doi: 10.1111/j.1420-9101.2008.01525.x

Molecular phylogeny and divergence dates for Australasian elapids and sea snakes (hydrophiinae): evidence from seven genes for rapid evolutionary radiations

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Keywords:

Bayesian inference;
Elapidae;
molecular divergence dating;
rapid radiation;
snakes.

Abstract

One of the most prolific radiations of venomous snakes, the Australo-Melanesian Hydrophiinae includes ~100 species of Australasian terrestrial elapids plus all ~60 species of viviparous sea snakes. Here, we estimate hydrophiine relationships based on a large data set comprising 5800 bp drawn from seven genes (mitochondrial: ND4, cytb, 12S, 16S; nuclear: rag1, cmos, myh). These data were analysed using parsimony, likelihood and Bayesian methods to better resolve hydrophiine phylogeny and provide a timescale for the terrestrial and marine radiations. Among oviparous forms, *Cacophis, Furina* and *Demansia* are basal to other Australian elapids (core oxyuranines). The Melanesian *Toxicocalamus* and *Aspidomorphus* group with *Demansia*, indicating multiple dispersal events between New Guinea and Australia. *Oxyuranus* and *Pseudonaja* form a robust clade. The small burrowing taxa form two separate clades, one consisting of *Vermicella* and *Neelaps calanotus*, and the other including *Simoselaps*, *Brachyurophis* and *Neelaps bimaculatus*. The viviparous terrestrial elapids form three separate groups: *Acanthophis*, the *Rhinoplocephalus* group and the *Notechis–Hemiaspis* group. True sea snakes (Hydrophiini) are robustly united with the *Notechis–Hemiaspis* group. Many of the retrieved groupings are consistent with previous molecular and morphological analyses, but the polyphyly of the viviparous and burrowing groups, and of *Neelaps*, are novel results. Bayesian relaxed clock analyses indicate very recent divergences: the ~160 species of the core Australian radiation (including sea snakes) arose within the last 10 Myr, with most inter-generic splits dating to between 10 and 6 Ma. The *Hydrophis* sea snake lineage is an exceptionally rapid radiation, with > 40 species evolving within the last 5 Myr.

Introduction

Hydrophiine snakes are taxonomically and morphologically the most diverse clade within the venomous Elapidae, with more than 160 species recognized in ~50 genera. This includes ~100 Australo-Melanesian terrestrial species, ~60 species of completely aquatic true sea snakes (Hydrophiini: Slowinski & Keogh, 2000), and six species of amphibious sea kraits (Laticaudini: Heatwole *et al.*, 2005). Such a prolific and diverse radiation presents an excellent model for evolutionary research and is of particular interest because the terrestrial forms and the true sea snakes are each known to represent rapid radiations with the marine forms nested within the terrestrial clade (Keogh, 1998; Lukoschek & Keogh, 2006). Numerous studies have attempted to reconstruct hydrophiine relationships using varied data sets of internal and/or external anatomical characters (McDowell, 1970; Shine, 1985; Wallach, 1985; Lee, 1997; Keogh, 1999; Rasmussen, 2002; Scanlon & Lee, 2004), karyotypes (Mengden, 1985), immunological distances

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(Schwaner *et al.*, 1985) and mitochondrial sequences (Keogh, 1998; Keogh *et al.*, 1998, 2000; Slowinski & Keogh, 2000; Scanlon & Lee, 2004; Lukoschek & Keogh, 2006).

These studies confirmed the monophyly of Hydrophiinae, Laticaudini, Oxyuraninae (all hydrophiines except Laticaudini: Sanders & Lee, 2008), Hydrophiini and several terrestrial genera (Hutchinson, 1990; Greer, 1997). Strong support was found for a clade including most viviparous Australian taxa plus the true sea snakes (e.g. McDowell, 1969; Scanlon & Lee, 2004; Lukoschek & Keogh, 2006), and a '*Notechis*' clade of at least *Notechis*, *Austrelaps*, *Hoplocephalus* and *Tropidechis* (e.g. Shine, 1985; Wallach, 1985; Keogh *et al.*, 1998, 2000; Scanlon & Lee, 2004). A large-bodied oviparous clade including at least *Pseudonaja*, *Oxyuranus*, *Demansia* and *Pseudechis* has been consistently recognized (Wallach, 1985; Keogh *et al.*, 1998; Keogh, 1999; Scanlon & Lee, 2004) and the Melanesian *Loveridgelaps*, *Salomonelaps*, *Ogmodon* and *Toxicocalamus* have generally been placed in basal positions relative to most Australian taxa (e.g. McDowell, 1970; Keogh, 1998; Keogh *et al.*, 1998; Scanlon & Lee, 2004). Despite these advances, previous analyses have been unable to resolve many, particularly basal, relationships within hydrophiines. Mitochondrial sequence data have recovered phylogenies characterized by a backbone of short, weakly supported internal branches that suggest a rapid evolutionary radiation (e.g. Keogh *et al.*, 1998; Scanlon & Lee, 2004; Lukoschek & Keogh, 2006).

In this paper, we assembled an expanded molecular data set for the hydrophiines, with additional mitochondrial and new nuclear gene sequences (~5800 bp) for 49 taxa, including 33 ingroup and two outgroup genera. Our sampling includes all genera of terrestrial forms and genera representing the deeper divergences in true sea snakes. Hydrophiine relationships are reconstructed using parsimony, likelihood and Bayesian phylogenetic inference. The analysis robustly resolves the affinities of the true sea snakes (Hydrophiini), and suggests polyphyly of the viviparous and fossorial terrestrial forms. We test whether several phylogenetic hypotheses (e.g. monophyly of fossorial taxa and of the viviparous clade) can be rejected based on our expanded molecular data set. Finally, a relaxed molecular clock analysis provides the first well-corroborated timescale for divergences across all major hydrophiine lineages. In particular, we provide dates for the initial terrestrial and marine radiations, demonstrating that these clades diversified extremely rapidly.

Methods

Taxon selection, DNA amplification and sequencing

Forty-nine taxa were sampled, representing 33 genera of terrestrial and marine hydrophiines and two elapines. Whole genomic DNA was isolated from blood, liver and skeletal muscle using standard proteinase K protocols (Sambrook *et al.*, 1989). Three nuclear and four mitochondrial fragments were selected to ensure a mix of relatively slow and fast loci. Nuclear loci were: ~1100 bp of RAG-1 (recombination reactivating gene 1); ~650 bp of c-*mos* (oocyte maturation factor); ~520 bp of MyHC-2 (myosin heavy chain 2) intron. Mitochondrial loci were ~700 bp of ND4 (NADH dehydrogenase subunit 4); ~1100 bp of cyt*b* (cytochrome *b*); ~510 bp of 16S rRNA (16S small subunit ribosomal RNA) and ~940 bp of 12S rRNA (12S small subunit ribosomal RNA). Standard PCR protocols with AmpliTaq Gold reagents (Perkin-Elmer/-Applied Biosystems, Norwalk, CT, USA) were used; thermal cycling parameters varied between loci and taxa. PCR products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and an ABI 3700 automated sequencer. Some additional sequencing was outsourced to a commercial firm (Macrogen, Seoul, South Korea). Sequences were edited manually; coding sequences were aligned by eye using reading frame in BioEdit Sequence Alignment Editor (Hall, 1999), RNA sequences were aligned using Clustal X (Thompson *et al.*, 1997) using default parameters and refined by eye. Unalignable regions were determined by eye and excluded from consideration; computer-based methods for constructing and evaluating alignments can yield counterintuitive results, which is why such alignments are still adjusted by eye in many studies. GenBank accession numbers and specimen details are listed in Appendix I; primer information is shown in Appendix II.

