heatwole 1999), and we included these eight marine species in our analyses. Most research on ecophysiology linked to marine life has been conducted on the dog-faced water snake *C. rynchops*, which has the widest distribution of any homalopsid, and

is the only homalopsid species known to possess a salt gland (pre-maxillary glands, Dunson and Dunson 1979, see Biogeography and salt gland function).

3) The Laticaudinae (sea kraits) are present in most coral reef areas of the Indian and Western-Pacific Oceans (Heatwole 1999). These amphibious snakes forage for fish in coral reef systems but return to land to perform all other activities (digestion, mating, egg-laying, Heatwole 1999, Brischoux and Bonnet 2009). Of the eight laticaudine species (Heatwole et al. 2005, Cogger and Heatwole 2006), we did not include the brackish water, lake-locked *L. crockeri* (derived from marine ancestors) in our analysis (Cogger et al. 1987). Laticaudine sea snakes have salt glands (Dunson and Taub 1967, Dunson et al. 1971, Babonis et al. 2009, see Biogeography and salt gland function), but often drink freshwater (on land during rainfall events; Guinea 1991, Bonnet and Brischoux 2008, Lillywhite et al. 2008).

4) The Hydrophiini (true sea snakes) include the majority of marine snake species. They are the most truly marine of all extant reptilian taxa, never voluntarily leaving the water (Heatwole 1999). Two secondarily-derived freshwater species were excluded from our analyses (Heatwole and Cogger 1993). We thus included 54 hydrophiine species that are found mostly on coral reef areas of the Indian and Western Pacific ocean (Heatwole 1999). Although most species are benthic foragers on coral reefs, one taxon (yellow-bellied sea snake *Pelamis platurus*) is truly pelagic, and hence is the only marine snake not associated with the benthic community (Marsh et al. 1993). *Pelamis platurus* is widespread over the tropical Indo-Pacific, feeding on small fish at the sea surface (Heatwole 1999, Brischoux and Lillywhite 2011). Salt glands have been described in five different genera of hydrophiines (see Biogeography and salt gland function), and it is likely that all hydrophiines possess such glands (Dunson 1968, Dunson and Dunson 1974).

Our analyses below are largely based on these four independent examples of the transition from terrestrial to marine life (acrochordids, homalopsids, laticaudines, and hydrophiines). Due to the unique life history of *P. platurus* (see above), we also performed our analyses on hydrophiines excluding *P. platurus*, and on *P. platurus* alone.

Geographic range data

Data on the distribution of each species of marine snake were taken from extent-of-occurrence range maps assembled by the IUCN Sea Snake Specialist Group (< www.iucnredlist. org/technical-documents/spatial-data >). These range maps provided detailed information on the distribution of the four phylogenetic groups (acrochordids, n = 8083 grid cells; homalopsids, n = 779; laticaudines, n = 9085; hydrophiines, n = 26975 [excluding *P. platurus*; n = 9898; and *P. platurus* alone, n = 17077]). Range maps for each group were converted to a 0.25° grid to match the resolution of our environmental data (see below). Because we were interested in the abiotic factors associated with the transition to marine life, we excluded all snake locations from freshwater environments.

Environmental data

We investigated whether six environmental variables were correlated with the presence of each snake group: 1) mean annual salinity, 2) standard deviation of mean monthly salinity, 3) mean annual temperature, 4) standard deviation of mean monthly temperature, 5) water depth, and 6) distance to the nearest shoreline. Temperature and salinity data (0.25° resolution) were taken from the World Ocean Atlas 2009 (Antonov et al. 2010, Locarnini et al. 2010). Bathymetry data (0.017° resolution) were extracted from the ETOPO1 global relief model (Amante and Eakins 2009), and re-sampled to match the resolution of the climate data. Both climate and bathymetry data were standardized to exclude terrestrial areas using a vector shoreline dataset (National Geospatial-Intelligence Agency 1990). This shoreline dataset was also used to calculate the distance from the centre of each grid cell to the nearest shoreline (using an equidistant cylindrical projection). Pair-wise correlations between environmental variables were generally low (r = 0.015–0.48), with the exception of mean temperature and variation in temperature, which were significantly correlated with each other (r = −0.77).

Statistical analyses

To determine which environmental variables influenced sea snake presence/absence, we used classification trees as implemented in the rpart and caret libraries in R 2.12.0 (R Development Core Team). Classification trees have the advantage of flexibly incorporating variable interactions and non-linear relationships, while producing models that are easy to interpret (De'ath and Fabricius 2000). These models attempt to explain variation in a categorical response variable (in this case, sea snake presence/absence) by repeatedly splitting the data into smaller, more homogenous groups. Splits in the tree divide the response variable into two mutually exclusive subsets (nodes) using a rule based on a single explanatory variable (e.g. mean temperature ≤ 20°C). Each resulting node is then further partitioned using this splitting procedure. The end result is a decision tree consisting of numerous nodes, each of which is defined by a threshold value of an explanatory variable, a typical value of the response, and the sample size within the group (De'ath and Fabricius 2000).

Classification trees require the selection of a tree-size that represents an optimal trade-off between model parsimony and classification error. To determine the optimal-sized tree, we used a cross-validation procedure based on the area under a receiver operating characteristic curve (AUC), which is a threshold-independent measure of classification accuracy that ranges from 0.5 (random) to 1.0 (perfect). Our validation procedure involved three steps. First, we grew a nested sequence of trees of increasing size. Second, we calculated the AUC of each tree based on leave one-group out cross-validation, whereby CT models were trained on 75% of the data, and tested on the remaining 25%. To reduce sampling errors, this step was repeated 50 times for each tree. Finally, we chose the tree that had the highest AUC that was within one standard error of the maximum AUC value (Breiman et al. 1984).

