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Snake evolution in Melanesia: origin of the Hydrophiinae (Serpentes, Elapidae), and the evolutionary history of the enigmatic New Guinean elapid *Toxicocalamus*

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The venomous snake subfamily Hydrophiinae includes more than 40 genera and approximately 200 species. Most members of this clade inhabit Australia, and have been well studied. But, because of poor taxon sampling of Melanesian taxa, basal evolutionary relationships have remained poorly resolved. The Melanesian genera *Ogmodon*, *Loveridgelaps*, and *Salomonelaps* have not been included in recent phylogenetic studies, and the New Guinean endemic, *Toxicocalamus*, has been poorly sampled and sometimes recovered as polyphyletic. We generated a multilocus phylogeny for the subfamily using three mitochondrial and four nuclear loci so as to investigate relationships among the basal hydrophiine genera and to determine the status of *Toxicocalamus*. We sequenced these loci for eight of the 12 described species within *Toxicocalamus*, representing the largest molecular data set for this genus. We found that a system of offshore island arcs in Melanesia was the centre of origin for terrestrial species of Hydrophiinae, and we recovered *Toxicocalamus* as monophyletic. *Toxicocalamus* demonstrates high genetic and morphological diversity, but some of the molecular diversity is not accompanied by diagnostic morphological change. We document at least five undescribed species that all key morphologically to *Toxicocalamus loriae* (Boulenger, 1898), rendering this species polyphyletic. Continued work on *Toxicocalamus* is needed to document the diversity of this genus, and is likely to result in the discovery of additional species. Our increased taxon sampling allowed us to better understand the evolution and biogeography of Hydrophiinae; however, several unsampled lineages remain, the later study of which may be used to test our biogeographic hypothesis.

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ADDITIONAL KEYWORDS: Australasia – Fiji – *Loveridgelaps* – *Ogmodon* – *Salomonelaps* – Solomon Islands.

INTRODUCTION

The Hydrophiinae Fitzinger, 1843 is one of two subfamilies within Elapidae Boie, 1827, and contains some of the most venomous snake species in the world, including taipans, tiger snakes, sea kraits, and sea snakes. There are more than 40 genera and close to 200 species currently recognized (Wallach, Williams & Boundy, 2014; The Reptile Database, 2015). Members of this subfamily are found terrestrially throughout Melanesia and Australia (Australasia), as well as in marine tropical and subtropical environments in the Indo-Pacific. The monophyly of Hydrophiinae has been well supported through morphological (McDowell, 1970; McCarthy, 1985) and genetic (Slowinski, Knight & Rooney, 1997; Keogh, 1998; Slowinski & Keogh, 2000;

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Sanders *et al.*, 2008; Metzger *et al.*, 2010) work. Also, *Laticauda* Laurenti, 1768 (sea kraits) has been well established as the basal lineage within Hydrophiinae, and has an Oriental origin (Keogh, 1998; Sanders *et al.*, 2008; Metzger *et al.*, 2010; Lane & Shine, 2011). Consequently, evidence points to an Oriental origin of the Hydrophiinae through marine invasion, followed by a terrestrial re-emergence in Melanesia (McDowell, 1970; Keogh, Shine & Donnellan, 1998; Scanlon & Lee, 2004); however, there is conflicting evidence as to whether all Melanesian taxa are basal to Australian taxa or whether there have also been reverse exchanges from Australia to Melanesia (Sanders *et al.*, 2008; Metzger *et al.*, 2010).

The evolutionary relationships and biogeographic origins of the basal hydrophiine genera have been difficult to assess because of incomplete taxon sampling (Scanlon, 2003; Scanlon & Lee, 2004; Pyron, Burbrink & Weins, 2013). Included among these poorly represented groups are five monotypic genera: *Micropechis* Boulenger, 1896 from New Guinea; *Ogmodon* Peters, 1864 from Fiji, and *Loveridgelaps* McDowell, 1970; *Salomonelaps* McDowell, 1970; and *Parapistocalamus* Roux, 1934 from the Solomon Islands. *Parapistocalamus* has never been included in a phylogenetic study. *Micropechis* has been represented by up to two individuals, and the other three monotypic genera have only been represented by one individual in molecular phylogenetic studies. For the four genera included, there was evidence that they were basal members of the clade (Keogh, 1998; Keogh *et al.*, 1998; Scanlon & Lee, 2004). In subsequent phylogenetic studies, *Ogmodon*, *Salomonelaps*, and *Loveridgelaps* were not included, and the basal lineages were poorly resolved within Hydrophiinae (Sanders *et al.*, 2008; Metzger *et al.*, 2010; Pyron *et al.*, 2013).

In addition, the unstable placement of the basal genera has been influenced by insufficient sampling within *Cacophis* Günther, 1863 and *Toxicocalamus* Boulenger, 1896;. *Cacophis* is found in the rainforests of eastern Australia, has been represented in phylogenetic studies by only one of the four species in the genus (*Cacophis squamulosus* Duméril, Bibron & Duméril, 1854), and its placement among the Hydrophiinae has been unstable (Keogh *et al.*, 1998; Scanlon, 2003; Scanlon & Lee, 2004; Sanders *et al.*, 2008; Metzger *et al.*, 2010; Pyron *et al.*, 2013). *Toxicocalamus*, endemic to New Guinea and adjacent islands to the north and south-east, has been represented by one or two of the 12 described species.

For *Toxicocalamus*, Sanders *et al.* (2008) used a single representative (*Toxicocalamus preussi* Sternfeld, 1913) and did not recover it among the basal Melanesian taxa of the Hydrophiinae. Rather, another New Guinean genus, *Micropechis*, was retrieved as basal. A second sample from a different species (*Toxicocalamus loriae* Boulenger, 1898) was added by Metzger *et al.* (2010), and was also used by Pyron *et al.* (2013). Both found that the two species did not cluster together, raising the possibility that *Toxicocalamus* is in fact polyphyletic, which would also be consistent with the prior assignment of its current contingent of species across three genera. Beyond this, evolutionary relationships of *Toxicocalamus* to other elapids remain poorly understood, and relationships within the genus have never been assessed.

Toxicocalamus consists of 12 named species of cryptozoic snakes (McDowell, 1969; Kraus, 2009; O'Shea, Parker & Kaiser, 2015). The genus was named by Boulenger (1896) to accommodate a single species, *Toxicocalamus longissimus*, endemic to Woodlark Island, off south-eastern New Guinea. Boulenger (1898), Lönnberg (1900), and Sternfeld (1913) later named *Apistocalamus*, *Pseudapistocalamus*, and *Ultrocalamus*, respectively, to contain related snake species newly named by them. Of these, *Pseudapistocalamus* was synonymized with *Toxicocalamus* and the other two taxa were subsumed within that genus as subgenera by McDowell (1969). These subgenera were recognized on the basis of major differences involving loss or fusion of assorted head scales, relative body width, and osteological and hemipenial features (McDowell, 1969); nonetheless, these names have not been used by subsequent authors. Indeed, the only systematic work on the genus subsequent to McDowell's (1969) revision has been the synonymization of *Vanapina lineata* (de Vis, 1905) with *T. longissimus* (Ingram, 1989), and the description of two new species by Kraus (2009) and one new species by O'Shea *et al.* (2015). Additional species require description (O'Shea, 1996; Kraus, 2009; O'Shea *et al.*, 2015; F. Kraus, unpubl. data): for example, snakes currently assigned to *T. loriae* are a sibling-species complex (Kraus, 2009; O'Shea *et al.*, 2015; F. Kraus, unpubl. data; and see below), and the western half of New Guinea has barely been surveyed for these snakes. Consequently, diversity in the genus will certainly be higher than is apparent from existing nomenclature.