Phylogenetic analyses

The aligned sequences were concatenated and analysed simultaneously. The incongruence length difference (=partition homogeneity) test (Farris *et al.*, 1994), implemented on the four loci, suggested extreme congruence ($P \geq 0.99$), and the well-supported nodes in separate analyses of each data set were highly consistent (results not shown). In any case, there are compelling arguments for simultaneous analyses even when data sets apparently conflict (see Kluge, 1989; Gatesy *et al.*, 1999). Phylogenetic analysis used parsimony and likelihood methods implemented in PAUP* v4.0b10 (Swofford, 2002) and Bayesian inference implemented in MrBayes v3.0 (Huelsenbeck & Ronquist, 2001), run on an IBM eServer 1350 Linux cluster with 129 dual 2.4 GHz nodes. The dating analyses (implemented in BEAST) also co-estimated phylogeny using Bayesian methods (see below). All trees were rooted using the elapines *Bungarus fasciatus* and *Naja naja* as outgroups; reciprocal monophyly of the elapine and hydrophiine subfamilies is strongly supported by molecular (Slowinski & Keogh, 2000; Scanlon & Lee, 2004) and morphological (McDowell, 1970) evidence. Maximum parsimony (MP) tree searches were heuristic with 1000 random step-wise sequence addition replicates and tree-bisection-reconnection (TBR) branch swapping.

All characters, and all substitution types, were weighted equally. Treerot (Sorenson, 1999) was used to calculate branch support and partitioned branch support, i.e. the support contributed by each locus (mt, myh, mos and rag) for each node (Baker & DeSalle, 1997). Maximum likelihood (ML) analysis was performed using 10 random taxon additions and TBR. The ML starting tree was the majority-rule consensus tree estimated by Bayesian analysis (see below); parameters were optimized on this tree and a new search performed with these parameter values fixed, cycles were repeated until topology did not change. As PAUP does not accommodate mixed models, the optimal single-partition model (GTRig) was used for the entire data set; ML trees and model parameters were re-estimated until negligible improvement in likelihood was observed. Nonparametric bootstrapping was used to assess support for nodes in the MP and ML analyses; the MP analyses used 1000 pseudoreplicates, ML analyses used 100 pseudoreplicates and optimal model parameters for the ML tree. Bootstraps > 70% were taken to indicate credible support.

For MRBAYES analyses, alternative partitioning strategies were assessed according to Bayes Factors using average values of likelihoods at stationarity; however, as the number of partitions (and thus parameters) increases, there is a trade-off between improvements in likelihood and difficulties achieving stationarity; hence, the standard deviation of posteriors for clades was also monitored. The relevant information was outputted from TRACER (Drummond & Rambaut, 2006) and MRBAYES. Partitioning strategies evaluated were all data combined; two partitions (mitochondrial and nuclear); four partitions (mt RNA, mt coding, myh, nuclear coding); six partitions (mt RNA, mt coding 1 + 2, mt coding 3, myh, nuclear coding 1 + 2, nuclear coding 3); seven partitions (mt coding 1, mt coding 2, mt coding 3, myh, nuclear coding 1 + 2, nuclear coding 3); eight partitions (mt coding 1, mt coding 2, mt coding 3, myh, nuclear coding 1, nuclear coding 2, nuclear coding 3). Alternative models were assigned best-fit substitution models using the Akaike information criterion implemented in MRMODELTEST v2.2 (Nylander, 2004) and PAUP (Swofford, 2002) and run with four incrementally heated chains for 2 000 000 generations (sampled every 100th generation). The seven-partition model was selected based on Bayes factors, and run with six chains for 5 000 000 generations (sampled every 100 generations) with all substitution parameters unlinked across partitions, and rates allowed to vary (branch lengths unlinked) across partitions. Likelihood stationarity was always reached by two million generations. On this basis, the first 20 000 sampled trees were discarded as burn-in and the remaining 30 000 were used to estimate posterior parameter and probability distributions.

We also tested some phylogenetic hypotheses to see if they were consistent with or rejected by the data: monophyly of the viviparious clade both with and without the problematic *Acanthophis* (Shine, 1985), the burrowing taxa (Scanlon & Lee, 2004), *Neelaps* (Cogger, 1975) and *Suta* (Greer, 1997). In addition, to test whether more than one colonization of Australia is supported, the monophyly of a clade containing all exclusively or primarily Australian genera was tested. The best tree consistent with each of these constraints was found using reverse constraints in PAUP (using the parsimony or likelihood settings above, as relevant); these trees were then compared with the best (unconstrained) tree. These tests were conducted in parsimony using the nonparametric test of Templeton (1983). In likelihood, the relevant test is uncertain. Trees retrieved by constrained searches are neither fully specified independent of the data, nor fully dependent on the data; so, it is unclear whether the Kishino & Hasegawa (1989) or the Shimodaira & Hasegawa (1999) test, or any variant, is most appropriate (see Lee & Hugall, 2003). Hence, both were used: The K–H test was used to compare each constrained tree with the best tree, and the Shimodaira & Hasegawa (1999) test was used to compare all seven trees simultaneously.

Divergence time estimation

To evaluate a molecular clock hypothesis for the hydrophiine data, likelihood scores for clock and nonclock ML trees were compared using a likelihood ratio (LR) test (Felsenstein, 1981; Sanderson, 1998). The LR was calculated as $2(\ln L_{\text{clock}} - L_{\text{nonclock}})$ (Nei & Kumar, 2000) and assumed to follow a chi-squared distribution with the number of degrees of freedom (d) equal to the number of taxa minus two (Sanderson, 1998). To assess levels of substitutional saturation, we plotted uncorrected pairwise distances against corrected ML pairwise distances for mitochondrial and nuclear ingroup sequences. Corrected distances were estimated using the best-fit model of sequence evolution found by MRMODELTEST v2.2 (Nylander, 2004).

Divergence times were estimated using Bayesian inference as implemented in BEAST v1.4 (Drummond & Rambaut, 2006). The reciprocal monophyly of the ingroup vs. outgroup (*Bungarus* and *Naja*) was assumed *a priori*; all other relationships were free to vary so that topological uncertainty was factored into the posterior divergence estimates. A Yule branching process (appropriate for interspecific data) with a uniform prior was adopted. A relaxed clock was used and rate variation across adjacent branches was initially assumed to be uncorrelated and log-normally distributed (Drummond *et al.*, 2006), preliminary results were then evaluated to ascertain whether a correlated model of rate variation was more appropriate. Bayes factors and preliminary BEAST runs suggested partitioning by loci and by codon (first + second vs. third), and best-fit models of nucleotide substitution for these partitions were identified using AIC implemented in MRMODELTEST v2.2 (Nylander,

2004) and PAUP (Swofford 2002): GTRig (mtDNA 1 + 2), GTRig (mtDNA 3), GTRig (mtRNA), GTRg (nDNA 1 + 2), GTRg (nDNA 3) and HKYg (myh). As the BEAST analysis includes more parameters (those relevant to divergence dating) than the MRBAYES analysis, a six- rather than seven-partition model was selected to facilitate convergence and stationarity. Model parameter values were unlinked (i.e. allowed to vary independently across partitions). The final analysis consisted of two independent MCMC analyses each run for 1 000 000 generations (with parameters sampled every 1000 steps). Both runs converged on the same results, and were combined using LOGCOMBINER v1.4 (Drummond & Rambaut, 2006). TRACER 1.2 (Drummond & Rambaut, 2006) was used to confirm acceptable mixing and likelihood stationarity of the MCMC chain, appropriate burn-in (25%) and adequate effective sample sizes (~200).