Species distribution models such as classification trees require information on locations where species are absent, in addition to where they are present. Because we lacked absence data, we randomly sampled 'pseudo-absences' (Elith et al. 2006) within the latitudinal and longitudinal extents occupied by sea snakes, which roughly corresponds to the distributions of the Indian and Pacific Oceans. The number of pseudo-absence records for each group was equal to five times the number of presence records.

In a second set of analyses, we used regression trees to explore environmental constraints on sea snake species richness. IUCN range maps were intersected with a 0.25° resolution grid, and the number of species in each grid cell was summed. The procedure used to select the optimal-sized tree which adequately predicted sea snake richness was the same as that used in our presence-absence analyses, except that R^2 was used as a measure of model fit instead of AUC. *Pelamis platurus* was excluded from these analyses due to its unique life-history and large geographic range.

Biogeography and salt gland function

Because marine snakes have evolved specific excretory structures that eliminate excess salt, the efficiency of such salt secreting structures may limit (and thus, predict) the salinity characteristics of the oceanic areas exploited by marine snakes. To test this hypothesis, data on maximum sodium (Na^+) excretion rates were collected from the literature for eight species belonging to the four lineages of marine snakes (Acrochordidae: *Acrochordus granulatus*, Dunson and Dunson 1973; Homalospidae: *Cerberus rynchops*, Dunson and Dunson 1979; Laticaudinae: *Laticauda semifasciata*, Dunson and Taub 1967; Hydrophiini: *Aipysurus laevis*, *Lapemis hardwickii*, *Hydrophis elegans*, *H. major*, Dunson and Dunson 1974, and *Pelamis platurus*, Dunson 1968). We then correlated these maximum Na^+ excretion rates with two measures of environmental tolerance: 1) the maximum salinity within each species' geographic range, and 2) geographic range size (calculated using cylindrical equal-area projections).

Results

All four groups of sea snakes were largely restricted to areas within 46 km from the nearest shoreline (Fig. 1). Within these areas, however, environmental constraints differed among the four groups.

The highest-ranked classification tree for Hydrophiini contained a secondary split on the distance to the nearest shoreline at 32.6 km (Fig. 1A), suggesting that this variable
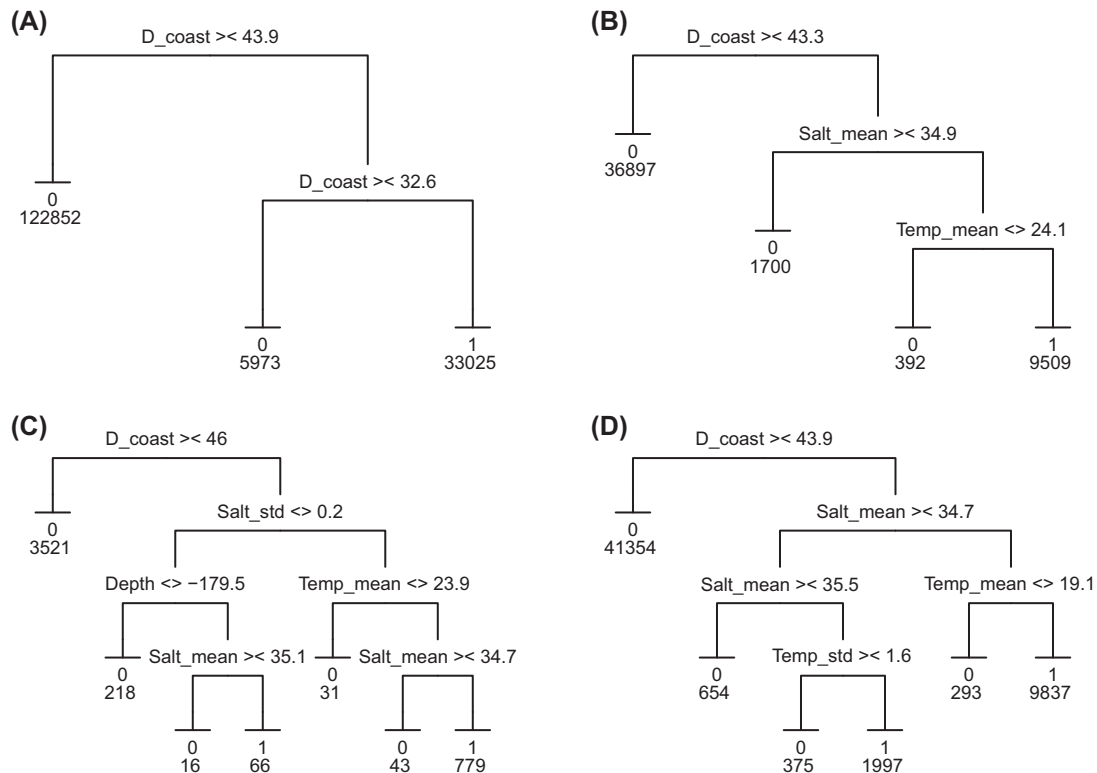

Figure 1. Highest-ranked classification trees predicting the distributions of hydrophiine (A), acrochordid (B), homalopsid (C), and laticaudine (D) sea snakes at a global scale. Trees are read from top to bottom. Each split in the tree attempts to divide the response variable (snake presence [1] or absence [0]) into homogenous groups according to a threshold value of an explanatory variable (shown above each split). A '<>' symbol indicates that cases with lower values go to the left, whereas a '><' symbol means that cases with lower values go to the right. Sea snake presence (1) or absence (0) and sample sizes (number of 0.25° grid cells) are given below each node. D_coast = distance to the nearest shoreline (km), Depth = ocean depth (m), Temp_mean = mean annual temperature (°C), Temp_std = standard deviation of mean monthly temperature, Salt_mean = mean annual salinity (according to the practical salinity scale), Salt_std = standard deviation of mean monthly salinity.

was the sole constraint on the distribution of this group. Results were qualitatively similar when the wide-ranging *P. platurus* was considered in isolation (Supplementary material Appendix 1). However, when *P. platurus* was excluded from the Hydrophiini (Supplementary material Appendix 1), the remaining hydrophiines were also more likely to occur in warm climates (annual temperatures $> 19.9°C$), suggesting that the broad thermal tolerance of *P. platurus* obscured the influence of temperature on the overall distribution of hydrophiines.