This sparse systematic treatment stems from the under-collected nature of the Papuan herpetofauna, generally, and the secretive habits of these snakes, specifically, both factors that have led to a scarcity of specimens to support biological studies (with '*T. loriae*' being the sole exception). Similarly, field studies of these snakes have been non-existent. In the almost 120 years since the genus was described, only two authors on the genus (F. Kraus and M.T. O'Shea) appear to have had experience with the species in the

field. Despite this, these snakes appear to be ecologically unusual among elapids in feeding primarily on earthworms (O'Shea, 1996; Shine & Keogh, 1996; Goodman, 2010; Calvete *et al.*, 2012; O'Shea *et al.*, 2015; F. Kraus, unpubl. data), although fly pupae and a land snail have also been reported among the stomach contents (Bogert & Matalas, 1945; McDowell, 1969). Beyond these ecological attributes, species of *Toxicocalamus* exhibit a range of morphological variation that is unusual within any snake genus. Some species are very thinly elongate, whereas others are of average snake habitus, and one is rather stout. A number of different fusions among the head and body scales has occurred. The fusion of head scales is common among fossorial snakes, but it usually involves consistent fusion of one or two pairs of scales. In *Toxicocalamus*, subcaudal scales may be single or divided, the anal scale may be single or divided, dorsal scale rows vary from 13 to 17, and five separate types of fusion have occurred among the head scales (McDowell, 1969; Kraus, 2009). The history of these evolutionary modifications and what may account for their variation remain unknown.

Most, if not all, species are also behaviourally inoffensive, being disinclined to bite: for example, one of us (F.K.) has handled 40 living animals of eight named and several unnamed species and has never witnessed any attempt to bite. Furthermore, it is doubtful that the small gapes and fangs of most species would allow for the envenomation of humans, or other larger vertebrates, should they attempt to bite. Despite this, *T. longissimus* – the only species examined to date – has very potent venom components (Calvete *et al.*, 2012), which would seem unnecessary for either capture of their earthworm prey or for effective defence, given their structural and behavioral limitations. Furthermore, *Toxicocalamus buergersi* Sternfeld, 1913 has a very elongated venom gland that extends posteriorly into the body cavity (McDowell, 1969), suggesting that it has the capacity to produce a large quantity of venom. Again, it is unclear what dietary or defensive use this ability could serve. It is possible that the highly toxic venom components of *T. longissimus* are merely phylogenetically conserved and retained from ancestors; however, it remains difficult to explain the large venom glands of *T. buergersi*.

Here, we conduct a molecular phylogenetic analysis to: (1) better understand the evolution of the basal genera within Hydrophiinae; (2) determine the phylogenetic placement of *Toxicocalamus* within the subfamily; and (3) determine the evolutionary relationships of the species within this peculiar genus. To address the basal instability, we include available sequence data from other hydrophiines, including the monotypic Melanesian genera *Micropechis*, *Ogmodon*, *Loveridgelaps*, and *Salomonelaps*; however, we were unable to include additional species from *Cacophis* within this study because of a lack of sample availability. We address the paucity of prior taxonomic sampling within *Toxicocalamus* by using eight of the 12 named species, as well as additional species that are currently undescribed. Of the four named species of *Toxicocalamus* missing from our data set, two are known only from holotypes (*Toxicocalamus grandis* Boulenger, 1914 and *Toxicocalamus ernstmayri* O'Shea *et al.*, 2015), another is know from two specimens (*Toxicocalamus spilolepidotus* McDowell, 1969), and the fourth is known from five specimens (*T. buergersi*). We were unsuccessful in obtaining DNA from preserved specimens of the latter two species, so we did not attempt to sample the holotypes.

MATERIAL AND METHODS

Taxon Sampling

To determine the evolutionary placement of *Toxicocalamus* within the Hydrophiinae, we used sequences on GenBank for 90 individuals from 68 species (Appendix). These 68 species include representatives from 40 of 44 genera within Hydrophiinae. The remaining four genera do not have sequences currently available. Two of these are sea snakes (*Kolpophis* Smith, 1926 and *Thalassophis* Schmidt, 1852), and are not likely to change the topology if they were included. *Antaioserpens* Wells & Wellington, 1985, is, according to Scanlon, Lee & Archer (2003), sister to *Simoselaps*, the placement of which has been stable in the phylogeny of Hydrophiinae (Sanders *et al.*, 2008; Metzger *et al.*, 2010; Pyron *et al.*, 2013). The final genus, *Parapistocalamus*, from the northern Solomon Islands would be a valuable addition to the phylogeny if tissues ever become available. In addition, we used six species from the other subfamily of Elapidae, Elapinae Boie, 1827, to root our phylogeny.

We collected 26 tissue samples of *Toxicocalamus* from 12 localities on New Guinea and surrounding islands. We also acquired two tissue samples of *Toxicocalamus* through tissue loan. In addition, there was one *T. preussi* sequence available on GenBank, and Scott Keogh provided sequence data for an additional *T. preussi* sample. These samples represent eight of the 12 currently named species, as well as samples from individuals of undescribed species (Fig. 1; Table 1).

DNA extraction, amplification, and sequencing

We used the DNEasy Blood and Tissue Kit (Qiagen) to extract total genomic DNA from all tissue samples. We performed gel electrophoresis on a 2.0%

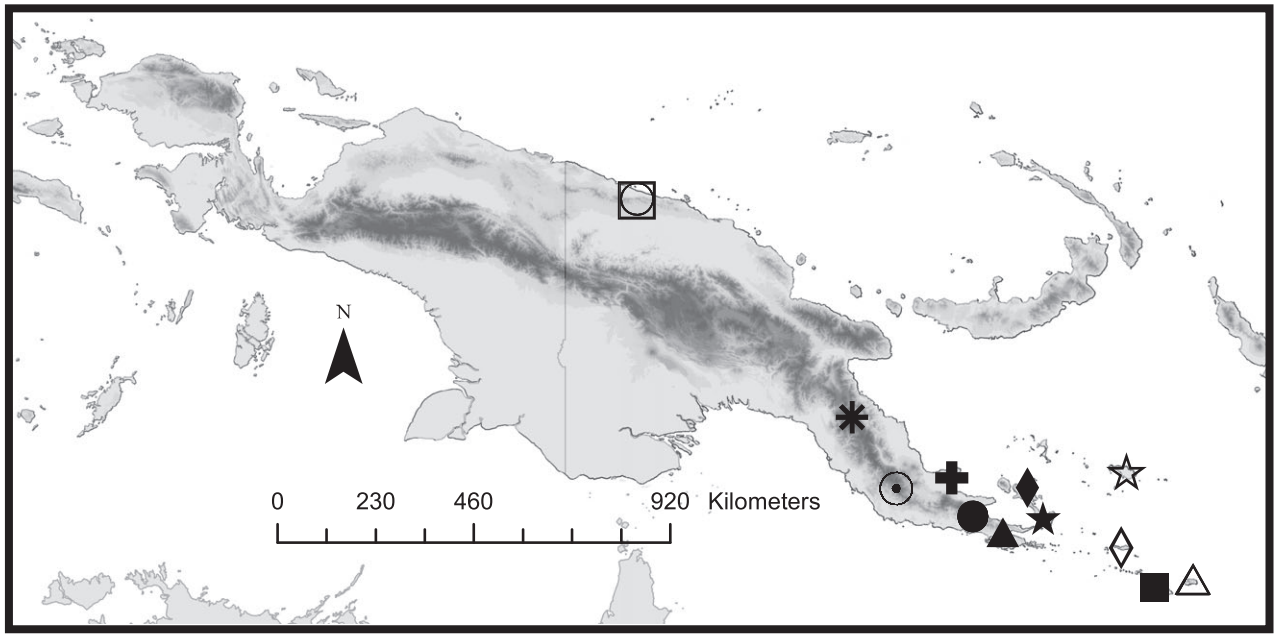

Figure 1. Topographic map of New Guinea and surrounding islands with *Toxicocalamus* sampling localities. Symbols correspond to species shown in Figure 3.

agarose gel to determine the quality of the extracted DNA. We attempted to sequence three mitochondrial loci and four nuclear loci for all individuals: 16S rRNA (*16S)*, cytochrome *b* (cyt*b*), NADH dehydrogenase (*ND4*), oocyte maturation factor (*c–mos*), recombination activating gene 1 (*RAG–1*), myosin heavy chain 2 intron (*MyHC–2*), and β–spectrin nonerythrocytic intron 1 (*SPTBN1*), using published or designed primers and standard polymerase chain reaction (PCR) conditions (Table 2). The PCR product was cleaned using Gel/PCR DNA Fragment Extraction Kit (IBI). Cleaned PCR product was sequenced in both directions at the University of Arizona Genetics Core Facility on an ABI 3730XL DNA Analyzer (Applied Biosystems Inc.).

