Abstract:

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, due to 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 twelve described species within *Toxicocalamus*, representing the largest molecular dataset for this genus. We found that a system of offshore island arcs in Melanesia was the center 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 *T. loriae*, rendering that species polyphyletic. Continued work on *Toxicocalamus* is needed to document the diversity of this genus and likely will result in additional species discovery. Our increased taxon sampling allowed us to better understand the evolution and biogeography of Hydrophiinae; however, several unsampled lineages remain, whose later study may be used to test our biogeographic hypothesis.

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ADDITIONAL KEYWORDS: Australasia – Fiji – Melanesia – Snake – Solomon Islands

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INTRODUCTION

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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 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. Monophyly of Hydrophiinae has been

well supported through morphological (McDowell, 1970; McCarthy, 1985) and genetic

(Slowinski, Knight & Rooney, 1997; Keogh, 1998; Slowinski & Keogh, 2000; 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

reemergence in Melanesia (McDowell, 1970; Keogh, Shine & Donnellan 1998; Scanlon & Lee,

2004). However, there is conflicting evidence whether all Melanesian taxa are basal to

Australian taxa or if there have been reverse exchanges from Australia to Melanesia as well

(Sanders *et al*. 2008; Metzger *et al*., 2010).

The evolutionary relationships and biogeographic origins of the basal hydrophiine genera

have been difficult to assess due to 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

61 studies, *Ogmodon*, *Salomonelaps*, and *Loveridgelaps* were not included, and the basal lineages

62 were poorly resolved within Hydrophiinae (Sanders *et al.*, 2008; Metzger *et al.*, 2010; Pyron, *et*

63 *al.*, 2013).

64   In addition, the unstable placement of the basal genera has been influenced by

65 insufficient sampling within *Cacophis* Günther, 1863, and *Toxicocalamus* Boulenger, 1896.

66 *Cacophis* is found in the rainforests of eastern Australia, has been represented in phylogenetic

67 studies by only one of the four species in the genus (*Cacophis squamulosus* Duméril, Bibron &

68 Duméril, 1854), and its placement among the Hydrophiinae has been unstable (Keogh *et al.*,

69 1998; Scanlon, 2003, Scanlon & Lee, 2004; Sanders *et al.*, 2008; Metzger *et al.*, 2010; Pyron *et*

70 *al.*, 2013). *Toxicocalamus*, endemic to New Guinea and adjacent islands to the north and

71 southeast, has been represented by one or two of the twelve described species.

72   For *Toxicocalamus*, Sanders *et al.* (2008) used a single representative (*T. preussi*

73 Sternfeld, 1913) and did not recover it among the basal Melanesian taxa of the Hydrophiinae.

74 Rather, another New Guinean genus, *Micropechis*, was retrieved as basal. A second sample from

75 a different species (*T. loriae* Boulenger, 1898) was added by Metzger *et al.* (2010) and also used

76 by Pyron *et al.* (2013). Both found that the two species did not cluster together, raising the

77 possibility that *Toxicocalamus* is in fact polyphyletic, which would also be consistent with the

78 prior assignment of its current contingent of species across three genera. Beyond this,

79 evolutionary relationships of *Toxicocalamus* to other elapids remain poorly understood, and

80 relationships within the genus have never been assessed.

81   *Toxicocalamus* consists of 12 named species of cryptozoic snakes (McDowell, 1969;

82 Kraus, 2009; O'Shea, Parker & Kaiser, 2015). The genus was named by Boulenger (1896) to

83 accommodate a single species, *T. longissimus*, endemic to Woodlark Island, off southeastern

84 New Guinea. Boulenger (1898), Lönnberg (1900), and Sternfeld (1913) later named

85 *Apistocalamus*, *Pseudapistocalamus*, and *Ultrocalamus*, respectively, to contain related snake

86 species newly named by them. Of these, *Pseudapistocalamus* was synonymized with

87 *Toxicocalamus* and the other two taxa subsumed within that genus as subgenera by McDowell

88 (1969). These subgenera were recognized on the basis of major differences involving loss or

89 fusion of assorted head scales, relative body width, and osteological and hemipenial features

90 (McDowell, 1969); nonetheless, these names have not been used by subsequent authors. Indeed,

91 the only systematic work on the genus subsequent to McDowell's (1969) revision has been the

92   synonymization of *Vanapina lineata* (de Vis, 1905) with *T. longissimus* (Ingram, 1989) and the

93   description of two new species by Kraus (2009) and one new species by O'Shea *et al.* (2015).

94   Additional species require description (O'Shea, 1996; Kraus, 2009; O'Shea *et al.* 2015; F. Kraus,

95   unpubl. data); for example, snakes currently assigned to *T. loriae* are a sibling-species complex

96   (Kraus, 2009, unpubl. data; O'Shea *et al.* 2015; and see below), and the western half of New

97   Guinea has barely been surveyed for these snakes. Consequently, diversity in the genus will

98   certainly be higher than apparent from existing nomenclature.

99   This sparse systematic treatment stems from the under-collected nature of the Papuan

100   herpetofauna generally and the secretive habits of these snakes specifically, both of which factors

101   have led to a scarcity of specimens to support biological studies (with "*T. loriae*" being the sole

102   exception). Similarly, field studies of these snakes have been non-existent. In the almost 120

103   years since the genus was described, only two authors on the genus (Kraus, O'Shea) appear to

104   have had experience with the species in the field. Despite this, these snakes appear to be

105   ecologically unusual among elapids in feeding primarily on earthworms (O'Shea, 1996; Shine &

106   Keogh, 1996; Goodman, 2010; Calvete *et al.*, 2012; O'Shea *et al.*, 2015; F. Kraus, unpubl. data),

107   although fly pupae and a land snail have also been reported among stomach contents (Bogert &

108   Matalas, 1945; McDowell, 1969). Beyond these ecological attributes, species of *Toxicocalamus*

109   exhibit a range of morphological variation that is unusual within any snake genus. Some species

110   are very thinly elongated, whereas others are of average snake habitus, and one is rather stout. A

111   number of different fusions among the head and body scales has occurred. Fusion of head scales

112   is common among fossorial snakes, but it usually involves consistent fusion of one or two pairs

113   of scales. In *Toxicocalamus*, subcaudal scales may be single or divided, the anal scale may be

114   single or divided, dorsal scale rows vary from 13-17, and five separate types of fusion have

115   occurred among the head scales (McDowell, 1969; Kraus, 2009). The history of these

116   evolutionary modifications and what may account for their variation remain unknown.