Environmental constraints on the distribution of Acrochordidae were more complex. Acrochordids occupied areas that were characterized by low annual salinities (< 34.9 PSS) and high annual temperatures ($> 24.1°C$; Fig. 1B).

Homalopsid occurrence was linked to monthly variation in salinity (Fig. 1C). In regions with high salinity variation (standard deviation > 0.2), homalopsids were most likely to occur in areas with warm annual temperatures ($> 23.9°C$) and low annual salinities (< 34.7 PSS). In areas with low variation in salinity (standard deviation < 0.2), homalopsids were more likely to be found in shallow waters (< 179.5 m) with low annual salinity (< 35.1 PSS).

Correlates of laticaudine distribution varied according to annual salinity levels (Fig. 1D). In low salinity regions (< 34.7 PSS), laticaudines were more likely to occur in areas with warm annual temperatures ($> 19.1°C$). However, under more saline conditions (> 34.7 PSS), laticaudines occupied areas with low annual salinities (< 35.5 PSS) and more stable thermal properties (variation in monthly temperatures < 1.6). Classification trees for all groups had extremely high predictive accuracy, with all models falling within the good to excellent category of Swets (1988) (Supplementary material Appendix 2).

The best regression tree of sea snake richness also had high explanatory power ($R^2 = 63.8\% \pm 0.0137$), but was structurally complex, containing eleven variable splits

(Fig. 2). Salinity emerged as the strongest predictor of sea snake richness, with annual salinity (Fig. 3A) and variation in monthly salinity (Fig. 3B) being the most frequently selected variables in the tree. In areas with low annual salinities (< 34.2 PSS), species richness was highest in areas with warm annual temperatures ($> 22.7°C$), shallow depths (> 117.5 m), and low annual salinities (< 33.3 PSS). Under more saline conditions (> 34.2 PSS), sea snake richness was highest in shallow areas (> 101.5 m) with low annual salinities (< 36 PSS) and high heterogeneity in monthly salinity (standard deviation > 0.2).

Maximum Na^+ excretion rates were positively correlated with maximum annual salinity within each species' oceanic range ($F_{1,6} = 9.63$, $R^2 = 0.61$, $p = 0.02$, Fig. 4A) and also with the oceanic range size of each species ($F_{1,6} = 5.93$, $R^2 = 0.49$, $p = 0.05$, Fig. 4B). That is, species with more effective salt-excreting glands penetrated into areas of higher salinity, and had broader geographic distributions.

Discussion

Collectively, our results support the hypothesis that oceanic salinity is a significant abiotic constraint on the current distributions of marine snakes. In turn, that result suggests that dealing with salinity has been a major evolutionary challenge during the transition from terrestrial to marine life in snakes. Data on oceanic salinity predicted the geographic distributions of three of our four lineages of snakes, and these effects were largely consistent with each group's degree of emancipation from the ancestral terrestrial environment. Homalopsids, acrochordids and laticaudines are restricted to estuarine habitats, mangroves, tidal mudflats, coastal waters and/or are amphibious, and thus may resemble early transitional forms along the gradient of habitat between the land and the ocean (e.g. laticaudines are amphibious and
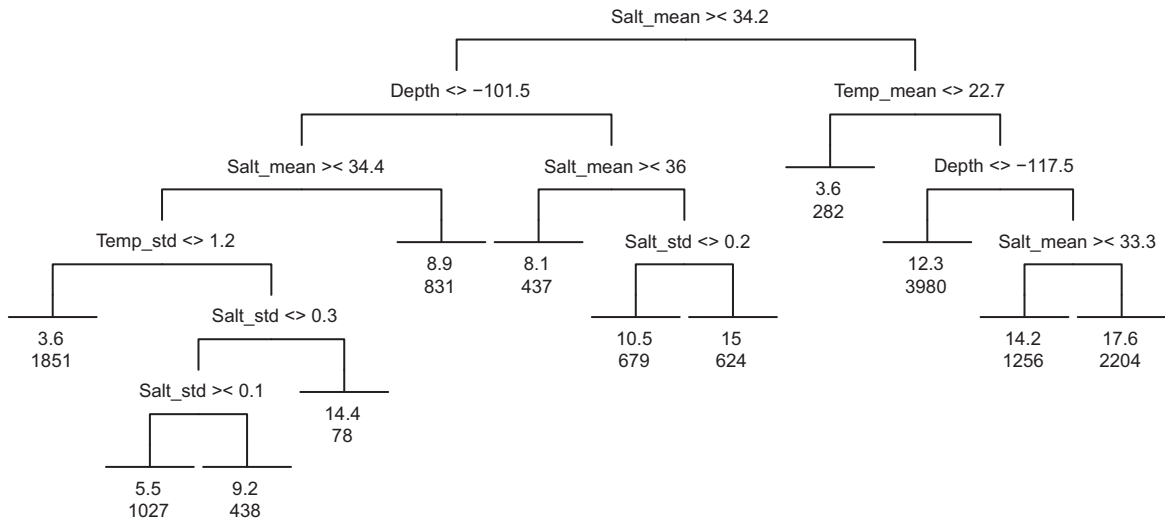