Sequence alignment and data analysis

To visualize and edit chromatograms, we used SEQUENCHER 5.1 (Gene Codes Corp.). Heterozygosities at nuclear loci were coded with the appropriate International Union of Pure and Applied Chemistry (IUPAC) ambiguity code. We used the MUSCLE alignment algorithm (Edgar, 2004) in MEGA 5.1 (Tamura *et al.*, 2011) with default settings to align sequences, and then verified the alignments by eye. Protein-coding sequences were translated into amino acids to ensure no stop codons were present. All other sequences used in this study are from GenBank (Appendix).

We calculated genetic distances within *Toxicocalamus* for all loci, and compared levels of genetic diversity among species of *Toxicocalamus* in MEGA 5.1 (Tamura *et al.*, 2011) using the Tamura and Nei (TrN) model (Tamura & Nei, 1993) for nucleotide substitution. To determine the appropriate partition and model of evolution for our loci, all possible partitions were considered for the protein-coding genes, whereas *16S*, *MyHC–2*, and *SPTBN–1* were left unpartitioned. We then used the Bayesian information criterion (BIC) and the greedy search scheme in PartitionFinder (Lanfear *et al.*, 2012) to generate the best partition and modelling scheme for all programs used in our phylogenetic analyses (Table 2).

Phylogenetic analyses

We used MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and RAxML 8.0.20 (Stamatakis, 2014) for phylogenetic analysis. For both programs, we generated a concatenated phylogeny of all loci used as well as individual gene trees for each locus.

We simultaneously ran MrBayes twice with one cold and three hot chains for 7 million generations each. The starting trees were independent between runs and randomly chosen. We sampled one out of every 1000 trees. The first 20 000 trees were discarded as burn-in, and then we used TRACER 1.6.0

Table 1. Species information and GenBank accession numbers for the loci used in this study for *Toxicocalamus*

Species	Museum no.	Collector no.	Latitude	Longitude	*c–mos*	*MyHC–2*	*SPTBN1*	*RAG–1*	*16S* rRNA	cyt*b*	*ND4*
T. pachysomus	BPBM 15771	FK 5368	−10.3471	150.2330	–	KU144952	KU172565	KU128756	KT968679	KT778530	KU128809
T. loriae (Clade 5)	BPBM 16544	FK 6288	−9.4263	150.8015	–	KU144953	KU172566	KU128757	KT968680	KT778531	KU128810
T. loriae (Clade 5)	BPBM 16545	FK 6388	−9.4562	150.5596	–	KU144954	KU172567	KU128758	KT968681	KT778532	KU128811
T. misimae	BPBM 17231	FK 7158	−10.6703	152.7206	KU128784	KU144955	KU172568	KU128759	KT968682	KT778533	KU128812
T. loriae (Clade 6)	BPBM 17987	FK 7523	−10.0145	149.597	GQ397225	GQ397216	GQ397193	GQ397197	GQ397235	GQ397170	GQ397211
T. loriae (Clade 6)	BPBM 17988	FK 7524	−10.0145	149.597	KU128785	–	KU172569	KU128760	KT968683	KT778534	KU128813
T. loriae (Clade 6)	BPBM 17989	FK 7665	−10.0171	149.6002	KU128786	–	KU172570	KU128761	KT968684	KT778535	KU128814
T. loriae (Clade 6)	BPBM 18164	FK 7694	−10.0171	149.6002	KU128787	–	KU172571	KU128762	KT968685	KT778536	KU128815
T. loriae (Clade 6)	BPBM 18166	FK 7710	−10.0171	149.6002	KU128788	KU144956	KU172572	KU128763	KT968686	KT778537	KU128816
T. loriae (Clade 1)	BPBM 19502	FK 8808	−9.4439	147.9838	KU128789	KU144957	KU172573	KU128764	KT968687	KT778538	KU128817
T. loriae (Clade 1)	BPBM 19503	FK 8877	−9.4447	148.0092	KU128790	KU144958	KU172574	KU128765	KT968688	KT778539	KU128818
T. loriae (Clade 1)	BPBM 19504	FK 8879	−9.4447	148.0092	KU128791	KU144959	KU172575	KU128766	KT968689	KT778540	KU128819
T. loriae (Clade 1)	BPBM 19505	FK 9258	−9.4439	147.9838	KU128792	KU144960	KU172576	KU128767	KT968690	KT778541	KU128820
T. loriae (Clade 1)	BPBM 19506	FK 9259	−9.4439	147.9838	KU128793	KU144961	KU172577	KU128768	KT968691	KT778542	KU128821
T. mintoni	BPBM 20822	FK 9717	−11.4961	153.4241	–	KU144962	KU172578	KU128769	KT968692	KT778543	KU128822
T. holopelturus	BPBM 20823	FK 10125	−11.3345	154.2239	KU128772	KU144939	KU172553	KU128744	KT968666	KT778515	KU128796
T. holopelturus	BPBM 20824	FK 10153	−11.3544	154.2232	KU128773	KU144940	KU172554	KU128745	KT968667	KT778516	KU128797
T. holopelturus	BPBM 20825	FK 10210	−11.3555	154.2246	KU128774	KU144941	KU172555	KU128746	KT968668	KT778517	KU128798
T. holopelturus	BPBM 20826	FK 10249	−11.3366	154.2236	KU128775	KU144942	KU172556	KU128747	KT968669	KT778518	KU128799
T. holopelturus	BPBM 20827	FK 10276	−11.3345	154.2239	KU128776	KU144943	–	KU128748	KT968670	KT778519	KU128800
T. stanleyanus	BPBM 23455	FK 11482	−3.4246	142.5189	KU128777	KU144944	KU172557	KU128749	KT968671	KT778520	KU128801
T. preussi	BPBM 23456	FK 11611	−3.3933	142.5283	KU128778	KU144945	KU172558	KU128750	KT968672	KT778521	KU128802
T. longissimus	BPBM 39702	FK 14989	−9.0844	152.8353	KU128779	KU144946	KU172559	KU128751	KT968673	KT778523	KU128803
T. loriae (Clade 3)	BPBM 39813	FK 16147	−9.2238	149.1561	KU128780	KU144947	KU172560	–	KT968674	KT778524	KU128804
T. longissimus	BPBM 42183	FK 16362	−9.0378	152.7440	KU128781	KU144948	KU172561	KU128752	KT968675	KT778526	KU128805
T. loriae (Clade 2)	BPBM 41390	AA 21153	−7.9538	147.0567	KU128770	KU144937	KU172551	KU128742	KT968664	KT778513	KU128794
T. loriae (Clade 2)	BPBM 41391	AA 21849	−7.9289	147.0458	KU128771	KU144938	KU172552	KU128743	KT968665	KT778514	KU128795
T. loriae (Clade 4)	UMMZ 242534	FK 16711	−10.06	151.0752	–	KU144950	KU172563	KU128754	KT968677	KT778528	KU128807
T. preussi (Slowinski & Keogh, 2000)	AM 135505	SAM 40321	−3.3933	142.5283	–	–	–	–	–	AF217825	–
T. preussi (Sanders *et al.*, 2008/Bolton *et al.*, unpublished)	AM 136279	ABTC:50506/ SAMARFJ126	–3.3933	142.5283	EU546909	EU546952	–	EU546870	EU547141/ KF736325	EU547043	EU547001

(Rambaut, Suchard & Drummond, 2013) to plot the log-likelihood scores against generation number to ensure stationarity was reached. A 50% majority-rule consensus tree was calculated using the posterior distribution of trees. Maximum-likelihood (ML) analyses in RAxML were performed with 1000 bootstrap pseudoreplicates. We visualized the phylogenetic trees with FigTree 1.4 (Rambaut & Drummond, 2012). Nodes with posterior probabilities (PPs) of ≥ 0.95 from Bayesian inference (BI) and nodes with bootstrap support (BS) $\geq 75\%$ from ML were considered to be strongly supported.