117   Most, if not all, species are also behaviorally inoffensive, being disinclined to bite – for

118   example, one of us (FK) has handled 40 living animals of eight named and several unnamed

119   species and has never witnessed any attempt to bite. Further, it is doubtful that the small gapes

120   and fangs of most species would allow for envenomation of humans or other larger vertebrates

121   should they attempt to bite. Despite this, *T. longissimus* – the only species examined to date – has

122   very potent venom components (Calvete *et al.*, 2012), which would seem unnecessary for either

123   capture of their earthworm prey or for effective defense, given their structural and behavioral

124   limitations. Furthermore, *T. buergersi* Sternfeld, 1913 has a very elongated venom gland that

125   extends posteriorly into the body cavity (McDowell, 1969), suggesting that it has the capacity to

126   produce a large quantity of venom. Again, it is unclear what dietary or defensive use this ability

127   could serve. It is possible that the highly toxic venom components of *T. longissimus* are merely

128   phylogenetically conserved and retained from ancestors. However, it remains difficult to explain

129   the large venom glands of *T. buergersi*.

130   Here we conduct a molecular phylogenetic analysis to 1) better understand the evolution

131   of the basal genera within Hydrophiinae, 2) determine *Toxicocalamus*' phylogenetic placement

132   within the subfamily, and 3) determine the evolutionary relationships of the species within this

133   peculiar genus. To address the basal instability, we include available sequence data from other

134   hydrophiines, including the monotypic Melanesian genera *Micropechis*, *Ogmodon,*

135   *Loveridgelaps*, and *Salomonelaps*. However, we were unable to include additional species from

136   *Cacophis* within this study due to lack of sample availability. We address the paucity of prior

137   taxonomic sampling within *Toxicocalamus* by utilizing eight of the 12 named species, as well as

138   additional species currently undescribed. Of the four named species of *Toxicocalamus* missing

139   from our dataset, two are known only from holotypes (*T. grandis* Boulenger, 1914 and *T.*

140   *ernstmayri* O'Shea, Parker & Kaiser, 2015), another from two specimens (*T. spilolepidotus*

141   McDowell, 1969), the fourth from five (*T. buergersi*). We were unsuccessful in obtaining DNA

142   from preserved specimens of the latter two species, so we did not attempt to sample the

143   holotypes.

144

145   MATERIAL AND METHODS

146   TAXON SAMPLING

147

148   To determine the evolutionary placement of *Toxicocalamus* within the Hydrophiinae, we

149   used sequences on GenBank for 90 individuals from 68 species (Appendix 1). These 68 species

150   include representatives of 40 of 44 genera within Hydrophiinae. The remaining four genera do

151   not have sequences currently available. Two of these (*Kolpophis* Smith, 1926 and *Thalassophis*

152   Schmidt, 1852) are seasnakes and likely would not change the topology if they were included.

153   *Antaioserpens* Wells & Wellington, 1985, is, according to Scanlon, Lee, & Archer (2003), sister

154 to *Simoselaps*, whose placement has been stable in the phylogeny of Hydrophiinae (Sanders *et*

155 *al.*, 2008; Metzger *et al.*, 2010; Pyron, *et al.*, 2013). The final genus, *Parapistocalamus*, from the

156 northern Solomon Islands would be a valuable addition to the phylogeny if tissues ever become

157 available. In addition, we used six species from the other subfamily of Elapidae, Elapinae Boie,

158 1827, to root our phylogeny.

159   We collected 26 tissue samples of *Toxicocalamus* from 12 localities on New Guinea and

160 surrounding islands. We also acquired two tissue samples of *Toxicocalamus* through tissue loan.

161 In addition, there was one *T. preussi* sequence available on GenBank, and Scott Keogh provided

162 sequence data for an additional *T. preussi*. These samples represent eight of the twelve currently

163 named species, as well as samples from individuals of undescribed species (Fig 1; Table 1).

164

165   DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

166

167   We used the DNEasy Blood and Tissue Kit (Qiagen) to extract total genomic DNA from

168 all tissue samples. We performed gel electrophoresis on a 2.0% agarose gel to determine quality

169 of the extracted DNA. We attempted to sequence three mitochondrial loci and four nuclear loci

170 for all individuals: 16S rRNA (*16S*), cytochrome b (*cyt-b*), NADH dehydrogenase (*ND4*), oocyte

171 maturation factor (*c-mos*), recombination activating gene 1 (*RAG-1*) myosin heavy chain 2 intron

172 (*MyHC-2*), and β-spectrin nonerythrocytic intron 1 (*SPTBN1*) using published or designed

173 primers and standard PCR conditions (Table 2). PCR product was cleaned using Gel/PCR DNA

174 Fragment Extraction Kit (IBI). Cleaned PCR product was sequenced in both directions at the

175 University of Arizona Genetics Core Facility on an ABI 3730XL DNA Analyzer (Applied

176 Biosystems Inc.).

177

178   SEQUENCE ALIGNMENT AND DATA ANALYSIS

179

180   To visualize and edit chromatograms, we used Sequencher 5.1 (Gene Codes Corp.).

181 Heterozygosities at nuclear loci were coded with the appropriate IUPAC ambiguity code. We

182 used the MUSCLE alignment algorithm (Edgar, 2004) in MEGA 5.1 (Tamura *et al.*, 2011) with

183 default settings to align sequences and then verified alignments by eye. Protein-coding sequences

184   were translated into amino acids to ensure no stop codons were present. All other sequences used

185   in this study are from GenBank (Appendix 1).