Figure 2. Highest-ranked regression tree predicting the richness of marine snakes at a global scale. The tree is read from top to bottom. Each split in the tree attempts to divide the response variable (snake richness) into homogenous groups according to a threshold value of an explanatory variable (shown above each split). A '<>' symbol indicates that cases with lower values go to the left, whereas a '><' symbol means that cases with lower values go to the right. Mean species richness and sample sizes (number of 0.25° grid cells) are given below each node. Variable abbreviations are the same as those used in Fig. 1.
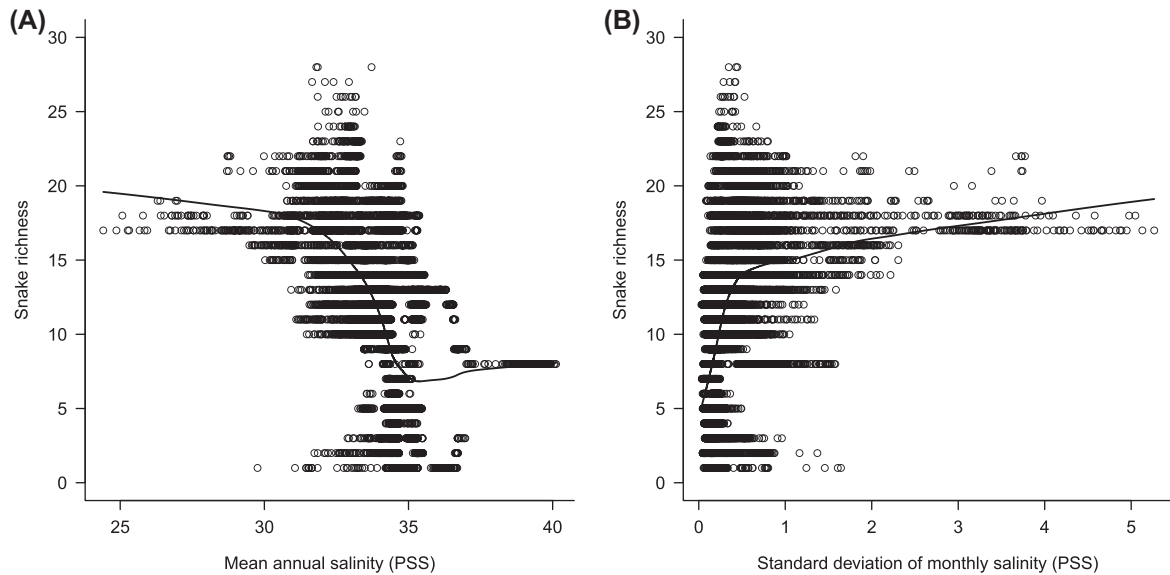
Figure 3. Relationships between sea snake richness and mean annual salinity (A), and monthly variation of salinity (B). PSS = practical salinity scale.

require extended time on land, Heatwole 1999). In contrast, salinity did not influence the distribution of hydrophiines (either with or without *P. platurus*), consistent with the fully marine habits of these species.

Geographic patterns in the species richness of marine snakes also were linked to oceanic salinity. Salinity was repeatedly included in the best regression tree of sea snake species richness, representing ∼64% of the variable splits (four occurrences of mean annual salinity, and three occurrences of salinity variation, Fig. 2). Overall, species richness of marine snakes was negatively linked

Figure 4. Maximum salinity within each species' geographic range (A) and geographic range size (B) vs the maximum Na+ excretion rate of each species. Different symbols represent different snake lineages: open squares = Acrochordidae, open circles = Homalopsidae, filled circles = Laticaudinae, filled squares = Hydrophiini.

to mean annual salinity, but positively related to variation in monthly salinity (Fig. 3). Although both parameters are somewhat correlated with one another, they provide complementary information. Low salinity levels should decrease the cost of osmotic maintenance through reduced salt gland functioning (Schmidt-Nielsen 1983), as well as decreased rates of water loss to the environment (Lillywhite et al. 2008, 2009). Similarly, high variation in salinity levels should allow regular access to brackish water over short time-scales (e.g. a month in our study), again decreasing the cost of osmotic maintenance, and dehydration rates. Perhaps more importantly, high variability in salinity levels is likely to reflect frequent, heavy rainfall events, during which marine species can drink fresh or slightly brackish water to restore their hydration state. Amphibious species have direct access to freshwater from precipitation on land (Bonnet and Brischoux 2008, Lillywhite et al. 2008), and snakes that are at sea (e.g. 'true' sea snakes or foraging sea kraits) also have access to fresh water lenses that form at the ocean surface (Lillywhite and Ellis 1994). Importantly, low and highly variable salinity levels would be expected to alleviate the energetic costs of osmoregulation even in species having very effective salt-secreting glands (e.g. many hydrophiines). In turn, the low osmoregulatory costs associated with such environmental conditions would have presented terrestrial watersnakes with an unoccupied niche which may have promoted rapid diversification, and ultimately led to higher species richness.