Character mapping

We mapped the relative width of ventrals, fusion of the preocular and prefrontal scales, anal plate divided/undivided, internasal fused to prefrontal, and subcaudals undivided onto our phylogeny. These five characters were chosen because they are important in *Toxicocalamus* species identification and McDowell (1969), Kraus (2009), and O'Shea *et al.* (2015) incorporated them into their dichotomous keys for *Toxicocalamus*. We used the most parsimonious character map to determine the ancestral state for the character. If two parsimonious trees were equally likely, we used the character state of *Ogmodon vitianus* Peters, 1864 as the out-group to determine which character map to present.

RESULTS

Taxon sampling

Several of our sampled undescribed species of *Toxicocalamus* key out morphologically to *T. loriae* (O'Shea, 1996; Kraus, 2009; O'Shea *et al.*, 2015), and are referred to as *T. loriae* in many museum collections; however, we retrieve these samples across a wide range of our phylogeny. For the sake of clarity in presenting our results, we will refer to each of these as '*T. loriae* clade 1, *T. loriae* clade 2, etc.', recognizing that these represent cryptic species that require further taxonomic elucidation but that they have remained morphologically undiagnosed and clustered under a single name (Kraus, 2009; O'Shea *et al.*, 2015).

Sequence data

We generated sequences for 28 individuals within *Toxicocalamus* and deposited them in GenBank (Table 1). In total, including GenBank sequences for out-group taxa, we analysed 126 individuals. The length of the concatenated alignment was 5843 base pairs: 1754 mitochondrial protein-coding, 521 rRNA, 1834 nuclear protein-coding, and 1734 nuclear intron (Tables 1and 2). Protein-coding genes did not contain frame shifts or internal stop codons. The genetic distances between species or clades of *Toxicocalamus* were in the following ranges: 0.06–0.29 for cyt*b*; 0.07–0.32 for *ND4*; 0.02–0.19 for *16S*; 0.01–0.06 for *MyHC–2*; 0.00–0.03 for *RAG1*; 0.00–0.01 for *c–mos*; and 0.00–0.04 for *SPTBN1*.

Phylogenetic relationships

We present the BI phylogenies of the concatenated data set and include the ML bootstrap support values on the nodes (Figs 2 and 3). Overall, the BI and ML trees were identical at all supported nodes (PPs of ≥ 0.95 from BI and/or nodes with BS $\geq 75\%$ from ML). The only differences in the topologies generated by the two algorithms were in the nodes without support, none of which change the relationships among the basal genera or the relationships among species within *Toxicocalamus*. Thus, our interpretations and the conclusions drawn are the same under each analysis.

Our results support Hydrophiinae as monophyletic and *Laticauda* as the basal member, as found in previous studies (Sanders *et al.*, 2008; Metzger *et al.*, 2010; Lane & Shine, 2011; Pyron *et al.*, 2013). Our phylogeny is also in general agreement with relationships found among the Australian genera and sea snakes (Scanlon & Lee, 2004; Wuster *et al.*, 2005; Lukoschek & Keogh, 2006; Sanders *et al.*, 2008); however, inclusion of *Ogmodon*, *Salomonelaps*, and *Loveridgelaps*, along with more representatives from *Toxicocalamus*, yielded a novel topology for these genera in relation to *Micropechis*, *Aspidomorphus* Fitzinger, 1843, *Demansia* Gray, 1842, and *Cacophis*. The included species from the Solomon Islands and Fiji are the basal terrestrial lineage within Hydrophiinae (PP = 1; BS = 99; Fig. 2), and *Toxicocalamus* is the next most-basal lineage, clearly supporting Melanesia as the origin of the terrestrial Hydrophiinae.

All analyses found *Toxicocalamus* to be monophyletic. Within *Toxicocalamus*, *Toxicocalamus stanleyanus* Boulenger, 1903 + *T. preussi* (PP = 1; BS = 99) is strongly supported as a clade basal to the remaining species. *Toxicocalamus holopelturus* McDowell, 1969 was strongly supported as sister to the remaining species (Fig. 3; PP = 1; BS = 93). Within the latter clade, *T. loriae* was found to be polyphyletic, although the placement of *T. loriae* clade 1 was only weakly supported (Fig. 3). As expected based on morphological similarity (Kraus, 2009), *Toxicocalamus misimae* McDowell, 1969 and *T. longissimus* are sister species (Fig. 3). This sister relationship is further corroborated by the geological

Table 2. Locus information used to infer the evolutionary history of *Toxicocalamus*

Locus	Forward primer	Reverse primer	Temp (°C)	MgCl (mM)	Size (bp)	Variable/ parsimony informative within *Toxicocalamus*	Model	Reference
c–mos	G303F 5′–ATTATGCCATCMCC TMTTCC–3′	G708R 5′–GCTACATCAG CTCTCCARCA–3′	53	2.5	726	25/14	GTR + G	Hugall *et al.* (2008)
MyHC-2	G240 5′–GAACACCAGCCTC ATCAACC–3′	G241 5′–TGGTGTCCTGCTC CTTCTTC–3′	55	2.5	525	64/42	HKY + I + G	Lyons *et al.* (1997)
	G240 5′–GAACACCAGCCTCA TCAACC–3′	MyHC2R413 5′–GTCCTAAACTC GCAGGCTAA–3′	50	2				Lyons *et al.* (1997) and This study
	MyHC2F60 5′–TCAGAAGTGG AAGAAGCTGTGCA–3′	G241 5′–TGGTGTCCTGCTCCT TCTTC–3′	50	2				This study and Lyons *et al.* (1997)
SPTBN1	SPTBN1-F1 5′–TCTCAAGACT ATGGCAAACA–3′	SPTBN1-R1 5′–CTGCCATCTC CCAGAAGAA–3′	54	2	1209	93/36	GTR + G	Matthee *et al.* (2001)
RAG–1	G396(R13) 5′–TCTGAATGGAA ATTCAAGCTGTT–3′	G397(R18) 5′–GATGCTGCCTC GGTCGGCCACCTTT–3′	55	2.5	1108	69/35	GTR + G	Groth & Barrowclough (1999)
	RAG1F122 5′–CTAAAGAAAAT GTGRCAGGATCTC–3′	RAG1R1054 5′–GGGCATCTCA AAACCAAATTGT–3′	50	2.5				This study
16S rRNA	16SF 5′–CGCCTGTTTATCAA AAACAT–3′	16SR 5′–CCGGTCTGAACTC AGATCACGT–3′	48	2.5	521	125/89	GTR + I + G	Kocher *et al.* (1989)
cyt*b*	L14910 5′–GACCTGTGATMTG AAAAACCAYCGTTGT–3′	H16064 5′–CTTTGGTTTACA AGAACAATGCTTTA–3′	48	2.5	1098	513/452	GTR + I + G	Burbrink, Lawson & Slowinski (2000)
	L14910 5′–GACCTGTGATMTG AAAAACCAYCGTTGT–3′	ToxcytbR493 5′–AAGCGGGTR AGGGTTGG–3′	55	2.5				Burbrink, Lawson & Slowinski (2000) and This study
	ToxcytbF380 5′–TGAGCAGCAA CATWATTACAAA–3′	ToxcytbR750 5′–GGTTAATGT GYTGTGGTGT–3′	48	2.5				This study
	ToxcytbF709 5′–TTAACGACCC YGAAAACTT–3′	H16064 5′–CTTTGGTTTACAA GAACAATGCTTTA–3′	48	2.5				This study and Burbrink *et al.* (2000)
ND4	ND4F 5′–TGACTACCAAAAGC TCATGTAGAAGC–3′	ND4 tRNA-Leu 5′–TACTTTTA CCTTGGATTTGCACCA–3′	48	2.5	656	327/298	GTR + I + G	Arevalo, Davis & Sites (1994)
	ND4F123 5′–TAACYTGCCTYC AACAAACAGA–3′	ND4R688 5′–TTGTCAAGRTC ACAGCTTGRTA–3′	50	2.5				This study