186   We calculated genetic distances within *Toxicocalamus* for all loci and compared levels of

187   genetic diversity among species of *Toxicocalamus* in MEGA 5.1 (Tamura *et al*., 2011) using the

188   Tamura and Nei (TrN) model (Tamura & Nei, 1993) for nucleotide substitution. To determine

189   the appropriate partition and model of evolution for our loci, all possible partitions were

190   considered for the protein-coding genes, while *16S*, *MyHC-2*, and *SPTBN-1* were left

191   unpartitioned. We then used the Bayesian Information Criterion (BIC) and the greedy search

192   scheme in PartitionFinder (Lanfear *et al*., 2012) to generate the best partition and modeling

193   scheme for all programs used in our phylogenetic analyses (Table 2).

194

195   PHYLOGENETIC ANALYSES

196

197   We used MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquest & Huelsenbeck,

198   2003) and RAxML 8.0.20 (Stamatakis, 2014) for phylogenetic analysis. For both programs, we

199   generated a concatenated phylogeny of all loci used, as well as individual gene trees for each

200   locus.

201   We simultaneously ran MrBayes two times with 1 cold and 3 hot chains for 7 million

202   generations each. The starting trees were independent between runs and randomly chosen. We

203   sampled one out of every 1000 trees. The first 20,000 trees were discarded as burn-in, and then

204   we used Tracer 1.6.0 (Rambaut, Suchard & Drummond, 2013) to plot the log-likelihood scores

205   against generation number to ensure stationarity was reached. A 50% majority-rule consensus

206   tree was calculated using the posterior distribution of trees. ML analyses in RAxML were

207   performed with 1000 bootstrap pseudoreplicates. We visualized the phylogenetic trees with

208   FigTree 1.4 (Rambaut & Drummond, 2012). Nodes with posterior probabilities (PP) of ≥ 0.95

209   from BI and nodes with bootstrap support (BS) $\geq 75\%$ from ML were considered strongly

210   supported.

211

212   CHARACTER MAPPING

213

214   We mapped relative width of ventrals, fusion of the preocular and prefrontal scales, anal

215   plate divided/undivided, internasal fused to prefrontal, and subcaudals undivided onto our

216   phylogeny. These five characters were chosen because they are important in *Toxicocalamus*

217   species identification and McDowell (1969), Kraus (2009), and O'Shea et al. (2015)

218   incorporated them into their dichotomous keys for *Toxicocalamus*. We used the most

219   parsimonious character map to determine the ancestral state for the character. If two

220   parsimonious trees were equally likely, we used the character state of *Ogmodon vitianus* as the

221   outgroup to determine which character map to present.

222

223   RESULTS

224   TAXON SAMPLING

225

226   Several of our sampled undescribed species of *Toxicocalamus* key out morphologically to

227   *T. loriae* (O'Shea, 1996; Kraus, 2009; O'Shea *et al*., 2015) and are referred to as *T. loriae* in

228   many museum collections. However, we retrieve these samples across a wide range of our

229   phylogeny. For the sake of clarity in presenting our results, we will refer to each of these as "*T.*

230   *loriae* Clade 1, *T. loriae* Clade 2, etc.", recognizing that these represent cryptic species that

231   require further taxonomic elucidation but that they have remained morphologically undiagnosed

232   and clustered under a single name (Kraus, 2009; O'Shea *et al*., 2015).

233

234   SEQUENCE DATA

235

236   We generated sequences for 28 individuals within *Toxicocalamus* and deposited them on

237   GenBank (Table 1). In total, including GenBank sequences for outgroup taxa, we analyzed 126

238   individuals. The length of the concatenated alignment was 5843 base pairs: 1754 mitochondrial

239   protein-coding, 521 rRNA, 1834 nuclear protein-coding, and 1734 nuclear intron (Table 1; Table

240   2). Protein-coding genes did not contain frameshifts or internal stop codons. Genetic distances

241   between species or clades with *Toxicocalamus* ranged from 0.06 – 0.29 for *cyt-b*, 0.07 – 0.32 for

242   *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,*

243   and 0.00 – 0.04 for *SPTBN1* sequences.

244

246

247   We present the BI phylogenies of the concatenated dataset and include the ML bootstrap

248   support values on the nodes (Fig. 2; Fig. 3). Overall, the BI and ML trees were identical at all

249   supported nodes (PP of ≥0.95 from BI and/or nodes with BS ≥75% from ML). The only

250   differences in the topologies generated by the two algorithms were in the nodes without support,

251   none of which change the relationships among the basal genera or the relationships among

252   species within *Toxicocalamus*. Thus, our interpretations and the conclusions drawn are the same

253   under each analysis.

254   Our results support Hydrophiinae as monophyletic and *Laticauda* as the basal member, as

255   found in previous studies (Sanders *et al*., 2008; Metzger *et al*., 2010; Lane & Shine, 2011; Pyron,

256   *et al.*, 2013). Our phylogeny is also in general agreement with relationships found among the

257   Australian genera and seasnakes (Scanlon & Lee, 2004; Wuster *et al*., 2005; Lukoschek &

258   Keogh, 2006; Sanders *et al.*, 2008). However, inclusion of *Ogmodon, Salomonelaps,* and

259   *Loveridgelaps*, along with more representatives from *Toxicocalamus*, yielded a novel topology

260   for these genera in relation to *Micropechis, Aspidomorphus* Fitzinger, 1843, *Demansia* Gray,

261   1842, and *Cacophis.* The included species from the Solomon Islands and Fiji are the basal

262   terrestrial lineage within Hydrophiinae (Fig. 2; PP=1/BS=99), and *Toxicocalamus* is the next-

263   most-basal lineage, clearly supporting Melanesia as the origin of the terrestrial Hydrophiinae.