At the species-level, salt gland function (maximum excretion rates of Na+) was linked to geographic distributions in eight species of snakes belonging to the four lineages of marine snakes that we studied. Both maximum salinity within a species' range, and the size of a species' oceanic range, were positively correlated with salt gland function (Fig. 4). Although causal links remain unclear, these results strongly suggest that a more efficient salt gland (i.e. being able to excrete higher salt loads) allows a
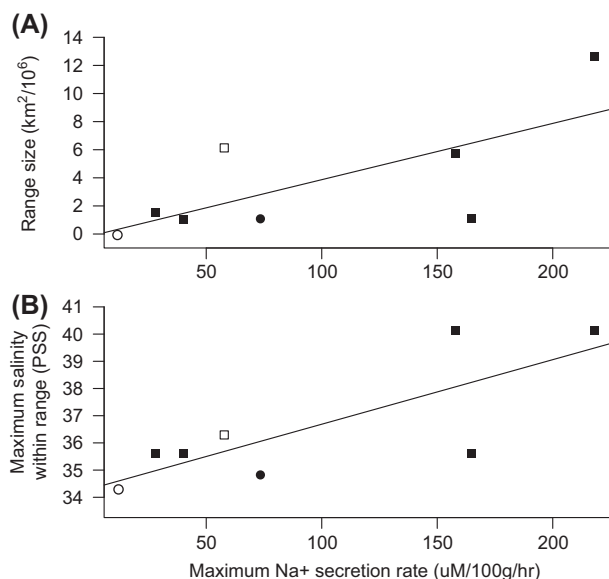
species to cope with more saline waters, and hence to exploit larger oceanic areas.

There are several caveats to our study, particularly relating to the resolution of the range maps used in our analyses. First, the IUCN range maps included several occurrences of sea snakes outside their core ranges (i.e. waifs). Inclusion of data points outside the range in which populations are viable might have introduced noise into our analyses. This is especially the case for extremely northern or southern locations (e.g. locations of *P. platurus* around the Cape of Good Hope). However, no obvious decision rules would have satisfactorily allowed us to remove potential waifs without biasing our results. Additionally, IUCN range maps were drawn solely over a coastal margin of 50 km, thereby potentially removing locations that shelter snake populations further at sea. However, this should produce a significant bias for three of the four lineages that we studied (Acrochordidae, Homalopsidae and Laticaudinae) because their ecologies limit their distribution to coastal waters. The situation is different for the totally marine Hydrophiini. In the case of benthic foragers (all species except *P. platurus*), geographic distributions should be constrained to waters ca 100 m deep (Heatwole 1999, Brischoux et al. 2007; Fig. 2). Although this bias is likely weak because shallow waters tend to be close to shore, the actual ranges of these species might be slightly underestimated in the available maps. For the pelagic *P. platurus*, available information suggests an extensive range covering the whole Indo-Pacific (Heatwole 1999). Clearly, the coastal range from IUCN range maps will underestimate the actual range of this species. This underestimation likely influenced our finding that distance to the nearest coast-line was such a prominent explanatory factor even for *P. platurus*. Because coastal waters

are less saline than offshore waters due to extended freshwater runoff from land, limiting the range of hydrophiine sea snakes to coastal waters might explain why we detected no effect of salinity on their geographic distribution. This possibility is supported by the link between salt gland function and species distributions (Fig. 4). However, this putative bias should be conservative as it concerns the most marine adapted lineage. In spite of these limitations, the relationships we found between coarse geographic information and independently gathered physiological data suggests that the effects of salinity we detected are likely to be robust to errors in our range maps.

Globally, our results suggest that salinity plays a significant role in the current distributions and richness of marine snakes, and does so more profoundly in species which are presumably analogous to the early transitional forms between the land and the sea (i.e. amphibious and near-coastal species). This result supports the fourth prediction in our Introduction, by suggesting that specific geographic areas may have offered favourable conditions for early transitional forms to cope with salinity constraints (Dunson and Mazzotti 1989). All four independent transitions to marine life in snakes may have taken place in a single area (between Malaysia, Indonesia and northern Australia) that currently contains representatives of all marine snake lineages. The highest values of marine snake species richness occur in this area (Fig. 5), across the Sunda and Sahul shelves (hereafter 'Indonesian Basin' for simplicity). The hypothesis that this single area has played a role in all four transitions to marine life in snakes is congruent with the geographic distributions of terrestrial outgroups identified by phylogenetic analyses of the marine snake groups (Keogh 1998, Keogh et al. 1998,
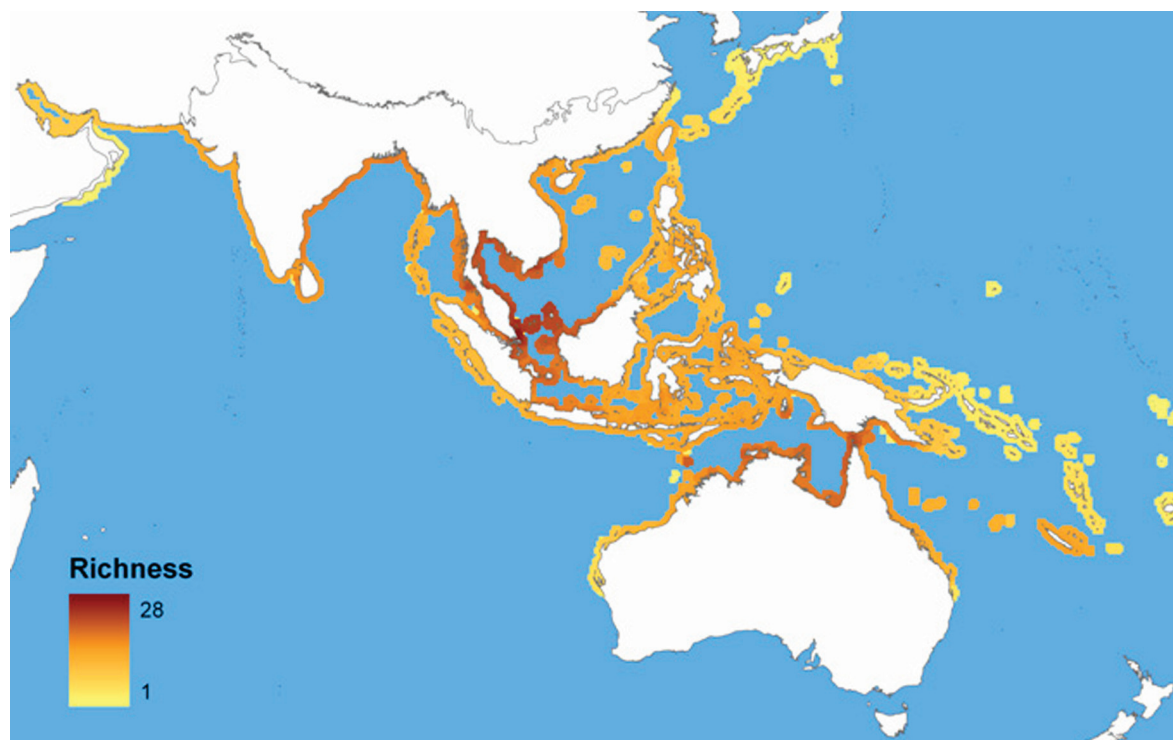