There are no elapid fossils that are both sufficiently old and can be placed with enough phylogenetic precision to use as molecular clock calibrations. Consequently, two well-supported divergences, with robust molecular dates estimated using long nuclear sequences and reliable squamate fossil calibrations (see Sanders & Lee, 2008), were used as secondary calibrations: *Laticauda* vs. all remaining hydrophiines (i.e. the oxyuranines), and *Micropechis* vs. other sampled oxyuranines. Using such secondary calibrations is problematic if the uncertainty in the original age estimates is not considered (Graur & Martin, 2004). Here, the secondary calibration priors were given log-normal distributions that represented the uncertainty in the original study and the asymmetrical bias in the fossil record (the true divergence date is more likely to be older than younger because of nonpreservation) (Table 1). The '*Micropechis* vs. other oxyuranines' calibration was assigned a hard lower bound of 4.5 Myr corresponding to the lowest value sampled in the posterior distribution of the original study; a prior mode of 10 Myr corresponding to the posterior mean; and a 95% confidence interval of 14.2 Myr corresponding to the 95% highest posterior distribution (HPD). Similarly, the '*Laticauda* vs. Oxyuraninae' calibration was assigned a hard lower bound of 6.5 Myr, a mode of 13 Myr and a 95% confidence interval of 18 Myr. As no additional outgroups beyond elapines were employed, the rooting position along the hydrophiine–elapine (ingroup–outgroup) branch is largely arbitrary. However, a hard upper bound (of 30 Myr following Sanders & Lee, 2008) was placed on this divergence to prevent overestimation of divergence dates through over-parameterization (Sanderson, 2003). These calibration constraints are consistent with the oldest unambiguous elapid fossils: isolated proteroglyphous fangs (Kuch *et al.*, 2006) that appear primitive with respect to modern elapids in retaining a longer opening on the venom groove (Sanders & Lee, 2008) and date the divergence of elapids from other colubroids at sometime before 20–23 Ma, with the hydrophiine–elapine divergence necessarily occurring afterwards. There is no unequivocal (e.g. good cranial) evidence for earlier elapids; the single older record is an isolated vertebra (Scanlon *et al.*, 2003) that deserves re-examination (Sanders & Lee, 2008; J. D. Scanlon, personal communication).

Table 1 Log-normal calibration priors and posterior probability estimates for node age (millions of years).

Node	Calibrations (priors) – log-normal prior distribution: mean [zero-offset, 95% CI]	Posteriors – posterior probability density: mean [95% HPD]
Micropechis vs. other oxyuranines	10.0 [4.5, 14.2]	11.5 [10.1, 15.6]
Laticauda vs. Oxyuraninae	13.0 [6.5, 18.0]	12.6 [9.1, 14.2]
Pseudechis vs. *Oxyuranus*	–	8.8 [6.7, 10.9]
Simoselaps group	–	8.7 [6.7, 11.2]
Acanthophis vs. *Pseudechis*	–	9.1 [7.3, 11.5]
Hemiaspis, sea snakes + *Notechis* group	–	8.4 [6.5, 10.6]
core *Notechis* group	–	4.9 [3.9, 6.5]
Aipysurus + *Hydrophis* sea snake groups	–	6.2 [4.7, 7.9]
Rhinoplocephalus group	–	8.4 [6.4, 10.8]

No prior age constraints were placed on nodes which were estimated (–).

Results

Phylogenetic analysis

The full data matrix consists of 5770 sites; 349 sites were excluded because of alignment ambiguity, leaving 5421 sites for analysis (3286 mitochondrial, 494 myh, 609 c-mos and 1032 rag). A total of 2102 sites were variable (1622, 160, 114 and 206) and 1531 (1318, 77, 46 and 90) were parsimony informative. Data were missing for RAG-1 for *Neelaps bimaculatus*, and c-mos for *Brachyurophis australis*. The final aligned data set with partition and exclusion commands is available in interleaved nexus format in supplementary material.

Parsimony analysis yielded a single tree of length 11 668, with a consistency index of 0.27 and retention index of 0.37 (Fig. 2); partitioned branch support was generally positive indicating concordant support (Fig. 2). The final ML tree was obtained after three optimizations using the GTR model (with gamma distributed rates and a proportion of invariant sites) and had a log-likelihood score of −55 694.594. The MRBAYES analysis converged on a set of trees with a harmonic mean of −53 879.77; the all compatible consensus is shown in Fig. 1. MP, ML and MRBAYES trees all exhibited weak support for most basal nodes and stronger support for many higher nodes. The MP and ML trees had the lowest resolution, with the fewest strongly supported nodes (19 < 70%), and the MRBAYES analysis had the highest resolution (39 nodes

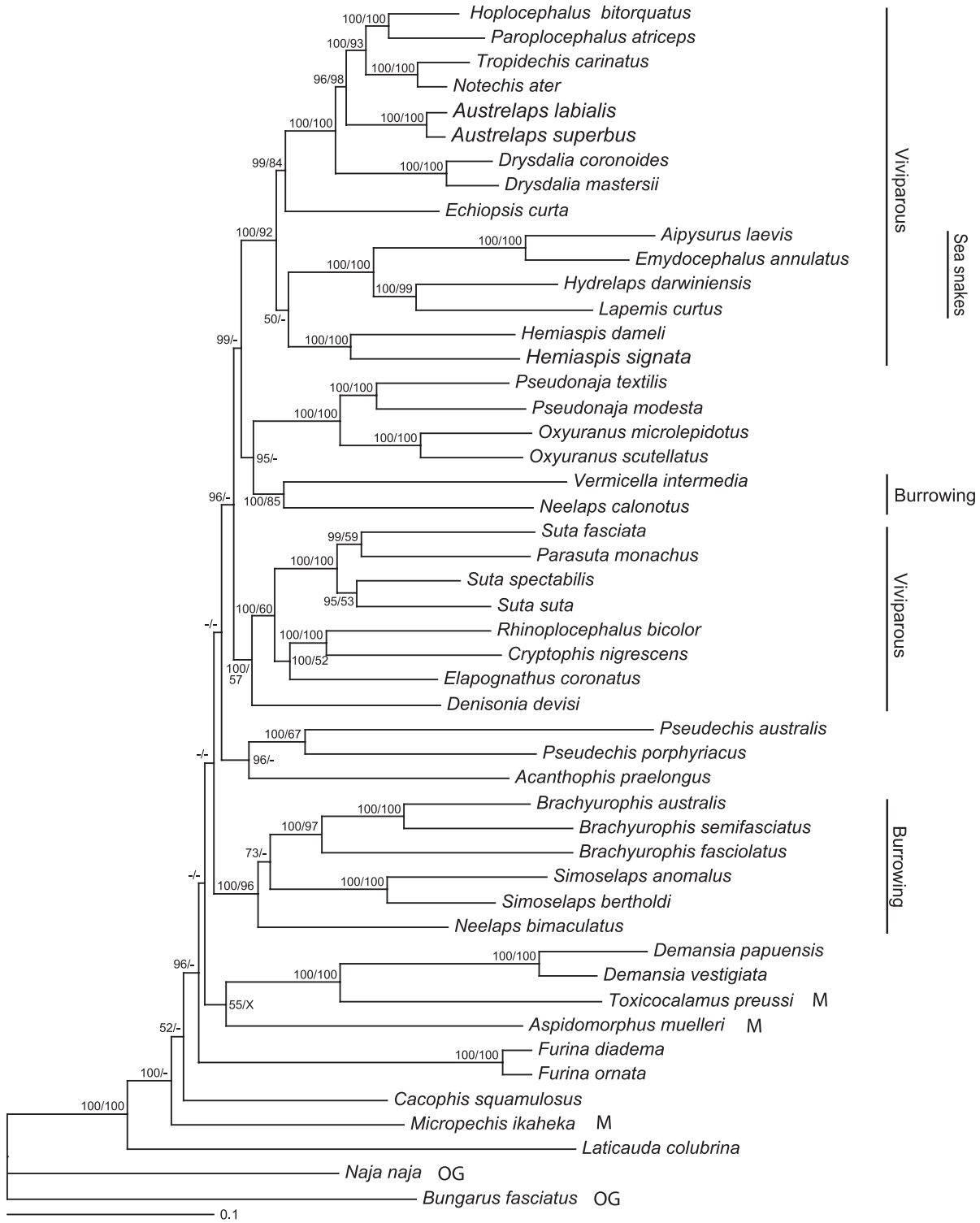

Fig. 1 MrBayes Bayesian all compatible consensus of 30 000 trees sampled after burn-in. Support values > 50% are shown for MrBayes and maximum-likelihood analyses respectively. A dash (–) indicates the clade appearing in the MrBayes or ML tree but with support less than 50%, and a cross (×) indicates the clade not appearing in the ML tree. All sampled species are Australian except for those labelled M (Melanesian) and OG (Old world outgroups).