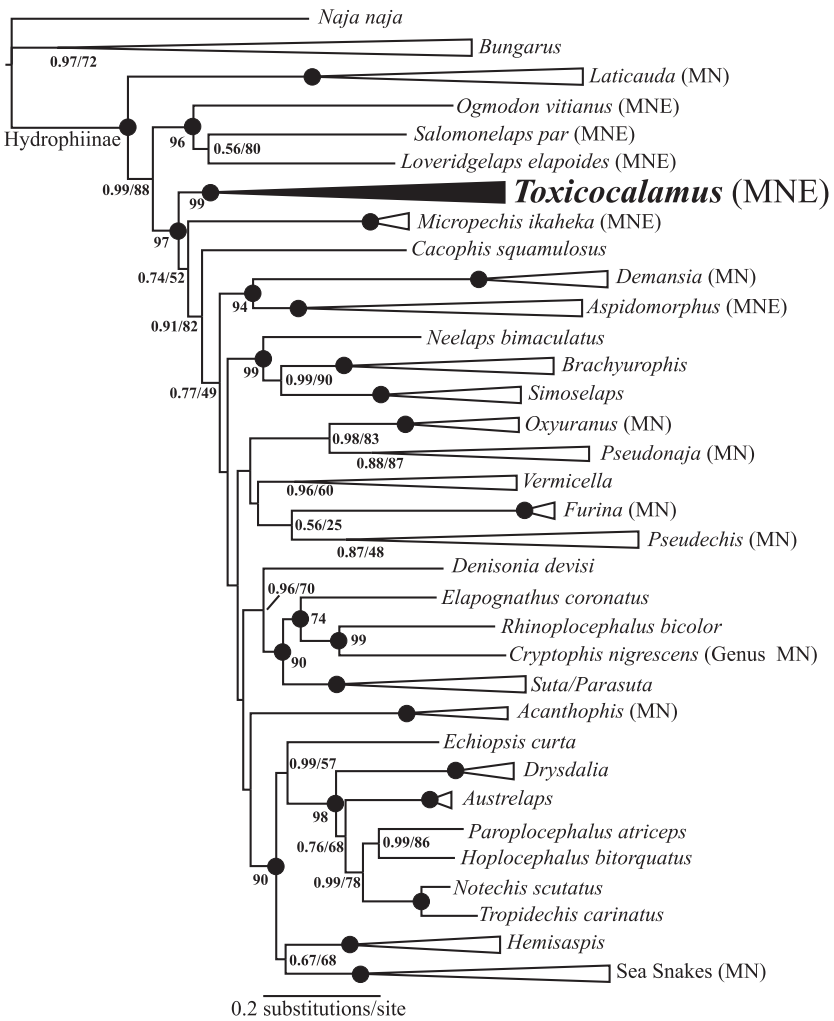

Figure 2. Concatenated Bayesian inference phylogeny of Hydrophiinae using three mitochondrial and four nuclear loci. Values on nodes represent posterior probability (PP) from MrBayes/bootstrap support (BS) values from RAxML. Dots on nodes represent PP of 1 and BS value of 100 unless given otherwise; nodes without values had PP < 0.5 and BS < 50. MNE, Melanesian endemic; MN, found in Melanesia.

history of the two islands that these species occupy. Misima Island and Woodlark Island are home to *T. misimae* and *T. longissimus*, respectively, and were connected as recently as 1.2 Mya, before the opening Woodlark Basin separated them (Taylor, Goodliffe & Martinez, 1999).

In analyses of *ND4* and cyt*b* gene trees, the position of *T. loriae* clade 1 was recovered as basal to the remaining lineage of '*T. loriae*' clades 2–6, *Toxicocalamus mintoni* Kraus, 2009; and *Toxicocalamus pachysomus* Kraus, 2009;. For this phylogenetic arrangement, *T. mintoni* and *T. pachysomus* render the '*T. loriae*' species complex paraphyletic. Nonetheless, both are morphologically very distinct from '*T. loriae*'. Several additional '*T. loriae*' specimens were found to form four strongly supported (clades 2, 3, 5, and 6) and one weakly supported (clade 4) lineages (Fig. 3).

Character mapping

We found that the ancestral state within *Toxicocalamus* was narrow ventrals, which is not seen in *Ogmodon*, *Salomonelaps*, or *Loveridgelaps*. This corresponds to a long and thin overall habitus, with the normal snake habitus being regained later either once or twice depending on the character-state reconstruction used (Fig. 4A). The state for *O. vitianus* is preocular unfused to prefrontal; therefore, if that is basal in *Toxicocalamus*, these scales have become fused three times independently (Fig. 4B). *Ogmodon vitianus* has a divided anal plate. Interpreting this

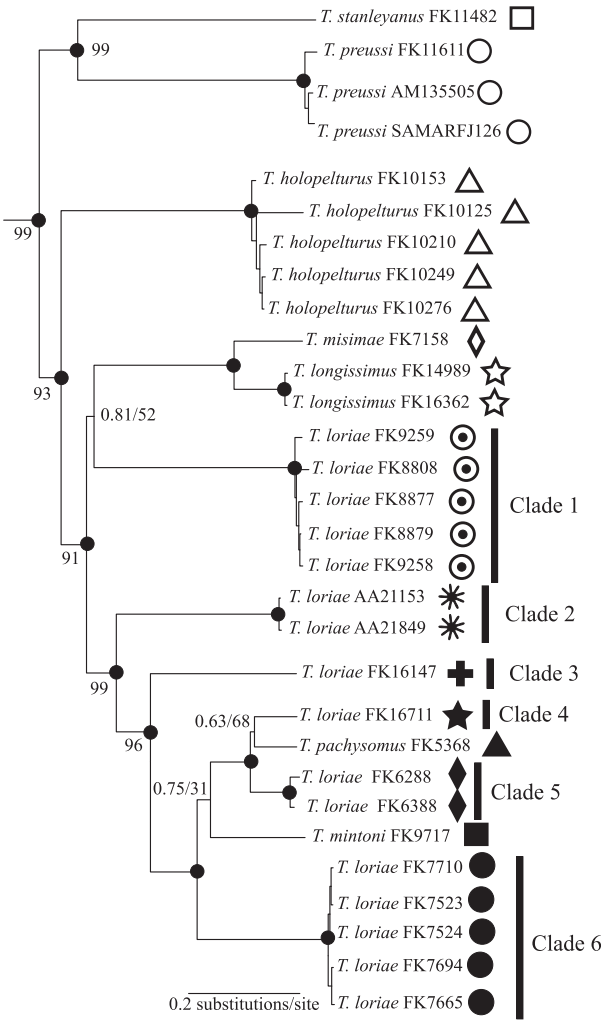

Figure 3. Concatenated Bayesian inference phylogeny of *Toxicocalamus* using three mitochondrial and four nuclear loci, expanded from Figure 2. Values on nodes represent posterior probability (PP) from MrBayes/bootstrap support (BS) values from RAxML. Dots on nodes represent PP of 1 and BS value of 100 unless given otherwise, nodes without values had PP < 0.5 and BS < 50, and symbols correspond to locations in Figure 1.

as ancestral, the anal plates have fused twice within *Toxicocalamus* (Fig. 4C). The character state of internasal fused to prefrontal is seen in *T. preussi* and *T. buergersi* (not in analysis), and having undivided subcaudals is an autapomorphy in *T. holopelterus* (Fig. 4D).

DISCUSSION

By including genera not used in prior phylogenetic analyses and representing *Toxicocalamus* by a majority of its species, we generated a well-supported phylogeny of the Hydrophiinae, clarifying the placement of basal taxa and shedding light on the species relationships within the enigmatic New Guinean endemic *Toxicocalamus*. Congruent with previous studies, we found Hydrophiinae to be monophyletic, with *Laticauda* basal to all other lineages (Keogh, 1998; Scanlon & Lee, 2004; Sanders *et al.*, 2008; Metzger *et al.*, 2010; Lane & Shine, 2011). Our results indicate that the five basalmost terrestrial genera in the subfamily are from Melanesia, and that the early ancestors of Hydrophiinae were likely to have been cryptozoic. Our study clearly demonstrates the adverse effects of inadequate taxon sampling on phylogenetic estimations. By using eight described and several undescribed species of *Toxicocalamus*, we determined that the genus is monophyletic, contrary to previous studies (Metzger *et al.*, 2010; Pyron *et al.*, 2013), and we confirm that species currently designated *T. loriae* represent a species complex in need of taxonomic revision (Kraus, 2009; O'Shea *et al.*, 2015). We also find *Toxicocalamus* to be basal to other New Guinean and Australian taxa within Hydrophiinae.