Figure 5. Map of sea snake richness at a 0.25° resolution (excluding *P. platurus*).

Alfaro et al. 2008, Sanders and Lee 2008, Sanders et al. 2008, 2010a, b). The inference that all of these transitions occurred in the same geographic area over an extended time frame (i.e. 11 My elapsed between the estimated minimum crown ages of Homalopsidae and Hydrophiinae) highlights the Indonesian Basin as offering unusually favourable environmental conditions for this major evolutionary transition. The Indonesian Basin is currently characterised by extensive interface environments between the land and the sea, such as large areas of shallow water, numerous islands and islets, as well as ragged coastlines and mangroves. In addition, the Indonesian Basin is a biodiversity hotspot, especially for coral reef ecosystems (e.g. the Coral Triangle, Green and Mous 2004). All of these biogeographic characteristics might have facilitated the transition from terrestrial to marine life in snakes. Although it is difficult to robustly infer the paleo-biogeographical history of this region, repeated marine transgressions and regressions over the Indonesian Basin during the Neogene, and a monsoonal climate, are likely to have offered somewhat similar conditions (Guo 1993, Voris 2000, Woodruff 2003, Hanebuth et al. 2011). The low salinity of the Indonesian Basin, as well as its high seasonal variation in salinity (due to the monsoonal regime) might have been critical in the evolutionary transition to marine life in snakes, providing an additional proximate cause as to why this region has served as a 'centre of origin' for biodiversity (Ekman 1953, Briggs 2000, Mora et al. 2003).

To conclude, our results suggest that salinity plays an important role in the current distributions of marine snakes in the tropical Indo-Pacific Ocean. The low and variable salinity of the Indonesian Basin is likely to have facilitated evolutionary transitions to marine life in snakes, and may indeed have been the location for all four of the transitions represented by extant marine snake species. More robust biogeographic inferences will require clarifying how salinity (among other parameters) affected rates of speciation, and extinction among marine snakes within a phylogenetic framework. Nevertheless, our findings suggest that the importance of salinity may have been underestimated in evolutionary and ecological studies of secondarily marine vertebrates (Gutiérrez et al. 2011). Future studies should examine the role of this environmental parameter in other lineages of secondarily marine vertebrates such as turtles, birds and mammals, all of which display osmoregulatory adaptations functionally similar to those of snakes. Additionally, studies on the likely impact of future climate change on marine vertebrates could usefully incorporate salinity and its forecasted changes. If oceanic salinity drives species distributions, and rainfall patterns and currents drive oceanic salinity, then changes in salinity may well mediate the impacts of climate-change on marine organisms.

References

Alfaro, M. E. et al. 2008. Phylogeny, evolutionary history, and biogeography of Oriental-Australian rear-fanged water snakes (Colubroidea: Homalopsidae) inferred from mitochondrial and nuclear DNA sequences. – Mol. Phylogenet. Evol. 46: 576–593.

Amante, C. and Eakins, B. W. 2009. ETOPO1 1 Arc-Minute Global Relief Model: procedures, data sources and analysis. – NOAA Technical Memorandum NESDIS NGDC-24.

Antonov, J. I. et al. 2010. World Ocean atlas 2009, Volume 2: salinity. – NOAA Atlas NESDIS 69, U.S. Government Printing Office, Washington, DC.

Babonis, L. S. et al. 2009. Immunolocalization of Na+/K+–ATPase and Na+/K+/2Cl− cotransporter in the tubular epithelia of sea snake salt glands. – Comp. Biochem. Physiol. A 154: 535–540.

Babonis, L. S. et al. 2011. Renal responses to salinity change in snakes with and without salt glands. – J. Exp. Biol. 214: 2140–2156.

Bonnet, X. and Brischoux, F. 2008. Thirsty sea snakes forsake refuge during rainfall. – Austral Ecol. 33: 911–921.

Boyd, I. L. 1997. The behavioural and physiological ecology of diving. − Trends Ecol. Evol. 12: 213–217.

Breiman, L. et al. 1984. Classification and regression trees. – Wadsworth International Group, Belmont, CA, USA.

Briggs, J. C. 2000. Centrifugal speciation and centres of origin. − J. Biogeogr. 27: 1183–1188.