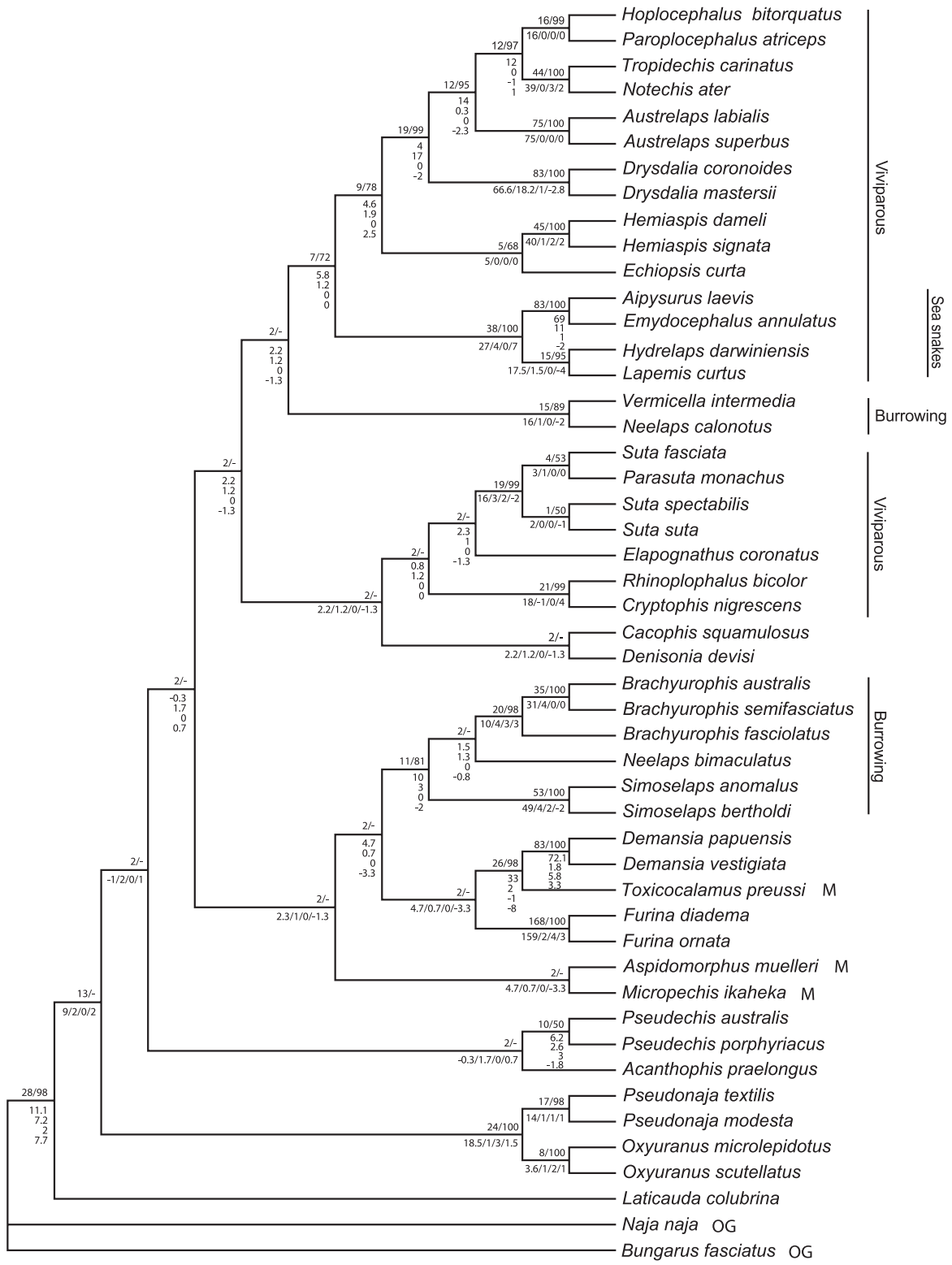

Fig. 2 Maximum parsimony tree obtained with heuristic searches and 1000 random step-wise sequence addition replicates. Nonparametric bootstrap supports (1000 replicates) are given above each node (dashes represent less than 50% bootstrap support); partitioned Bremer supports are below each node in the order mitochondrial/myh/c-*mos*/rag. Melanesian taxa are labelled M and Old world outgroups are labelled OG.

> 95% posterior probability). The ML and MrBayes trees were very similar, with the exception that *Furina* and *Toxicocalamus–Demansia* are placed as sister lineages in the ML tree (Fig 1). Parsimony recovered many of the same clades as the MrBayes and ML analyses, with most differences confined to lower nodes, such as the placement of *Pseudonaja–Oxyuranus* and *Pseudechis* as the most basal Australian taxa (vs. *Cacophis* and *Furina* in the MrBayes and ML trees). There were no strongly supported clades in parsimony (> 70%) that conflicted with any of the model-based trees.

None of the six groupings tested was retrieved as monophyletic in the optimal trees (Figs 1 and 2). Under parsimony, nonparametric Templeton tests indicated that monophyly of the hypothesized clade containing all primarily or exclusively Australian genera could be rejected ($P = 0.04$), but not any other hypothesized clade (*Suta* $P = 0.37$, *Neelaps* $P = 0.14$, burrowers $P = 0.61$, viviparous excluding *Acanthophis* $P = 0.86$, viviparous including *Acanthophis* $P = 0.33$–36 over four trees). Under likelihood, K–H tests rejected monophyly of the Australian clade ($P << 0.01$), *Suta* ($P = 0.04$) and *Neelaps* ($P = 0.03$), but not the monophyly of the other clades (burrowers $P = 0.30$, viviparous $P = 0.38$, viviparous including *Acanthophis* $P = 0.28$). The S–H test (with a pool of plausible trees containing the best tree and the six constrained trees) only rejected the Australian clade ($P = 0.01$), but not any other clade (*Suta* $P = 0.72$, *Neelaps* $P = 0.11$, burrowers $P = 0.76$, viviparous $P = 0.79$, viviparous including *Acanthophis* $P = 0.71$). However, the results of the S–H test changed greatly if the included pool of 'plausible' trees was varied; furthermore, the test appeared to be overly liberal, failing to reject clades that appeared highly inconsistent with the data. For instance, ML analyses grouped *Neelaps calonotus* and *Vermicella* (bootstrap 85%) and *N. bimaculatus* with *Simoselaps* plus *Brachyurophis* (bootstrap 96%); yet the S–H test under the same ML model failed to reject the monophyly of *Neelaps* ($P = 0.11$). For this reason, we focus on the K–H tests when discussing the likelihood results.

Divergence times

Comparison of likelihood scores for clock and nonclock trees confirmed that a molecular clock could be rejected for hydrophiines (LR = 64.02, d.f. = 47, $P = 0.01$), justifying the use of the relaxed clock model. Plots of uncorrected pairwise distances against maximum-likelihood distances (mitochondrial: GTRig and nuclear: GTRg) showed much higher levels of inferred saturation (nonlinearity with increasing genetic distance) in the mitochondrial vs. nuclear sequences (Fig. 3). The combined BEAST MCMC runs yielded high effective sample sizes (> 500) for branch length and topological parameters. Levels of rate heterogeneity were moderate (coefficients of branch rate variation 0.22); and there was only weak correlation of rates between adjacent branches

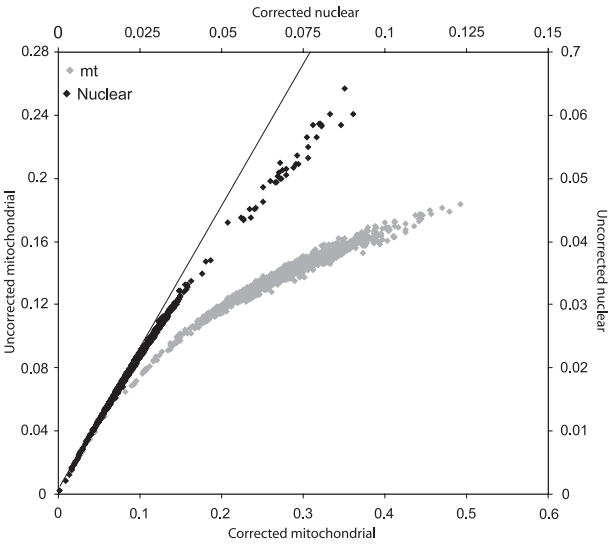

Fig. 3 Saturation plot of corrected pairwise distances vs. uncorrected distances for mitochondrial (grey diamonds) and nuclear (black diamonds) ingroup sequences. Corrected distances were calculated using best-fit models estimated using AIC implemented in MrModeltest v2.2 (Nylander, 2004).