264   All analyses found *Toxicocalamus* to be monophyletic. Within *Toxicocalamus*, *T.

265   stanleyanus* Boulenger, 1903 + *T. preussi* (PP=1/BS=99) is strongly supported as a clade basal to

266   the remaining species. *Toxicocalamus holopelturus* McDowell, 1969 was strongly supported as

267   sister to the remaining species (Fig. 3; PP=1/BS=93). Within the latter clade, *T. loriae* was found

268   to be polyphyletic, though *T. loriae* Clade 1's placement was only weakly supported (Fig. 3). As

269   expected based on morphological similarity (Kraus, 2009), *T. misimae* McDowell, 1969 and *T.

270   longissimus* are sister species (Fig. 3). This sister relationship is further corroborated by the

271   geological history of the two islands these species occupy. Misima Island and Woodlark Island

272   are home to *T. misimae* and *T. longissimus,* respectively, and were connected as recently as 1.2

273   million years ago, before the opening Woodlark Basin separated them (Taylor, Goodliffe, &

274   Martinez, 1999).

275   In analyses of *ND4* and *cyt-b* gene trees, the position of *T. loriae* Clade 1 was recovered

276   as basal to the remaining lineage of "*T. loriae*" Clades 2-6, *T. mintoni* Kraus, 2009, and *T.*

277   *pachysomus* Kraus, 2009. For this phylogenetic arrangement, *T. mintoni* and *T. pachysomus*

278   render the "*T. loriae*" species complex paraphyletic. Nonetheless, both are morphologically very

279   distinct from "*T. loriae*". Several additional "*T loriae*" specimens were found to form four

280   strongly supported (Clades 2, 3, 5, 6) and one weakly supported (Clade 4) lineages (Fig 3).

281

282   CHARACTER MAPPING

283

284   We found that the ancestral state within *Toxicocalamus* was narrow ventrals which is not

285   seen in *Ogmodon, Salomonelaps, or Loveridgelaps*. This corresponds to a long and thin overall

286   habitus with the normal snake habitus being regained later either once or twice depending on the

287   character-state reconstruction used (Fig. 4A). The state for *Ogmodon vitianus* is preocular

288   unfused to prefrontal; therefore, if that is basal in *Toxicocalamus,* these scales have become

289   fused three independent times (Fig. 4B). *Ogmodon vitianus* has a divided anal plate. Interpreting

290   this as ancestral, the anal plates have fused twice within *Toxicocalamus* (Fig. 4C). The character

291   state of internasal fused to prefrontal is seen in *T. preussi* and *T. buergersi* (not in analysis), and

292   having undivided subcaudals is an autapomorphy in *T. holopelterus* (Fig. 4D).

293

294   DISCUSSION

295

296   By including genera not used in prior phylogenetic analyses and representing

297   *Toxicocalamus* by a majority of its species, we generated a well-supported phylogeny of the

298   Hydrophiinae, clarifying placement of basal taxa and shedding light on the species relationships

299   within the enigmatic New Guinean endemic *Toxicocalamus*. Congruent with previous studies,

300   we found Hydrophiinae to be monophyletic, with *Laticauda* basal to all other lineages (Keogh,

301   1998; Scanlon & Lee, 2004; Sanders *et al.* 2008; Metzger *et al*., 2010; Lane & Shine, 2011). Our

302   results indicate that the five basal-most terrestrial genera in the subfamily are from Melanesia

303   and that the early ancestors of Hydrophiinae were likely cryptozoic. Our study clearly

304   demonstrates the adverse effects of inadequate taxon sampling on phylogenetic estimations. By

305   utilizing eight described and several undescribed species of *Toxicocalamus,* we determined that

306   the genus is monophyletic, contrary to previous studies (Metzger *et al*., 2010; Pyron, *et al.*,

307   2013), and we confirm that species currently designated *Toxicocalamus loriae* represent a

308   species complex in need of taxonomic revision (Kraus, 2009; O'Shea *et al*., 2015). We also find

309   *Toxicocalamus* to be basal to other New Guinean and Australian taxa within Hydrophiinae.

310   The basal relationships within Hydrophiinae, including the placement of *Toxicocalamus,*

311   have been difficult to determine due to incomplete taxon sampling, which has led to different

312   nomenclatures for the subfamilial taxonomy. We follow most authors in defining the subfamily

313   Hydrophiinae to contain all marine and terrestrial Australasian taxa (Slowinski & Keogh, 2000;

314   Castoe *et al*. 2007; Metzger *et al*., 2010), with the basal member of this subfamily being

315   *Laticauda* (Fig. 2). Some authors have elevated Hydrophiinae to family status and divided it into

316   two separate subfamilies, the Laticaudinae, including only *Laticauda*, and the Oxyuraninae,

317   which includes the remaining genera (Sanders *et al*., 2008; Kelly *et al*., 2009). However,

318   *Parapistocalamus*, a genus endemic to the Solomon Islands, has not been represented within any

319   molecular phylogenies, and its morphological placement in relation to *Laticauda* and the other

320   genera is uncertain. Based on the movement of the palatine bone during swallowing McDowell

321   (1970) differentiated Elapids into two groups: "palatine erectors", which includes all Elapids

322   outside Hydrophiinae, as well as *Laticauda* and *Parapistocalamus*, and "palatine draggers",

323   which includes the remaining hydrophiines (Deufel & Cundall, 2009). McDowell (1985) later

324   described *Laticauda* and *Parapistocalamus* as intermediates between the two phenotypes

325   because they lack the palatine choanal process like other Australasian elapids. If a tissue sample

326   can be acquired for *Parapistocalamus hedigeri* Roux, 1934, then it would be possible to test this

327   nomenclatural hypothesis further and determine *Parapistocalamus*' placement among the other

328   monotypic basal genera *Ogmodon*, *Loveridgelaps*, and *Salomonelaps* in Melanesia. We predict

329   that *Parapistocalamus* would be the next most-basal genus after *Laticauda*. The complete

330   "palatine dragger" phenotype would then be a synapomorphy for the remaining hydrophiines,

331   with *Ogmodon, Loveridgelaps*, and *Salomonelaps* being the basal members with that character

332   state.