Brischoux, F. and Bonnet, X. 2009. Life history of sea kraits in New Caledonia. – In: Grandcolas, P. (ed.), Zoologia Neocaledonica 7. Mémoires du Muséum national d'Histoire naturelle, pp. 133–147.

Brischoux, F. and Lillywhite, H. B. 2011. Light- and flotsam-dependent "float-and-wait" foraging in pelagic sea snakes (*Pelamis platurus*). – Mar. Biol. 158: 2343–2347.

Brischoux, F. and Shine, R. 2011. Morphological adaptations to marine life in snakes. – J. Morphol. 272: 566–572.

Brischoux, F. et al. 2007. Snakes at sea: diving performances of free-ranging sea kraits. – Proceedings of the 11th Annual Meeting on Health, Science and Technology, Tours Univ., France.

Brischoux, F. et al. 2008. Allometry of diving capacities: ectothermy versus endothermy. – J. Evol. Biol. 21: 324–329.

Butler, P. J. and Jones, D. R. 1997. Physiology of diving of birds and mammals. – Physiol. Rev. 77: 837–899.

Cogger, H. and Heatwole, H. 2006. *Laticauda frontalis* (De Vis, 1905) and *Laticauda* n. sp. Nov. from Vanuatu and New Caledonia (Serpentes: Elapidae: Laticaudinae), a new lineage of sea kraits. – Rec. Aust. Mus. 58: 245–256.

Cogger, H. et al. 1987. The status and natural history of the Rennell Island sea krait, *Laticauda crockeri* (Serpentes: Laticaudidae). – J. Herpetol. 21: 255–266.

Costa, D. P. 2002. Osmoregulation. – In: Perrin, W. F. et al. (eds), Encyclopedia of marine mammals. Academic Press, pp. 337–342.

De'ath, G. and Fabricius, K. E. 2000. Classification and regression trees: a powerful yet simple technique of ecological data analysis. – Ecology 81: 3178–3192.

Dunson, W. A. 1968. Salt gland secretion in the pelagic sea snake *Pelamis*. – Am. J. Physiol. 215: 1512–1517.

Dunson, W. A. 1976. Salt glands in reptiles. – In: Gans, C. and Dawson, W. R. (eds), Biology of the Reptilia. Physiology A. Vol. 5. Academic Press, pp. 413–445.

Dunson, W. A. and Taub, A. M. 1967. Extrarenal salt excretion in sea snakes. – Am. J. Physiol. 213: 975–982.

Dunson, W. A. and Dunson, M. K. 1973. Convergent evolution of sublingual salt glands in the marine file snake and the true sea snakes. – J. Comp. Physiol. 86: 193–208.

Dunson, W. A. and Dunson, M. K. 1974. Interspecific differences in fluid concentration and secretion rate of sea snake salt glands. – Am. J. Physiol. 227: 430–438.

Dunson, W. A. and Dunson, M. K. 1979. A possible new salt gland in a marine homalopsid snake (*Cerberus rynchops*). – Copeia 1979: 661–672.

Dunson, W. A. and Mazzotti, F. J. 1989. Salinity as a limiting factor in the distribution of retiles in Florida Bay: a theory for the estuarine origin of marine snakes and turtles. – Bull. Mar. Sci. 44: 229–244.

Dunson, W. A. et al. 1971. Sea snakes: an unusual salt gland under the tongue. – Science 173: 437–441.

Ekman, S. 1953. Zoogeography of the sea. – Sidgwick and Jackson.

Elith, J. et al. 2006. Novel methods improve prediction of species' distributions from occurrence data. – Ecography 29: 129–151.

Fernández, M. and Gasparini, Z. 2008. Salt glands in the Jurassic metriorhynchid *Geosaurus*: implications for the evolution of osmoregulation in Mesozoic crocodyliforms. – Naturwissenschaften 95: 79–84.

Green, A. and Mous, P. J. 2004. Delineating the Coral Triangle, its ecoregions and functional seascapes. – The Nature Conservancy Expert Workshop, South East Asia Center for Marine Protected Areas, Bali, Indonesia.

Greer, A. E. 1997. The biology and evolution of australian snakes. – Surrey Beatty.

Guinea, M. L. 1991. Rainwater drinking by the sea krait *Laticauda colubrina*. – Herpetofauna 21: 13–14.

Guo, S.-X. 1993. The evolution of the Cenozoic tropical monsoon climate and monsoon forests in southwestern China. Evolving landscapes and evolving biotas of east Asia since the Mid-Tertiary. – In: Jablonski, N. G. (ed.), Proc. 3rd. Confr. Evolution of the East Asian Environment. Centre for Asian Studies, Univ. of Hong Kong, Hong Kong, pp. 123–135.

Gutiérrez, J. S. et al. 2011. Understanding the energetic costs of living in saline environments: effects of salinity on basal metabolic rate, body mass and daily energy consumption of a long-distance migratory shorebird. – J. Exp. Biol. 214: 829–835.

Gyi, K. K. 1970. A revision of colubrid snakes of the subfamily Homalopsinae. – Univ. Kansas Mus. Nat. Hist. Publ. 20: 47–223.

Halsey, L. G. et al. 2006. A phylogenetic analysis of the allometry of diving. – Am. Nat. 167: 276–287.

Hanebuth, T. J. J. et al. 2011. Formation and fate of sedimentary depocentres on southeast Asia's Sunda Shelf over the past sea-level cycle and biogeographic implications. – Earth Sci. Rev. 104: 92–110.

Heatwole, H. 1999. Sea snakes. – Australian Natural History Series, Univ. of New South Wales, NSW.

Heatwole, H. and Cogger, H. G. 1993. Family Hydrophiidae. – In: Walton, D. (ed.), Fauna and flora of Australia, Volume 2. Australian Government Publishing Service, Canberra, pp. 310–318.