(rate covariance ~0.05); so, the uncorrelated model was retained. The maximum credibility tree retrieved by TreeAnnotator v1.4 (Drummond & Rambaut, 2006) (Fig. 4) is similar to the MrBayes tree in topology and posterior support values. However, as BEAST accounts for implied rate changes when evaluating topology, some differences to the MrBayes tree were apparent and these concerned taxa with unusual branch lengths in the MrBayes tree (e.g. *Cacophis*, true sea snakes; see Discussion).

Mean posterior estimates for calibration nodes are close to the priors: 12.6 Myr (95% HPD: 10.1–15.6) for *Laticauda* vs. Oxyuraninae, and 11.5 Myr (95% HPD: 9.1–14.2) for *Micropechis* vs. other sampled oxyuranines. Most subsequent splits are estimated to have occurred within the next 6 Myr, with nearly all intergeneric splits occurring by 5 Ma. Divergence date estimates for selected, well-supported clades are shown in Table 1. The most recent common ancestor of the core Australian oxyuranine radiation is dated at 10.3 Ma (95% HPD: 8.0–17.7). The *Pseudechis-Oxyuranus* clade is dated at 8.8 Myr old (95% HPD: 6.7–10.9), with *Acanthophis* diverging from this clade 9.1 Ma (95% HPD: 7.3–11.5). The fossorial *Simoselaps* group (excluding *Vermicella* and *Neelaps calanotus*) is dated at 8.7 Myr old (95% HPD: 6.7–11.2) and the *Rhinoplocephaus* group at 8.4 Myr old (95% HPD: 6.4–10.8). The *Notechis–Hemiaspis* and sea snake clade is dated at 8.4 (6.5–10.6) Myr old and the core *Notechis* group at only 4.9 Myr old (95% HPD: 3.9–6.5). The *Aipysurus* and *Hydrophis* sea snake groups *sensu* Smith (1926) are estimated to have diverged 6.2 Ma (95% HPD: 4.7–7.9).

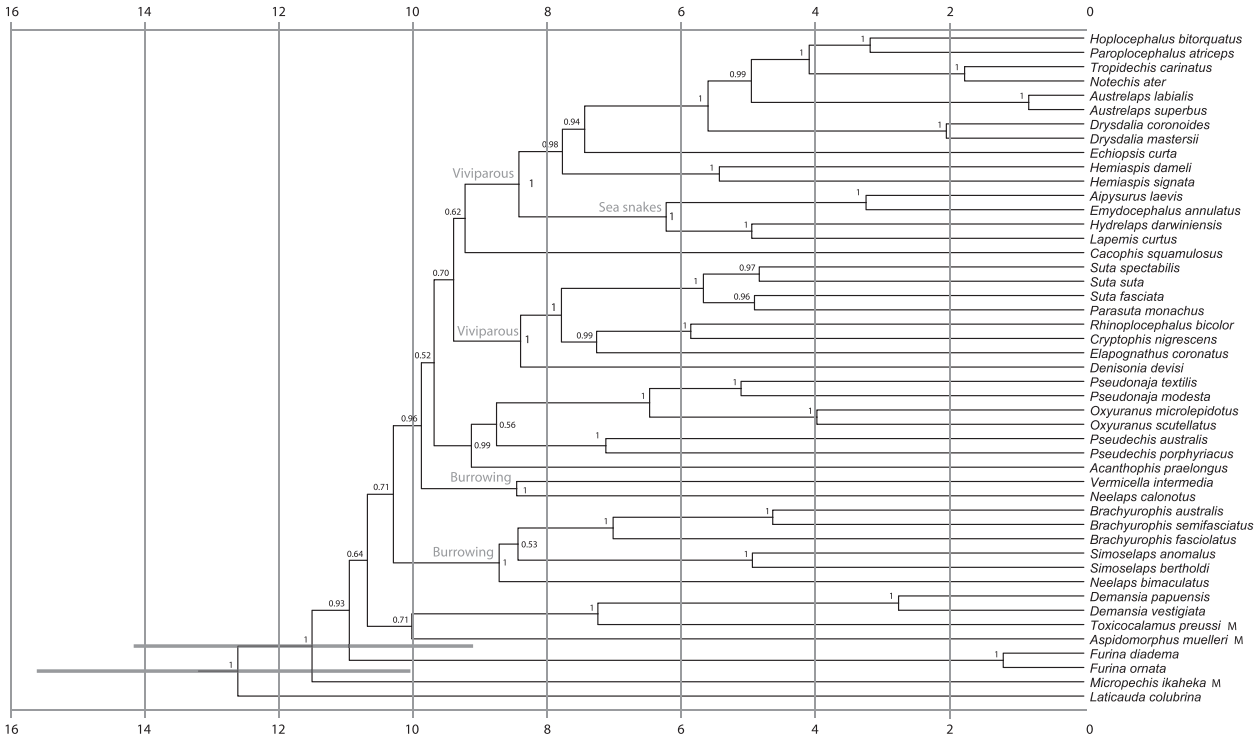

Fig. 4 BEAST maximum credibility ultrametric tree for ingroup taxa. Node bars indicate 95% highest posterior distributions for calibration points. Timescale is in millions of years before present. Posterior probability support values are shown for each node. Melanesian taxa are labelled M.

Discussion

The large molecular data set of four mitochondrial and three nuclear genes provides a robust estimate of the phylogenetic relationships among the terrestrial and marine hydrophiine elapids, and helps to date the major components of this diverse radiation. The data provide support for a series of previously poorly supported clades and also identify several novel relationships. The phylogenetic implications are first discussed, with reference to previous studies, followed by the implications of the estimated divergence dates for rapid evolutionary radiations.

Across-lineage rate heterogeneity and phylogenetic reconstruction

The likelihood-based trees (ML, MRBAYES and BEAST) were much more similar to each other, and better resolved, than were the parsimony trees. One notable difference between the model-based trees concerned differences in the MRBAYES and BEAST results. MRBAYES does not consider across-lineage rate variability, whereas BEAST explicitly incorporates this into tree construction (penalizing extreme values through the log-normal prior). Therefore, it is expected that taxa on anomalously long or short branches in the MRBAYES analysis might be repositioned in the BEAST analysis to reduce rate heterogeneity. This seems to be the case. *Cacophis* is the ingroup taxon with the shortest tip (root-to-tip path length) in the MRBAYES analysis, and is moved into a more nested position in the BEAST analysis. A more nested position would cause *Cacophis* to have a longer root-to-tip path length in any retrieved (non-ultrametric) tree, and thus even out the tips. Conversely, true sea snakes are among the longest tips in the MRBAYES analyses, and are moved into a slightly more basal position (below *Hemiaspis*) in the BEAST analyses, resulting in a shorter path length. In general, where the optimal tree (ignoring rate variability) contains very uneven tips but an alternative tree with more even tips is almost as well-supported, incorporation of rate variability into tree reconstruction might favour the second tree.