333   *Ogmodon vitianus* Peters, 1864 from Fiji, and *Loveridgelaps elapoides* Boulenger, 1890,

334   and *Salomonelaps par* Boulenger, 1884 from the Solomon Islands, were initially included in

335   molecular phylogenetic studies and found to be among the basal members of Hydrophiinae

336   (Keogh, 1998; Keogh *et al*., 1998). More recent studies have not included these data, preventing

337 a complete evolutionary understanding of this subfamily (Sanders *et al*., 2008; Metzger *et al*.,

338 2010; Pyron *et al*., 2013). Including these genera in our phylogeny, we determined that they form

339 a monophyletic assemblage basal to the New Guinean and Australian species (Fig. 2). This

340 phylogenetic arrangement supports Melanesia as the evolutionary origin of terrestrial

341 hydrophiines, which is further supported by the next two basal-most lineages (*Toxicocalamus*,

342 *Micropechis*) also being Melanesian.

343   *Toxicocalamus* was recovered as monophyletic and not sister to any single currently

344 recognized genus. Metzger *et al*. (2010) recovered a paraphyletic *Toxicocalamus* when using the

345 *T. loriae* and *T. preussi* sequences available on GenBank as representatives of the genus, and

346 Pyron *et al*. (2013) obtained the same results using the same dataset. Our results indicate that this

347 conclusion likely resulted from two things. First, few of the outgroup taxa used in this study were

348 also used by them. Second, they utilized two highly divergent taxa as the only representatives for

349 *Toxicocalamus*. These omissions presumably led to poor resolution and long-branch attraction at

350 the base of the phylogeny. Previous studies had suggested *Toxicocalamus* to be closely related to

351 *Aspidomorphus, Demansia,* or *Micropechis* (Metzger *et al*., 2010; Sanders *et al*., 2008), but our

352 study does not support those findings either. Rather, we found *Micropechis* to be basal to the

353 remaining Hydrophiinae, followed by *Cacophis*. All of the basal terrestrial genera are cryptozoic,

354 spending much of their time under logs and rocks and in leaf-litter (McDowell, 1970; Zug &

355 Ineich, 1993; Shine & Keogh, 1996), although most also forage actively on the forest floor,

356 either diurnally or nocturnally (McCoy, 2006; F. Kraus, pers. obs.).

357   These basal relationships within the Hydrophiinae are consistent with the geological

358 history of the region. Kelly *et al*. (2009) estimated the Hydrophiinae to have originated

359 ~23MYA, and the oldest fossil elapid, interpreted as a *Laticauda*, is of the same age (Scanlon et

360 al., 2003). This coincides in time with the formation of island arcs in the western Pacific that

361 include parts of what are now the Solomon Islands, Fiji, and New Guinea (Hall, 2002, 2012).

362 Our results suggest that the early terrestrial hydrophiines originated on these islands, which could

363 only have been colonized by an early marine ancestor like *Laticauda*. The Solomon and Fiji

364 islands are parts of the Outer Melanesian Arc, which arose ca. 40 MYA, prior to the origin of the

365 Hydrophiinae (Hall, 2002, 2012; Colley, 2009; Davies, 2009). A separate and more northerly

366 island arc formed on the margin of the Caroline Plate at approximately the same time, was

367 rotated into adjacency to the Outer Melanesian Arc, and continued rotating to the south and west

368 to accrete sequentially onto the northern margin of New Guinea 20-5 MYA (Davies et al., 1997;

369 Hall, 2002; Hall, 2012). Judging from the present distribution of the basal lineages in this clade,

370 terrestrial hydrophiines seem likely to have arisen on islands of these arc systems when they

371 were placed so as to form a single continuous chain ca. 30-20 MYA (cf.

372 http://searg.rhul.ac.uk/current_research/plate_tectonics/plate_tectonics_SE_Asia%200-

373 55Ma.html). Separation of the northern (and western) arc from the Outer Melanesian Arc and its

374 subsequent accretion onto New Guinea would have led to the rapid invasion and speciation of

375 elapids in New Guinea and Australia (the former being merely the northern portion of the latter

376 continent plus accreted islands of these former arc systems), as inferred by the very short branch

377 lengths among basal taxa (Fig. 2; Keogh *et al*., 1998; Scanlon & Lee, 2004; Lukoschek &

378 Keogh, 2006).

379 The remaining phylogeny of Hydrophiinae was not fully resolved, but there was support

380 for invasions from New Guinea to Australia and reinvasions back to New Guinea. For example,

381 *Aspidomorphus* and *Demansia* are well supported as sister genera. *Aspidomorphus* is endemic to

382 New Guinea while *Demansia* is found in both Australia and New Guinea. The only Australian

383 endemic found among the basal genera was *Cacophis*, with moderate support in both our BI and

384 ML phylogenies (Fig. 2). In previous phylogenetic analysis, *Cacophis* has been hypothesized to

385 be sister to *Notechis* Boulenger, 1896 (Keogh *et al*., 1998), sister to *Aspidomorphus* and/or

386 *Demansia* (Scanlon & Lee, 2003), related to *Furina* Duméril, 1853 (Sanders *et al*., 2008), among

387 the basal Hydrophiinae (Metzger *et al*., 2010), or among Australian taxa other than *Notechis* or

388 *Furina* (Pyron *et al*., 2013). Using morphological data, Scanlon (2003) was unable to determine

389 its placement within Hydrophiinae. To better determine if *Cacophis* is related to other Australian

390 taxa or to the fossorial Melanesian taxa requires further taxon sampling within that genus.