The basal relationships within Hydrophiinae, including the placement of *Toxicocalamus*, have been difficult to determine because of incomplete taxon sampling, which has led to different nomenclatures for the subfamilial taxonomy. We follow most authors in defining the subfamily Hydrophiinae to contain all marine and terrestrial Australasian taxa (Slowinski & Keogh, 2000; Castoe *et al.*, 2007; Metzger *et al.*, 2010), with the basal member of this subfamily being *Laticauda* (Fig. 2). Some authors have elevated Hydrophiinae to family status and divided it into two separate subfamilies, the Laticaudinae, including only *Laticauda*, and the Oxyuraninae, with the remaining genera (Sanders *et al.*, 2008; Kelly *et al.*, 2009); however, *Parapistocalamus*, a genus endemic to the Solomon Islands, has not been represented within any molecular phylogenies, and its morphological placement in relation to *Laticauda* and the other genera is uncertain. Based on the movement of the palatine bone during swallowing, McDowell (1970) differentiated Elapids into two groups: 'palatine erectors', with all Elapids outside Hydrophiinae, as well as *Laticauda* and *Parapistocalamus*; and 'palatine draggers', with the remaining hydrophiines (Deufel & Cundall, 2010). McDowell (1985) later described *Laticauda* and *Parapistocalamus* as intermediates between the two phenotypes because they lack the palatine choanal process, like other Australasian elapids. If a tissue sample can be acquired for *Parapistocalamus hedigeri* Roux, 1934, then it would be possible to test this nomenclatural hypothesis further, and to determine the placement of *Parapistocalamus* among the other monotypic

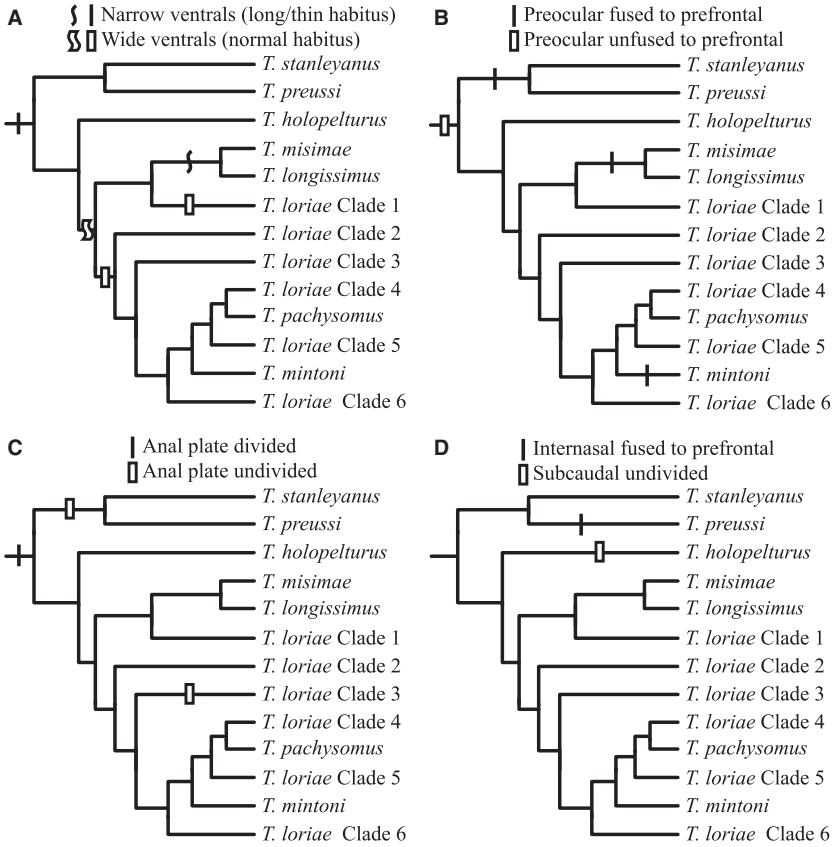

Figure 4. Mapping of morphological characters used to distinguish species of *Toxicocalamus* by McDowell (1969), Kraus (2009), and O'Shea *et al*. (2015) onto our topology of hypothesized relationships from Figure 3. (A) Two most parsimonious character-state reconstructions for ventral width with ancestral condition as narrow ventrals: straight symbols denote the reconstruction with two origins of wide ventrals; curved symbols denote the reconstruction with evolution of wide ventrals followed by reversion to narrow ventrals. (B) Most parsimonious state changes for preocular and prefrontal fusion, (C) most parsimonious state changes for anal plate division, and (D) map depicting two unrelated character states: fusion of the internasal with prefrontal (seen in *T. buergersi* as well) and autapomorphy of subcaudals undivided.

basal genera *Ogmodon*, *Loveridgelaps*, and *Salomonelaps* in Melanesia. We predict that *Parapistocalamus* would be the next most-basal genus after *Laticauda*. The complete 'palatine dragger' phenotype would then be a synapomorphy for the remaining hydrophiines, with *Ogmodon*, *Loveridgelaps*, and *Salomonelaps* being the basal members with that character state.

Ogmodon vitianus from Fiji, and *Loveridgelaps elapoides* Boulenger, 1890, and *Salomonelaps par* Boulenger, 1884 from the Solomon Islands, were initially included in molecular phylogenetic studies and found to be among the basal members of Hydrophiinae (Keogh, 1998; Keogh *et al.*, 1998). More recent studies have not included these data, preventing a complete evolutionary understanding of this subfamily (Sanders *et al.*, 2008; Metzger *et al.*, 2010; Pyron *et al.*, 2013). Including these genera in our phylogeny, we determined that they form a monophyletic assemblage basal to the New Guinean and Australian species (Fig. 2). This phylogenetic arrangement supports Melanesia as the evolutionary origin of terrestrial hydrophiines, which is further supported by the next two basalmost lineages (*Toxicocalamus* and *Micropechis*) also being Melanesian.

Toxicocalamus was recovered as monophyletic and not sister to any single currently recognized genus. Metzger *et al.* (2010) recovered a paraphyletic *Toxicocalamus* when using the *T. loriae* and *T. preussi* sequences available on GenBank as representatives of the genus, and Pyron *et al.* (2013) obtained the same results using the same data set. Our results indicate that this conclusion probably resulted from two things. First, few of the out-group taxa used in this study were also used by Pyron *et al.* (2013). Second, they used two highly divergent taxa as the only

representatives for *Toxicocalamus*. These omissions presumably led to poor resolution and long-branch attraction at the base of the phylogeny. Previous studies had suggested *Toxicocalamus* to be closely related to *Aspidomorphus*, *Demansia*, or *Micropechis* (Sanders *et al.*, 2008; Metzger *et al.*, 2010), but our study does not support those findings either. Rather, we found *Micropechis* to be basal to the remaining Hydrophiinae, followed by *Cacophis*. All of the basal terrestrial genera are cryptozoic, spending much of their time under logs and rocks and in leaf litter (McDowell, 1970; Zug & Ineich, 1993; Shine & Keogh, 1996), although most also forage actively on the forest floor, either diurnally or nocturnally (McCoy, 2006; F. Kraus, pers. observ.).

These basal relationships within the Hydrophiinae are consistent with the geological history of the region. Kelly *et al.* (2009) estimated the Hydrophiinae to have originated ~23 Mya, and the oldest fossil elapid, interpreted as a *Laticauda*, is of the same age (Scanlon *et al.*, 2003). This coincides in time with the formation of island arcs in the western Pacific that include parts of what are now the Solomon Islands, Fiji, and New Guinea (Hall, 2002, 2012). Our results suggest that the early terrestrial hydrophiines originated on these islands, which could only have been colonized by an early marine ancestor like *Laticauda*. The Solomon and Fiji islands are parts of the Outer Melanesian Arc, which arose *c*. 40 Mya, prior to the origin of the Hydrophiinae (Hall, 2002, 2012; Colley, 2009; Davies, 2009). A separate and more northerly island arc, formed on the margin of the Caroline Plate at approximately the same time, was rotated into adjacency to the Outer Melanesian Arc, and continued rotating to the south and west to accrete sequentially onto the northern margin of New Guinea between 20 and 5 Mya (Davies *et al.*, 1997; Hall, 2002, 2012). Judging from the present distribution of the basal lineages in this clade, terrestrial hydrophiines seem likely to have arisen on islands of these arc systems when they were placed, so as to form a single continuous chain *c.* 30–20 Mya (cf. http://searg.rhul.ac.uk/current_research/plate_tectonics/plate_tectonics_SE_Asia%200-55Ma.html). Separation of the northern (and western) arc from the Outer Melanesian Arc and its subsequent accretion onto New Guinea would have led to the rapid invasion and speciation of elapids in New Guinea and Australia (with New Guinea being merely the northern portion of the Australian continent plus accreted islands of these former arc systems), as inferred by the very short branch lengths among basal taxa (Fig. 2; Keogh *et al.*, 1998; Scanlon & Lee, 2004; Lukoschek & Keogh, 2006).