Even though the ML and Bayesian trees were most similar, none of the well-corroborated ML and Bayesian clades was strongly contradicted by the parsimony analyses (where parsimony retrieves conflicting clades, these are invariably poorly supported, with bootstraps well below 70%). Most genera are retrieved here as monophyletic, and are already well diagnosed in the literature (Hutchinson, 1990; Greer, 1997). For this reason, the discussion will focus on suprageneric groupings, and on the two genera that do not emerge as monophyletic (*Neelaps* and *Suta*). Support values

are indicated in the following order (MrBayes/beast/ML/MP). As heterodox molecular results are (often rightfully) treated with scepticism if inconsistent with all other evidence, we also briefly discuss diagnostic morphological traits consistent with the current molecular results; the exact taxonomic distribution of these traits mentioned is presented in Scanlon & Lee (2004).

Laticauda

As in previous analyses (McDowell, 1970; Keogh, 1998; Scanlon & Lee, 2004), *Laticauda* is the sister group to all other hydrophiines (oxyuranines). The strong molecular support for this arrangement is consistent with a classic morphological feature: all oxyuranines have reduced the choanal process of the palatine and lost the lateral process, leading to novel jaw movements (palatine dragging: McDowell, 1970). The sole exception is *Cacophis*, which retains a full choanal process (Greer, 1997; Scanlon, 2003).

Basal oxyuranine divergences – *Demansia* and *Cacophis*

Of the sampled taxa, the Melanesian *Micropechis* is robustly retrieved as sister to the remaining oxyuranines, and most other basal forms are exclusively (*Aspidomorphus* and *Toxicocalamus*) or partly *(Furina* and *Demansia*) Melanesian, consistent with previous studies (McDowell, 1970; Keogh *et al.*, 1998; Scanlon & Lee, 2004). This pattern of predominantly mesic, basal Melanesian forms is found in other Australasian squamates (e.g. agamids: Hugall *et al.*, 2008) and mammals (e.g. murids: Aplin, 2006) and supports a 'stepping stone' scenario, in which Australia was colonized from the north by rainforest or wet sclerophyll taxa (e.g. McDowell, 1970; Keogh, 1998; Keogh *et al.*, 1998). The exclusively Melanesian *Toxocalamus* and *Aspidomorphus* are successive outgroups to the Australo-Melanesian *Demansia*, suggesting colonization of Australia within *Demansia*. This would be further supported if phylogenetic analysis reveals that basal species in *Demansia* are Melanesian. Our data do not support a close association of *Demansia* with other Australian genera (Wallach, 1985; Keogh, 1999; Scanlon & Lee, 2004). Consistent with the strong support for a *Toxicocalamus–Demansia* clade (1.0/1.0/100/99), the K–H test in ML (and Templeton test in MP) rejects the monophyly of an Australian clade.

Cacophis, a small cryptic oviparous taxon, is retrieved as a very basal oxyuranine lineage in the MrBayes analysis (0.96), with adjacent taxa (*Micropechis, Furina* and *Aspidomorphus*) having broadly similar ecologies. Surprisingly, *Cacophis* is placed with very dissimilar, viviparous forms in the ML, MP and beast analyses. The first position appears to be more plausible given that *Cacophis* shares several distinctive, possibly synapomorphic traits with *Furina*: apical awns on the hemipenis (Keogh, 1999) and a diastema in the tooth row, and possesses many plesiomorphic features which are found in many or most Melanesian forms, but are rare among Australian forms: white skin between scales, at least partial retention of the choanal process of the palatine, and lack of a posterior reduction in scale row number. Although none of these characters is unique and unreversed, together they suggest a basal position within oxyuranines (McDowell, 1970).

Core Australian oxyuranines

With the exception of *Cacophis*, *Furina* and *Demansia* (which are interdigitated with Melanesian taxa), the remaining Australian genera plus sea snakes form a clade, here termed 'core Australian oxyuranines'. The few Melanesian species in *Oxyuranus, Pseudonaja* and *Pseudechis* probably have resulted from secondary northwards dispersal during periods of low sea-levels when Australia and New Guinea were a single landmass. Basal relationships within core Australian oxyuranines are poorly resolved, but the following well-supported groupings arise from the basal polytomy.

Burrowing clades: *Simoselaps* group and *Vermicella* group

The Australian burrowing taxa form two distinct clades: the *Simoselaps* group (*Simoselaps, Brachyurophis* and *N. bimaculatus*), which appears as the most basal lineage of core Australian oxyuranines and the *Vermicella* group (*Vermicella* and *N. calanotus*), which is variously positioned higher in the tree. This contradicts evidence for monophyly of burrowing forms, including several shared traits that are absent or rare in terrestrial oxyuranines: a wide premaxilla (also in *Acanthophis*), loss of haemapophyses in tail, reduced rod-shaped post-orbital, reduced palatine teeth (Scanlon & Lee, 2004). The first two traits are correlates of head-first burrowing, whereas the last two are bone and tooth reductions which often occur in miniaturized burrowing taxa (Lee, 1998). Thus, there is little morphological evidence for monophyly of burrowing forms independent of highly adaptive, correlated traits. However, the molecular data suggesting diphyly is still weak: the K–H test does not reject monophly of all burrowing forms ($P = 0.30$).

Neelaps does not emerge as monophyletic: the two species sampled are each basal to one of the burrowing lineages, and the K–H test rejects the monophyly of *Neelaps* ($P = 0.03$). The two *Neelaps* species have been recognized as close relatives because of their strong phenetic resemblance and similar (principally western) distributions (e.g. Cogger, 1975; Wallach, 1985; Greer, 1997). However, the morphological characters supporting their monophyly are not overly convincing: separation of preocular and second supralabial (which also occurs in *Simoselaps* and *Furina*), and very similar colour patterns consisting of dark head and nuchal bands with

an orange or reddish body (several burrowing *Vermicella, Brachyurophis* and *Simoselaps* species have similar head and nuchal bands and/or body colouration). If the phylogeny retrieved here is correct, *Neelaps* represents a grade of relatively generalized burrowers lacking the specializations of other fossorial taxa (e.g. the shovel nose of *Brachyurophis*).

Pseudonaja and Oxyuranus

The sister group relationship of *Pseudonaja* and *Oxyuranus* recognized by previous authors (e.g. Wallach, 1985; Keogh *et al*., 1998; Keogh, 1999; Scanlon & Lee, 2004) is strongly corroborated by all analyses. These large oviparous taxa have very similar ecologies (active, fast, aggressive, feeding on small mammal prey) and toxicology (venom highly potent to mammals) and share several distinctive traits absent in other core Australian oxyuranines: parietal and lower post-ocular scales in contact, and a short hyoid (Scanlon & Lee, 2004).

Pseudechis and Acanthophis

Pseudechis and *Acanthophis* do not have well-supported close relatives. They group with moderate support (0.96) in the MrBayes tree, but not in the BEAST, ML or MP trees. The generalized nature of *Pseudechis* and the highly autapomorphic nature of *Acanthophis* have also confounded morphological analyses, and the position of these two genera remains enigmatic. However, in none of our analyses does *Acanthophis* cluster with other livebearers. This contradicts previous analyses, which placed *Acanthophis* in the viviparous clade, either as basal to all other viviparous forms (Keogh *et al*., 2000) or as sister to the *Echiopsis* (Mengden, 1985; Greer, 1997) or *Notechis* lineages (Wallach, 1985).

Viviparous clade 1: *Rhinoplocephalus* group

The viviparous Australian taxa (excluding one species of *Pseudechis*) and hydrophiin sea snakes have previously been suggested to form a monophyletic group, based on the highly distinctive character of single subcaudal (undivided) scales. These taxa, excluding sea snakes and *Hemiaspis*, are further united by a single anal scale (Shine, 1985). Our optimal trees place the viviparous taxa in two separate clades, with *Acanthophis* (which has partially single subcaudals) remote from both these clades. However, a clade of viviparous forms (excluding *Acanthophis*), and a clade of viviparous forms including *Acanthophis*, are not rejected by K–H tests ($P = 0.38$ and 0.28 respectively).