391 It is important to note that two of the nomina that McDowell (1969) used as subgenera of

392 *Toxicocalamus* are polyphyletic. The type species for *Apistocalamus* is *T. loriae*, but McDowell

393 (1969) included *T. holopelturus* in that subgenus. Those taxa do not form a monophyletic clade.

394 The type species for *Toxicocalamus* is *T. longissimus*, but McDowell (1969) included *T.*

395 *stanleyanus* in that subgenus. Once again, they are not monophyletic. The third subgenus,

396 *Ultrocalamus*, included just *T. preussi* (type species) and *T. buergersi*, which were grouped by

397 McDowell (1969) based on the shared fusion of the internasal and prefrontal. We could not

398 obtain a sample of *T. buergersi*, and, therefore, we cannot test the validity of *Ultrocalamus*.

399 However, on the basis of our results, there is no current justification for recognizing subgenera

400 within *Toxicocalamus* – recognition of any two or more of them would render the others

401 paraphyletic (Fig. 3). Furthermore, taxonomy and species diversity within the genus remain

402 imperfectly known, with several species remaining to be diagnosed and the western half of New

403 Guinea remaining to be even modestly sampled for the genus. Thus, for a truly complete

404 understanding of this genus, further study, with emphasis on increased taxon sampling, will be

405 required.

406   *Toxicocalamus* species mostly come in two different body forms. The first are extremely

407 thin and elongate animals having narrow ventral scales; the second have a more normal snake

408 habitus and width to the ventral scales (*T. pachysomus* is an outlier of stouter habitus, cf. Kraus,

409 2009). Our results indicate that the elongate body form is ancestral within this genus (Fig. 4A).

410 All such species (*T. holopelturus*, *T. longissimus*, *T. misimae*, *T. preussi*, and *T. stanleyanus*) are

411 placed basally in the tree, and the "normal" snake habitus is re-gained later in evolution (Fig.

412 4A). Scalational fusions occur in several different species within *Toxicocalamus*, and

413 relationships are largely inconsistent with this variation (Fig. 4). Species that share particular

414 head-scale fusion patterns are not retrieved as monophyletic, suggesting that these features have

415 arisen multiple times (Fig. 4B,C). Also, our genetically divergent clades morphologically

416 assigned to *T. loriae* make clear that morphological divergence has not mirrored all substantial

417 genetic divergence or speciation patterns in the complex, a pattern also evident from

418 consideration of color patterns of living animals (F. Kraus, unpubl. obs.). Some of these more

419 derived populations have already been described, but most are currently recognized as "*T.

420 loriae*", a "species" that clearly requires taxonomic revision, as previously indicated (Kraus,

421 2009; O'Shea *et al.*, 2015).

422   At minimum, our phylogenetic analyses indicate that *T. loriae* as currently defined

423 morphologically is polyphyletic. There is considerable genetic distance between the two most

424 distant clades (1 and 6) based on *cyt-b* (0.21), *ND4* (0.16), and *16S* (0.10) data. *Toxicocalamus

425 loriae* Clade 1's position as part of a *T. longissimus* + *T. misimae* clade was only weakly

426 supported, and *ND4* and *cyt-b* d trees did not support this conclusion, nor do morphological data

427 (McDowell, 1969; Kraus, 2009). *Toxicocalamus loriae* Clade 1 occurs approximately 80 km

428 from the type locality for *T. loriae* on Mt. Victoria and represents our best estimate of true *T.

429 loriae*. To confirm this, re-collection on Mt. Victoria is needed so that molecular data from

430   individuals from that locality may be integrated into our phylogeny. *Toxicocalamus loriae* is

431   reported to occur throughout much of New Guinea, but it is unknown what range of genetic

432   variation is encompassed across this distribution because of the historical difficulty of collecting

433   in the western half of the island. If the trends apparent from this study apply throughout the

434   entirety of its range, then it is very likely that many species currently recognized as *T. loriae*

435   represent independent lineages and require systematic revision.

436   Despite remaining deficiencies in taxon sampling, we have presented evidence for

437   undocumented genetic diversity within *Toxicocalamus.* Our best-supported phylogeny infers

438   strong evidence for at least 13 distinct clades, five of which would appear to represent currently

439   undescribed species. Moreover, much of New Guinea remains unexplored. Hydrophiinae is a

440   speciose group and represents a relatively recent rapid radiation in the Australasian region

441   (Slowinski & Keogh, 2000; Sanders & Lee, 2008; Sanders *et al*., 2008). Discerning the true

442   evolutionary history of the genera contained within it will require extensive sampling effort

443   across both species and genetic markers. Understanding the relationships among the

444   Hydrophiinae has been a challenge for decades, but resolving the phylogeny of this group may

445   lead to a much better understanding of the biogeographic history of the region. Future work on

446   *Toxicocalamus* will lead to several species descriptions (F. Kraus, ongoing), but documentation

447   of the species distributions across New Guinea remains sorely needed.

448

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Appendix 1. List of species and accession numbers used to generate the Hydrophiinae phylogeny in Figure 2.

Outgroup Species	CytB	RAG1	ND4	SPTBN1	MyHC2	Cmos	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
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	EU547153