Heatwole, H. et al. 2005. Geographic variation in sea kraits of the *Laticauda colubrina* complex (Serpentes: Elapidae: Hydrophiinae: Laticaudini). – Herpetol. Monogr. 19: 1–136.

Houser, D. S. et al. 2005. Ecology of water relations and thermoregulation. – eLS.

Keogh, J. S. 1998. Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. – Biol. J. Linn. Soc. 63: 177–203.

Keogh, J. S. et al. 1998. Phylogenetic relationships of terrestrial Australo-Papuan elapid snakes (subfamily Hydrophiinae) based on cytochrome b and 16S rRNA sequences. – Mol. Phylogenet. Evol. 10: 67–81.

Kooyman, G. 1989. Diverse divers: physiology and behavior. – Springer.

Lillywhite, H. B. and Ellis, T. M. 1994. Ecophysiological aspects of the coastal-estuarine distribution of acrochordid snakes. – Estuaries 17: 53–61.

Lillywhite, H. B. et al. 2008. Sea snakes (*Laticauda* spp.) require fresh drinking water: implication for the distribution and persistence of populations. – Physiol. Biochem. Zool. 81: 785–796.

Lillywhite, H. B. et al. 2009. Water exchange and permeability properties of the skin in three species of amphibious sea snakes (*Laticauda* spp.). – J. Exp. Biol. 212: 1921–1929.

Locarnini, R. A. et al. 2010. World Ocean atlas 2009, Volume 1: temperature. – NOAA Atlas NESDIS 68, U.S. Government Printing Office, Washington, DC.

Marsh, H. et al. 1993. Conserving marine mammals and reptiles in Australia and Oceania. – In: Moritz, C. and Kikkawa, J. (eds), Conservation biology in Australia and Oceania. Surrey Beatty, pp. 225–244.

Mazin, J.-M. and de Buffrénil, V. 2001. Secondary adaptation of tetrapods to life in water. – Dr. Friedrich Pfeil.

McDowell, S. B. 1979. A catalogue of the snakes of New Guinea and the Solomons, with special reference to those in the Bernice P. Bishop Museum. Part III. Boinae and Acrochordoidea (Reptilia, Serpentes). – J. Herpetol. 13: 1–92.

Modesto, S. P. 2006. The cranial skeleton of the Early Permian aquatic reptile *Mesosaurus tenuidens*: implications for relationships and palaeobiology. – Zool. J. Linn. Soc. 146: 345–368.

Mora, C. et al. 2003. Patterns and processes in reef fish diversity. – Nature 421: 933–936.

National Geospatial-Intelligence Agency 1990. World vector shoreline (1:250 000) data. – <http://shoreline.noaa.gov/data/datasheets/wvs.html>.

Ortiz, R. M. 2001. Osmoregulation in marine mammals. – J. Exp. Biol. 204: 1831–1844.

Peaker, M. and Linzell, J. 1975. Salt glands in birds and reptiles. – Cambridge Univ. Press.

Sanders, K. L. and Lee, M. S. Y. 2008. Molecular evidence for a rapid late-Miocene radiation of Australasian venomous snakes (Elapidae, Colubroidea). – Mol. Phylogenet. Evol. 46: 1180–1188.

Sanders, K. L. et al. 2008. Molecular phylogeny and divergence dates for Australasian elapids and sea snakes (hydrophiinae): evidence from seven genes for rapid evolutionary radiations. – J. Evol. Biol. 21: 682–695.

Sanders, K. L. et al. 2010a. Phylogeny and divergence times of filesnakes (*Acrochordus*): inferences from morphology, fossils and three molecular loci. – Mol. Phylogenet. Evol. 56: 857–867.

Sanders, K. L. et al. 2010b. Uncoupling ecological innovation and speciation in sea snakes (Elapidae, Hydrophiinae, Hydrophiini). – J. Evol. Biol. 23: 2685–2693.

Schmidt-Nielsen, K. 1983. Animal physiology: adaptations and environments. – Cambridge Univ. Press.

Seymour, R. S. and Webster, M. E. D. 1975. Gas transport and blood acid-base balance in diving sea snakes. – J. Exp. Zool. 91: 169–182.

Swets, J. A. 1988. Measuring the accuracy of diagnostic systems. – Science 240: 1285–1293.

Voris, H. K. 2000. Maps of Pleistocene sea levels in southeast Asia: shorelines, river systems and time durations. – J. Biogeogr. 27: 1153–1157.

Voris, H. K. and Glodek, G. S. 1980. Habitat, diet and reproduction of the file snake, *Acrochordus granulatus*, in the straits of Malacca. – J. Herpetol. 14: 108–111.

Voris, H. K. et al. 2002. Phylogenetic relationships of the Oriental-Australian rear-fanged water snakes (Colubridae: Homalopsinae) based on mitochondrial DNA sequences. – Copeia 2002: 906–915.

Witmer, L. M. 1997. The evolution of the antorbital cavity of archosaurs: a study in soft-tissue reconstruction in the fossil record with an analysis of the function of pneumaticity. – Soc. Vertebrate Palaeontol. Mem. 3: 1–73.

Woodruff, D. S. 2003. Neogene marine transgressions, palaeogeography and biogeographic transitions on the Thai–Malay Peninsula. – J. Biogeogr. 30: 551–567.

Young, M. T. et al. 2010. The evolution of Metriorhynchoidea (mesoeucrocodylia, thalattosuchia): an integrated approach using geometric morphometrics, analysis of disparity, and biomechanics. – Zool. J. Linn. Soc. 158: 801–859.