The first viviparous clade includes *Densonia*, *Rhinoplocephalus, Cryptophis, Elapognathus, Suta* and *Parasuta*. Membership largely corresponds to the '*Rhinoplocephalus* group' recognized (but not internally resolved) in previous studies (e.g. Wallach, 1985; Keogh *et al*., 1998, 2000; Scanlon & Lee, 2004). Most members of this group (except *Elapognathus*) share distinctive morphological novelties that are absent in other viviparous taxa, and rare within oviparous forms: a distal projection in crotch of the hemipenis sulcus (Keogh, 1999) and diverging ascending vomer processes (Scanlon & Lee, 2004). *Denisonia* is sister to all other members of the group, and *Parasuta* is nested within *Suta*. The monophyly of *Suta* is further rejected by K–H tests ($P = 0.04$). Greer (1997) distinguished *Suta* and *Parasuta* on the basis of iris colour (pale orange-brown in the former and dark brown in the latter), but notes that this character varies considerably, even within single *Suta* species.

Viviparous clade 2: the *Notechis* group, sea snakes (Hydrophiini) and *Hemiaspis*

All analyses recovered strong support for the 'core *Notechis* group' (*Austrelaps*, *Hoplocephalus*, *Notechis* and *Tropidechis*) and successive sister lineages *Drysdalia* and *Echiopsis*; together these comprise the *Notechis* group *sensu lato*. These taxa share a single anal scale, interpreted in the current tree as convergently derived in the *Rhinoplocephalus* group and *Oxyuranus*. The *Notechis* clade was recovered by Keogh *et al*. (1998, 2000) but with *Echiopsis* as sister to *Hoplocephalus* (Keogh *et al*., 2000). The morphological characters diagnosing this part of the tree are discussed in Keogh *et al*. (2000) and Scanlon & Lee (2004). We add here that most members of the core *Notechis* group possess two traits unusual among hydrophiines: at least partially arboreal habits and a temporolabial separated from the lip margin; *Drysdalia* shares with the core *Notechis* group the unusual karytopic character of 14 microchromosomes (otherwise found only in *Denisonia*).

Although earlier mitochondrial data sets suggested that *Hemiaspis* might represent the closest living relative to the true sea snakes (Keogh *et al*., 1998; Keogh, 2002), our analysis is the first molecular study to robustly resolve the association of true sea snakes with the *Notechis* group and *Hemiaspis* (1.0/1.0/92/74). The close relationship of sea snakes to all viviparous oxyuranines has previously been suggested based on shared possession of viviparity and single anal scales (McDowell, 1969), immunological distances (Cadle & Gorman, 1981; Schwaner *et al*., 1985) and mitochondrial sequences (Keogh, 1998; Keogh *et al*., 1998). The current analysis is consistent with this but unites the sea snakes robustly with a subgroup of the viviparous forms. However, relationships within this subgroup are unstable, with sea snakes either sister to *Hemiaspis* (MrBayes, ML) or the *Notechis* group (MP, BEAST). Many members of the *Notechis* group and *Hemiaspis* are unusual among oxyuranines in foraging and feeding aquatically, a predisposition greatly elaborated in sea snakes (Greer, 1997; Heatwole, 1999). However, unlike the predominantly southern *Notechis* group, *Hemiaspis* 'swamp snakes' have an adjacent distribution to the region of peak sea snake diversity (north-eastern Australia: Greer, 1997) and might repre-

sent the closest living relatives of sea snakes (Keogh *et al.*, 1998; Keogh, 2002). The molecular support for monophyly of the true sea snakes (1.0/1.0/100/100), along with unique (posterior, valvular nostril; fused nasals and internasals) and unusual (anteriorly expanded palatine) traits (Scanlon & Lee, 2004), strongly refute the idea of multiple marine invasions (Rasmussen, 2002). Support is also found for the *Aipysurus* and *Hydrophis* sister groups (1.0/1.0/100/100) recognized by Smith (1926) and Lukoschek & Keogh (2006).

Divergence dates and rapid radiations

For both calibration nodes, the posteriors (11.5 and 12.6 Myr old) are very similar to the priors (10 and 13 Myr old), indicating that the priors are broadly concordant under the present data set (see Sanders & Lee, 2007). However, the posteriors are slightly closer together in time than are the priors; this suggests that basal hydrophiine divergences (including the two calibration nodes) in the current analysis are closer together (relative to the rest of the tree), compared with the previous study. This is consistent with the observation that the current analysis is dominated by mtDNA data, whereas the previous analysis used exclusively nuclear genes. The mtDNA data set is more prone to saturation-driven compression of basal branches than is the nuclear data (Fig. 3), which would produce the pattern observed here. However, the relative similarity of posteriors to priors suggests that the saturation effect is not very great and that the divergence dates obtained are not problematic.

Our phylogeny corroborates earlier studies that demonstrate that the terrestrial Australo-Melanesian elapids represent a rapid radiation that diverged once their ancestors reached the Australo-Melanesian region (Keogh, 1998; Keogh *et al.*, 1998; Scanlon & Lee, 2004), but our dating analysis (see also Sanders & Lee, 2008) strongly supports the view that this radiation is much younger than previously assumed (cf. Schwaner *et al.*, 1985; Keogh, 1998; Keogh *et al.*, 2005). Most inter-generic divergences are estimated to have occurred between 10 and 6 Ma (Fig. 4, Table 1), a recent and relatively short time window that might account for the poor resolution among basal oxyuranine divergences found in this and previous studies. Our timescale indicates that the viviparous group that includes the '*Notechis* clade', *Hemiaspis* and the sea snakes is 8.4 Myr old (95% HPD: 6.5–10.6), slightly older than Shine's (1985, 1991) estimate of 5 Myr old. Divergences within the core *Notechis* group appear to be particularly recent, e.g. the *Hoplocephalus–Paroplocephalus* and *Notechis–Tropidechis* divergences are estimated to have occurred less than 3 Ma. By contrast, *Acanthophis* is estimated to have split from its closest sister lineage 9.1 Ma (95% HPD: 7.3–11.5), a relatively early divergence consistent with the wide range (southern Australia to eastern Indonesia) and extreme ecological specialization of this taxon (Greer, 1997; Scanlon & Lee, 2004).

The crown radiation of true sea snakes is dated at only 6.2 Myr old (95% HPD: 4.7–7.9), an extremely brief interval to generate ~60 species of great ecological and morphological diversity. This is consistent with a second radiation into vacant ecospace (Sepkoski, 1998; Lukoschek & Keogh, 2006; McPeek & Brown, 2007); true sea snakes are the only fully marine squamates to have existed in the last 30 Myr (since the extinction of the giant palaeophiids in the Eocene), and with elongate bodies and potent venom hydrophiines may have been pre-adapted for swimming and feeding efficiently in marine environments. The implied diversification rate within the *Hydrophis* group is particularly impressive, with a basal divergence (Lukoschek & Keogh, 2006) that is estimated to have occurred only 4.9 Ma (95% HPD: 3.6–6.5). The *Hydrophis* lineage comprises at least 40 extant species, including a diverse array of dietary (e.g. burrowing eels and fish eggs) and habitat (e.g. the pelagic *Pelamis*) specialists (Heatwole, 1999; Lukoschek & Keogh, 2006).

Acknowledgments

The authors' work was supported by the Australian Research Council grants to MSY Lee and JS Keogh. We thank the South Australian Partnership for Advanced Computing (SAPAC), who provided access to supercomputer resources, and Andrew F. Hugall, Adam Skinner, John Scanlon, Ian Scott and Christine Hayes for their valued input.

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Received 10 December 2007; revised 5 February 2008; accepted 10 February 2008

Appendix

Appendix I: GenBank accessions for all sequences.