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

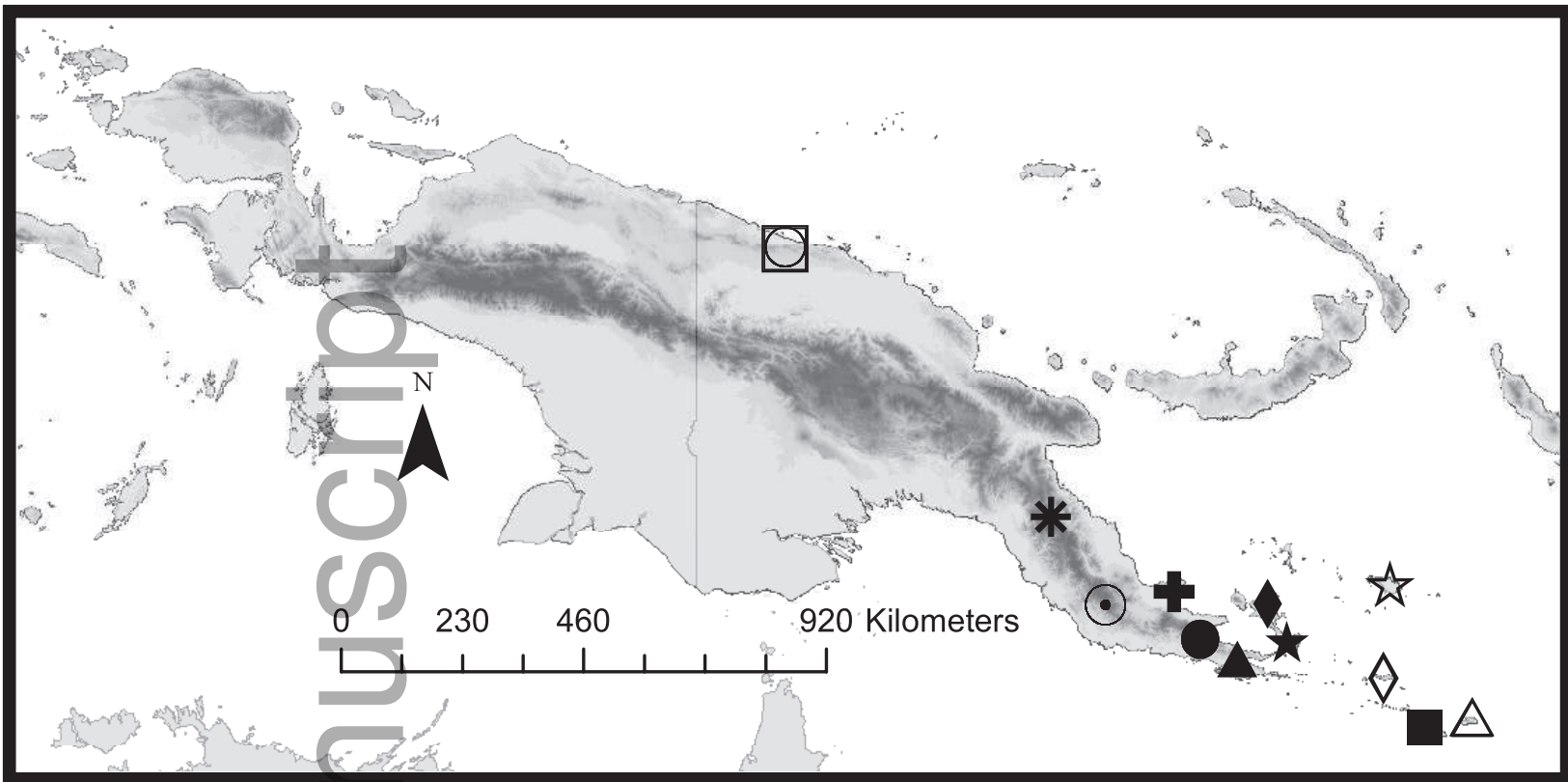| Species	Museum #	Collector #	Latitude	Longitude	C-MOS	MyHC-2	SPTBN1	RAG-1	16S rRNA	cyt-b	ND4
T. pachysomus	BPBM 15771	FK 5368	-10.3471	150.2330	—	KU144952	KU172565	KU128756	KT968679	KT778530	KU128809
T. loriae (Clade 5)	BPBM 16544	FK 6288	-9.4263	150.8015	—	KU144953	KU172566	KU128757	KT968680	KT778531	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	—

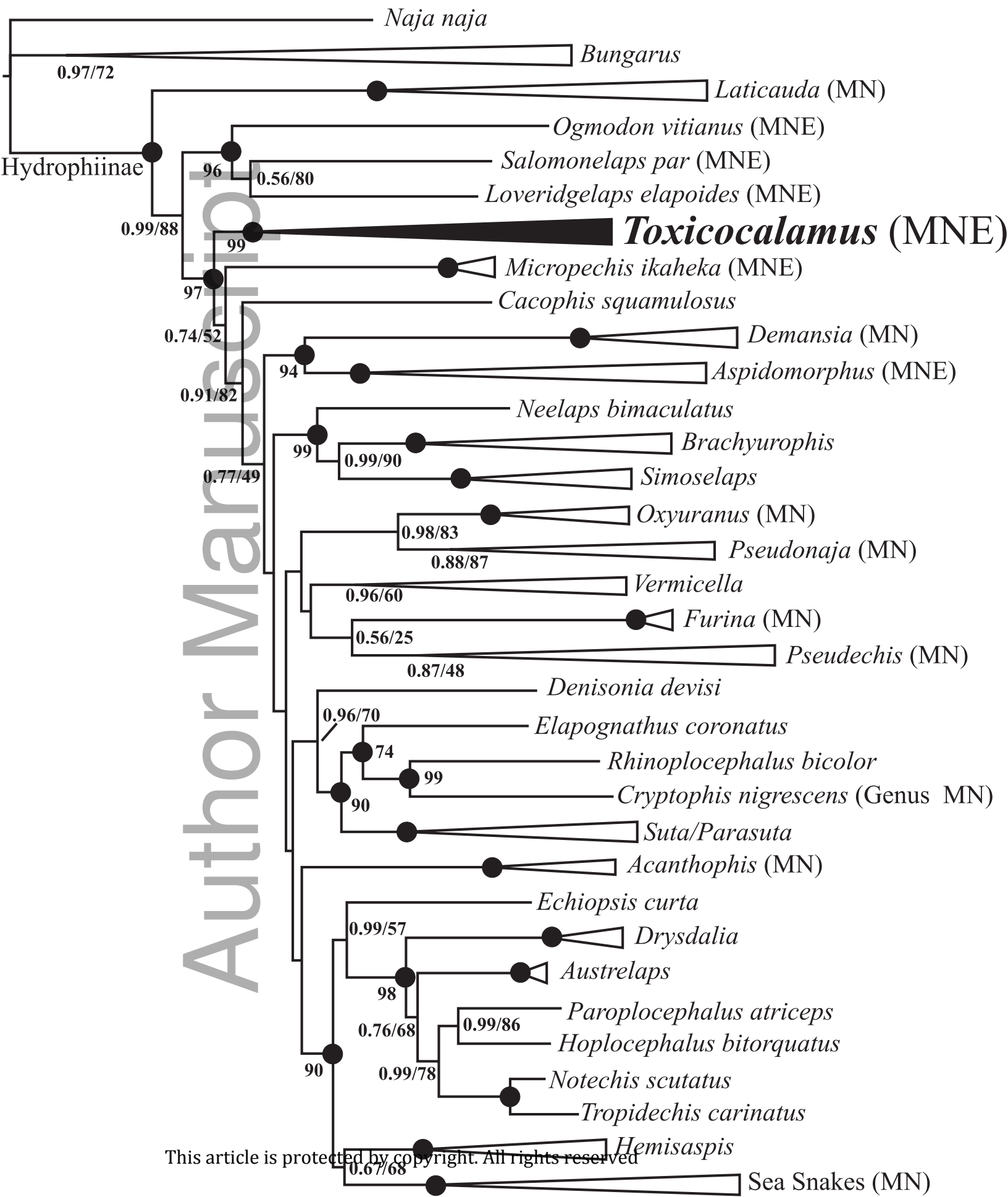| T. preussi (Sanders et al., 2008/Bolton et al., unpublished) | AM 136279 | ABTC:50506/ SAMARFJ126 | -3.3933 | 142.5283 | EU546909 | EU546952 | — | EU546870 | EU547141/ KF736325 | EU547043 | EU547001 |