The remaining phylogeny of Hydrophiinae was not fully resolved, but there was support for invasions from New Guinea to Australia and reinvasions back to New Guinea. For example, *Aspidomorphus* and *Demansia* are well supported as sister genera. *Aspidomorphus* is endemic to New Guinea whereas *Demansia* is found in both Australia and New Guinea. The only Australian endemic found among the basal genera was *Cacophis*, with moderate support in both our BI and ML phylogenies (Fig. 2). In previous phylogenetic analysis, *Cacophis* has been hypothesized to be sister to *Notechis* Boulenger, 1896 (Keogh *et al.*, 1998), sister to *Aspidomorphus* and/or *Demansia* (Scanlon *et al.*, 2003), related to *Furina* Duméril, 1853 (Sanders *et al.*, 2008), among the basal Hydrophiinae (Metzger *et al.*, 2010), or among Australian taxa other than *Notechis* or *Furina* (Pyron *et al.*, 2013). Using morphological data, Scanlon (2003) was unable to determine its placement within Hydrophiinae. To better determine whether *Cacophis* is related to other Australian taxa or to the fossorial Melanesian taxa requires further taxon sampling within that genus.

It is important to note that two of the nomina that McDowell (1969) used as subgenera of *Toxicocalamus* are polyphyletic. The type species for *Apistocalamus* is *T. loriae*, but McDowell (1969) included *T. holopelturus* in that subgenus. Those taxa do not form a monophyletic clade. The type species for *Toxicocalamus* is *T. longissimus*, but McDowell (1969) included *T. stanleyanus* in that subgenus. Once again, they are not monophyletic. The third subgenus, *Ultrocalamus*, included just *T. preussi* (type species) and *T. buergersi*, which were grouped by McDowell (1969) based on the shared fusion of the internasal and prefrontal. We could not obtain a sample of *T. buergersi*, and, therefore, we cannot test the validity of *Ultrocalamus*. On the basis of our results, however, there is no current justification for recognizing subgenera within *Toxicocalamus*: the recognition of any two or more of them would render the others paraphyletic (Fig. 3). Furthermore, taxonomy and species diversity within the genus remain imperfectly known, with several species remaining to be diagnosed and the western half of New Guinea remaining to be even modestly sampled for the genus. Thus, for a truly complete understanding of this genus, further study, with an emphasis on increased taxon sampling, will be required.

Toxicocalamus species mostly come in two different body forms. The first are extremely thin and elongate animals with narrow ventral scales; the second have a more normal snake habitus and width to the ventral scales (*T. pachysomus* is an outlier of stouter habitus; cf. Kraus, 2009). Our results indicate that the elongate body form is ancestral within this genus (Fig. 4A). All such species (*T. holopelturus*, *T. longissimus*, *T. misimae*, *T. preussi*, and

T. stanleyanus) are placed basally in the tree, and the 'normal' snake habitus is re-gained later in evolution (Fig. 4A). Scalational fusions occur in several different species within *Toxicocalamus*, and relationships are largely inconsistent with this variation (Fig. 4). Species that share particular head-scale fusion patterns are not retrieved as monophyletic, suggesting that these features have arisen multiple times (Fig. 4B, C). Also, our genetically divergent clades morphologically assigned to *T. loriae* make clear that morphological divergence has not mirrored all substantial genetic divergence or speciation patterns in the complex, a pattern also evident from the consideration of colour patterns of living animals (F. Kraus, pers. observ.). Some of these more derived populations have already been described, but most are currently recognized as '*T. loriae*', a 'species' that clearly requires taxonomic revision, as previously indicated (Kraus, 2009; O'Shea *et al.*, 2015).

At a minimum, our phylogenetic analyses indicate that *T. loriae* as currently defined morphologically is polyphyletic. There is considerable genetic distance between the two most distant clades (1 and 6) based on cyt*b* (0.21), *ND4* (0.16), and *16S* (0.10) data. The position of *T. loriae* clade 1 as part of a *T. longissimus* + *T. misimae* clade was only weakly supported, and *ND4* and cyt*b* trees did not support this conclusion, nor do the morphological data (McDowell, 1969; Kraus, 2009). *Toxicocalamus loriae* clade 1 occurs approximately 80 km from the type locality for *T. loriae* on Mount Victoria, and represents our best estimate of true *T. loriae*. To confirm this, recollection on Mount Victoria is needed so that molecular data from individuals from that locality may be integrated into our phylogeny. *Toxicocalamus loriae* is reported to occur throughout much of New Guinea, but it is unknown what range of genetic variation is encompassed across this distribution because of the historical difficulty of collecting in the western half of the island. If the trends apparent from this study apply throughout the entirety of its range, then it is very likely that many species currently recognized as *T. loriae* represent independent lineages and require systematic revision.

Despite remaining deficiencies in taxon sampling, we have presented evidence for undocumented genetic diversity within *Toxicocalamus*. Our best-supported phylogeny infers strong evidence for at least 13 distinct clades, five of which would appear to represent currently undescribed species. Moreover, much of New Guinea remains unexplored. Hydrophiinae is a speciose group and represents a relatively recent rapid radiation in the Australasian region (Slowinski & Keogh, 2000; Sanders & Lee, 2008; Sanders *et al.*, 2008). Discerning the true evolutionary history of the genera contained within it will require extensive sampling effort across both species and genetic markers. Understanding the relationships among the Hydrophiinae has been a challenge for decades, but resolving the phylogeny of this group may lead to a much better understanding of the biogeographic history of the region. Future work on *Toxicocalamus* will lead to several species descriptions (F. Kraus, unpubl. data), but documentation of the species distributions across New Guinea remains sorely needed.

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APPENDIX

List of species and accession numbers used to generate the Hydrophiinae phylogeny in Figure 2