Genus	Species	RAG-1	C-*MOS*	MyHC-2	12S rRNA	16S rRNA	ND4	Cyt*b*
Naja	*naja*	EU366432	EU366445	EU546948	EU547088	EU547137	EU546997	EU547039
Bungarus	*fasciatus*	EU366438	EU366447	EU546995	EU547135	EU547184	EU547037	EU547086
Laticauda	*colubrina*	EU366433	EU366446	EU546949	EU547089	EU547138	EU546998	EU547040
Micropechis	*ikaheka*	EU366435	EU366449	EU546951	EU547091	EU547140	EU547000	EU547042
Aspidomorphus	*muelleri*	EU366434	EU366449	EU546950	EU547090	EU547139	EU546999	EU547041
Toxicocalamus	*preussi*	EU546870	EU546909	EU546952	EU547092	EU547141	EU547001	EU547043
Demansia	*papuensis*	EU546871	EU546910	EU546953	EU547093	EU547142	EU547002	EU547044
Demansia	*vestigiata*	EU546872	EU546911	EU546954	EU547094	EU547143	EU547003	EU547045
Pseudechis	*australis*	EU546873	EU546912	EU546955	EU547095	EU547144	EU547004	EU547046
Pseudechis	*porphyriacus*	EU546874	EU546913	EU546956	EU547096	EU547145	EU547005	EU547047
Pseudonaja	*textilis*	EU546875	EU546914	EU546957	EU547097	EU547146	EU547006	EU547048
Pseudonaja	*modesta*	EU546876	EU546915	EU546958	EU547098	EU547147	EU547001	EU547049
Oxyuranus	*microlepidotus*	EU366439	EU366450	EU546959	EU547099	EU547148	EU547002	EU547050
Oxyuranus	*scutellatus*	EU546877	EU546916	EU546960	EU547100	EU547149	EU547003	EU547051
Cacophis	*squamulosus*	EU366440	EU366451	EU546961	EU547101	EU547150	EU547007	EU547052
Furina	*diadema*	EU546878	EU546917	EU546962	EU547102	EU547151	EU547008	EU547053
Furina	*ornata*	EU546879	EU546918	EU546963	EU547103	EU547152	EU547009	EU547054
Vermicella	*intermedia*	EU546880	EU546919	EU546964	EU547104	EU547153	EU547010	EU547055
Brachyurophis	*australis*	EU546881	-	EU546965	EU547105	EU547154	EU547011	EU547056
Brachyurophis	*fasciolatus*	EU546882	EU546921	EU546966	EU547106	EU547155	EU547012	EU547057
Brachyurophis	*semifasciatus*	EU546883	EU546922	EU546967	EU547107	EU547156	EU547013	EU547058
Neelaps	*bimaculatus*	–	EU546920	EU546968	EU547108	EU547157	EU547007	EU547059
Neelaps	*calonotus*	EU546884	EU546923	EU546969	EU547109	EU547158	EF210841	EU547060
Simoselaps	*anomalus*	EU546885	EU546924	EU546970	EU547110	EU547159	EU547014	EU547061
Simoselaps	*bertholdi*	EU546886	EU546925	EU546971	EU547111	EU547160	EU547015	EU547062

Appendix I: Continued.

Genus	Species	RAG-1	C-*MOS*	MyHC-2	12S rRNA	16S rRNA	ND4	Cyt*b*
Acanthophis	*praelongus*	EU546887	EU546926	EU546972	EU547112	EU547161	EU547016	EU547063
Suta	*fasciata*	EU546888	EU546927	EU546973	EU547113	EU547162	EU547017	EU547064
Suta	*spectabilis*	EU546889	EU546928	EU546974	EU547114	EU547163	EU547018	EU547065
Suta	*suta*	EU366436	EU366452	EU546975	EU547115	EU547164	EU547019	EU547066
Parasuta	*monachus*	EU546890	EU546929	EU546976	EU547116	EU547165	EU547020	EU547067
Rhinoplocephalus	*bicolor*	EU546891	EU546930	EU546977	EU547117	EU547166	EU547021	EU547068
Elapognathus	*coronatus*	EU546892	EU546931	EU546978	EU547118	EU547167	EU547022	EU547069
Cryptophis	*nigrescens*	EU546893	EU546932	EU546979	EU547119	EU547168	EU547023	EU547070
Denisonia	*devisi*	EU546894	EU546933	EU546980	EU547120	EU547169	EU547024	EU547071
Echiopsis	*curta*	EU546895	EU546934	EU546981	EU547121	EU547170	EU547014	EU547072
Drysdalia	*coronoides*	EU546898	EU546937	EU546984	EU547124	EU547173	EU547027	EU547075
Drysdalia	*mastersii*	EU546899	EU546938	EU546985	EU547125	EU547174	EU547028	EU547076
Austrelaps	*labialis*	EU546900	EU546939	EU546986	EU547126	EU547175	EU547029	EU547077
Austrelaps	*superbus*	EU546901	EU546940	EU546987	EU547127	EU547176	EU547030	EU547078
Hoplocephalus	*bitorquatus*	EU546902	EU546941	EU546988	EU547128	EU547177	EU547031	EU547079
Paroplocephalus	*atriceps*	EU546903	EU546942	EU546989	EU547129	EU547178	EU547032	EU547080
Tropidechis	*carinatus*	EU546904	EU546943	EU546990	EU547130	EU547179	EU547033	EU547081
Notechis	*ater*	EU546905	EU546944	EU546991	EU547131	EU547180	EU547034	EU547082
Hemiaspis	*dameli*	EU546896	EU546935	EU546982	EU547122	EU547171	EU547025	EU547073
Hemiaspis	*signata*	EU546897	EU546936	EU546983	EU547123	EU547172	EU547026	EU547074
Aipysurus	*laevis*	EU546906	EU546945	EU546992	EU547132	EU547181	EF506673	EU547083
Hydrelaps	*darwiniensis*	EU546907	EU546946	EU546993	EU547133	EU547182	EU547035	EU547084
Lapemis	*curtus*	EU366437	EU366453	EU546994	EU547134	EU547183	EU547036	EU547085
Emydocephalus	*annulatus*	EU546908	EU546947	EU546996	EU547136	EU547185	EU547038	EU547087

Appendix II: Primer information

Gene(s)	Primer	Reference
RAG-1	G396 (R13) 5′-TCT GAA TGG AAA TTC AAG CTG TT-3′ G397 (R18) 5′-GATGCTGCCTCGGTCGGCCACCTTT-3′	Groth & Barrowclough (1999)
C-*MOS*	G303 5′-ATT ATG CCA TCM CCT MTT CC-3′ G74 5′-TGA GCA TCC AAA GTC TCC AAT C-3′ G708 5′-GCT ACA TCA GCT CTC CAR CA-3′	Saint *et al.* (1998) and Hugall *et al.* (2008)
MyHC-2	G240 5′-GAA CAC CAG CCT CAT CAA CC-3′ G241 5′-TGG TGT CCT GCT CCT TCT TC-3′	Lyons *et al.* (1997)
12S rRNA	tRNA-Phe 5′-AAA GTA TAG CAC TGA AAA TGC TAA GAT GG-3′ tRNA-Val 5′-GTC GTG TGC TTT AGT CTA AGC TAC-3′	Keogh *et al.* (1998)
16S rRNA	5′-CGC CTG TTT ATC AAA AAC AT-3′ 5′-CCG GTC TGA ACT CAG ATC ACG T-3′	Kocher *et al.* (1989)
ND4 + tRNA	ND4 5′-TGA CTA CCA AAA GCT CAT GTA GAA GC-3′ tRNA-Leu 5′-TAC TTT TACC TTG GAT TTG CAC CA-3′	Arévalo *et al.* (1994)
Cyt*b* + tRNA	tRNA-Glu 5′-TGATMTGAAAACCACCGTTG Elapid Cytb Lb 5′-GGACAAATATCATTCTGAGCAGCAACAG Elapid Cytb H 5′-TTGTAGGAGTGATAGGGATGAAATGG	Lukoschek & Keogh (2006)