Outgroup Species	cyt*b*	*RAG–1*	*ND4*	*SPTBN1*	*MyHC2*	*c–mos*	*16S*
Acanthophis antarcticus	AF217813	–	AY340162	–	–	–	–
Acanthophis laevis	–	–	AY340165	–	–	–	–
Acanthophis praelongus	EU547063	EU546887	AY340164	–	EU546972	EU546926	EU547161
Acanthophis pyrrhus	–	–	AY340168	–	–	–	–
Acanthophis rugosus	–	–	AY340152	–	–	–	–
Aipysurus laevis	EU547083	FJ587087	EF506638	–	EU546992	EU546945	DQ233998
Aspidomorphus lineaticollis	GQ397132	GQ397199	GQ397212	GQ397173	GQ397219	GQ397229	GQ397239
Aspidomorphus lineaticollis	GQ397131	GQ397198	GQ397205	GQ397174	GQ397217	GQ397227	GQ397237
Aspidomorphus lineaticollis FK16621	KT778527	KU128753	KU128806	KU172562	KU144949	KU128782	KT968676
Aspidomorphus lineaticollis FK16959	KT778529	KU128755	KU128808	KU172564	KU144951	KU128783	KT968678
Aspidomorphus muelleri	GQ397163	GQ397203	GQ397206	GQ397188	GQ397222	GQ397232	GQ397242
Aspidomorphus muelleri	GQ397161	GQ397202	GQ397213	GQ397187	GQ397221	GQ397231	GQ397241
Aspidomorphus muelleri	GQ397153	GQ397195	GQ397207	GQ397183	GQ397214	GQ397224	GQ397233
Aspidomorphus muelleri	AF217814	EU366434	EU546999	GQ397184	EU546950	EU366448	KF736326
Aspidomorphus muelleri FK14215	KT778522	–	–	–	–	–	–
Aspidomorphus muelleri FK16281	KT778525	–	–	–	–	–	–
Aspidomorphus schlegeli	GQ397169	GQ397200	GQ397210	GQ397189	GQ397218	GQ397228	GQ397238
Aspidomorphus schlegeli	GQ397167	GQ397196	GQ397204	GQ397190	GQ397215	GQ397223	GQ397234
Aspidomorphus schlegeli	GQ397168	–	–	GQ397191	–	–	–
Austrelaps labialis	EU547077	EU546900	EU547029	–	EU546986	EU546939	EU547175
Austrelaps superbus	EU547078	EU546901	EU547030	–	EU546987	EU546940	EU547176
Brachyurophis australis	EU547056	EU546881	EU547010	–	EU546965	–	KF736316
Brachyurophis semifasciata	EU547057	EU546882	EU547012	–	EU546966	EU546922	KF736318
Bungarus fasciatus	EU547086	JF357954	EU547037	–	–	AY058924	JN687935
Bungarus flaviceps	AJ749351	–	–	–	–	–	–
Bungarus multicinctus	AJ749327	–	–	–	–	AF435021	HM439979
Bungarus niger	AJ749304	–	–	–	–	–	–
Bungarus sindanus	AJ749346	–	–	–	–	–	–
Cacophis squamulosus	EU547052	EU366440	EU547007	–	EU546961	EU366451	EU547150
Cryptophis nigrescens	EU547070	EU546893	EU547022	–	EU546979	EU546932	EU547168
Demansia papuensis	EU547044	EU546871	EU547002	–	EU546953	EU546910	EU547142
Demansia psammophis	GQ397172	GQ397201	GQ397209	GQ397192	GQ397220	GQ397230	GQ397240
Demansia vestigiata	EU547045	EU546872	EU547003	–	EU546954	EU546911	EU547143
Denisonia devisi	EU547071	EU546894	EU547023	–	EU546980	EU546933	EU547169
Drysdalia coronoides	EU547075	EU546898	GU062856	–	–	EU546937	EU547173
Drysdalia mastersii	EU547076	EU546899	GU062869	–	EU546985	EU546938	EU547174
Echiopsis curta	EU547072	EU546895	EU547024	–	EU546981	EU546934	EU547170
Elapognathus coronata	EU547069	EU546892	EU547021	–	EU546978	EU546931	EU547167
Emydocephalus annulatus	EU547087	FJ587094	FJ593195	–	EU546996	EU546947	EU547185
Ephalophis greyae	JX002976	FJ587095	FJ593197	–	–	FJ587173	FJ587208
Furina diadema	EU547053	EU546878	EU547008	–	EU546962	EU546917	EU547151
Furina ornata	EU547054	EU546879	EU547009	–	EU546963	EU546918	KF736324
Hemiaspis damelii	EU547073	EU546896	FJ593193	–	–	EU546935	DQ233979
Hemiaspis signata	EU547074	EU546897	EU547026	–	EU546983	EU546936	EU547172
Hoplocephalus bitorquatus	EU547079	EU546902	EU547031	–	EU546988	EU546941	EU547177
Hydrelaps darwiniensis	EU547084	FJ587098	FJ593200	–	EU546993	EU546946	DQ234047
Hydrophis atriceps	JQ217206	KC014270	KC014471	–	–	KC014291	JQ217152

Continued

Outgroup Species	cyt*b*	*RAG–1*	*ND4*	*SPTBN1*	*MyHC2*	*c–mos*	*16S*
Hydrophis brookii	DQ233943	FJ587110	KC014474	–	–	FJ587188	DQ234028
Hydrophis peronii	JQ217200	FJ587102	FJ593204	–	–	FJ587180	KC014311
Hydrophis curtus	EU547085	FJ587123	FJ593227	–	EU546994	FJ587200	KJ653937
Hydrophis coggeri	JQ217207	KC014267	JQ217217	–	–	KC014295	JQ217153
Hydrophis schistosa	KC014393	JX987181	JX987171	–	–	KC014290	JX987140
Laticauda colubrina	AF217834	EU366433	FJ606513	–	EU546949	AF544702	EU547138
Laticauda colubrina	EU547040	–	AY058977	–	–	EU366446	–
Laticauda colubrina	–	–	FJ606508	–	–	AY058932	–
Laticauda frontalis	–	FJ587080	FJ606515	–	–	FJ587157	FJ587206
Laticauda frontalis	–	EU366433	FJ593190	–	–	FJ587156	FJ587205
Laticauda guineai	–	–	FJ606516	–	–	–	–
Laticauda laticaudata	AB701327	FJ587082	FJ593192	–	–	FJ587159	FJ587203
Laticauda laticaudata	AB701328	–	FJ606532	–	–	FJ587158	FJ587204
Laticauda laticaudata	AB701325	–	FJ606537	–	–	–	–
Laticauda laticaudata	FJ587153	–	FJ606526	–	–	–	–
Laticauda laticaudata	FJ587154	–	FJ606536	–	–	–	–
Laticauda saintgironsi	–	–	FJ606506	–	–	–	–
Laticauda saintgironsi	–	–	FJ606501	–	–	–	–
Laticauda semifasciata	AB701339	–	–	–	–	–	–
Laticauda semifasciata	AB701336	–	–	–	–	–	–
Loveridgelaps elapoides	S. Keogh	–	S. Keogh	–	–	–	S. Keogh
Microcephalophis gracilis	KC014419	KC014271	KC014494	–	–	KC014299	KC014341
Micropechis ikaheka	EU547042	EU366435	EU547000	–	EU546951	FJ587160	EU547140
Micropechis ikaheka	EU547042	–	–	–	–	EU366449	FJ587207
Micropechis ikaheka	GQ397171	–	GQ397208	GQ397194	–	GQ397226	GQ397236
Naja naja	EU547039	EU366432	EU546997	–	EU546948	AF435020	EU547137
Neelaps bimaculatus	EU547059	–	EU547013	–	EU546968	EU546920	KF736345
Notechis scutatus	AF217836	EU546905	AY058981	–	EU546991	EU546944	EU547180
Ogmodon vitianus	S. Keogh	–	S. Keogh	–	–	–	KF736310
Oxyuranus microlepidotus	EU547050	EU366439	EF210823	–	EU546959	EU366450	EU547148
Oxyuranus scutellatus	EU547051	EU546877	EF210826	–	EU546960	EU546916	EU547149
Parasuta monachus	EU547067	EU546890	EU547019	–	EU546976	EU546929	EU547165
Paroplocephalus atriceps	EU547080	EU546903	EU547032	–	EU546989	EU546942	EU547178
Pseudechis australis	EU547046	EU546873	AY340177	–	–	EU546912	EU547144
Pseudechis australis	AF217824	–	AY343092	–	–	–	AJ749377
Pseudechis porphyriacus	–	–	AY340170	–	–	–	–
Pseudonaja modesta	EU547049	EU546876	–	–	EU546958	EU546915	EU547147
Pseudonaja nuchalis	–	–	EF210839	–	–	–	–
Pseudonaja textilis	EU547048	EU546875	–	–	EU546957	EU546914	EU547146
Rhinoplocephalus bicolor	EU547068	EU546891	EU547020	–	EU546977	EU546930	EU547166
Salomonelaps par	S. Keogh	–	S. Keogh	–	–	–	S. Keogh
Simoselaps anomalus	EU547061	EU546885	EU547014	–	EU546970	EU546924	KF736315
Simoselaps bertholdi	EU547062	EU546886	EU547015	–	EU546971	EU546925	EU547160
Suta fasciata	EU547064	EU546888	EU547016	–	EU546973	EU546927	EU547162
Suta spectabilis	EU547065	EU546889	EU547017	–	EU546974	EU546928	EU547163
Suta suta	EU547066	EU366436	EU547018	–	EU546975	EU366452	EU547164
Tropidechis carinatus	EU547081	EU546904	EU547033	–	EU546990	EU546943	EU547179
Vermicella calonotus	EU547060	EU546884	EF210841	–	EU546969	EU546923	EU547158
Vermicella intermedia	EU547055	–	EF210842	–	–	EU546919	