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

Locus	Forward Primer	Reverse Primers	Temp (°C)	MgCl (mM)	Size (bp)	Variable/Parsimony informative within Toxicocalamus	Model	Reference
C-MOS	G303F 5'-ATT ATG CCA TCM CCT MTT CC-3'	G708R 5'-GCT ACA TCA GCT CTC CAR CA-3'	53	2.5	726	25/14	GTR + G	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'	55	2.5	525	64/42	HKY + I + G	Lyons et al. (1997)
	G240 5'-GAA CAC CAG CCT CAT CAA CC-3'	MyHC2R413 5'-GTC CTA AAC TCG CAG GCT AA-3'	50	2				Lyons et al. (1997) and This study
	MyHC2F60 5'-TCA GAA GTG GAA GAA GCT GTG CA-3'	G241 5'-TGG TGT CCT GCT CCT TCT TC-3'	50	2				This study and Lyons et al. (1997)
SPTBN1	SPTBN1-F1 5'-TCT CAA GAC TAT GGC AAA CA-3'	SPTBN1-R1 5'-CTG CCA TCT CCC AGA AGA A-3'	54	2	1209	93/36	GTR + G	Matthee et al. (2001)
RAG-1	G396(R13) 5'-TCT GAA TGG AAA TTC AAG CTG TT-3'	G397(R18) 5'-GAT GCT GCC TCG GTC GGC CAC CTT T-3'	55	2.5	1108	69/35	GTR + G	Groth & Barrowclough (1999)
	RAG1F122 5'-CTA AAG AAA ATG TGR CAG GAT CTC-3'	RAG1R1054 5'-GGG CAT CTC AAA ACC AAA TTG T-3'	50	2.5				This study
16S rRNA	16SF 5'-CGC CTG TTT ATC AAA AAC AT-3'	16SR 5'-CCG GTC TGA ACT CAG ATC ACG T-3'	48	2.5	521	125/89	GTR + I + G	Kocher et al. (1989)
cyt-b	L14910 5'-GAC CTG TGA TMT GAA AAA CCA YCG TTG T-3'	H16064 5'-CTT TGG TTT ACA AGA ACA ATG CTT TA-3'	48	2.5	1098	513/452	GTR + I + G	Burbrink, Lawson & Slowinski (2000)
	L14910 5'-GAC CTG TGA TMT GAA AAA CCA YCG TTG T-3'	ToxcytbR493 5'-AAG CGG GTR AGG GTT GG-3'	55	2.5				Burbrink et al. (2000) and This study
	ToxcytbF380 5'-TGA GCA GCA ACA TWA TTA CAA A-3'	ToxcytbR750 5'-GGT TAA TGT GYT GTG TGT-3'	48	2.5				This study
	ToxcytbF709 5'-TTA ACG ACC CYG AAA ACT T-3'	H16064 5'-CTT TGG TTT ACA AGA ACA ATG CTT TA-3'	48	2.5				This study and Burbrink et al. (2000)
ND4	ND4F 5'-TGA CTA CCA AAA GCT CAT GTA GAA GC-3'	ND4 tRNA-Leu 5'-TAC TTT TACC TTG GAT TTG CAC CA-3'	48	2.5	656	327/298	GTR + I + G	Arevalo, Davis & Sites (1994)
	ND4F123 5'-TAA CYT GCC TYC AAC AAA CAG A-3'	ND4R688 5'-TTG TCA AGR TCA CAG CTT GRT A-3'	50	2.5				This study

zoj_12423_f1.eps

Naja naja

Bungarus

0.97/72

Laticauda (MN)

Ogmodon vitianus (MNE)

Hydrophiinae

96

Salomonelaps par (MNE)

0.56/80

Loveridgelaps elapoides (MNE)

0.99/88

99

Toxicocalamus (MNE)

Micropechis ikaheka (MNE)

97

Cacophis squamulosus

0.74/52

Demansia (MN)

94

Aspidomorphus (MNE)

0.91/82

Neelaps bimaculatus

99

Brachyurophis

0.99/90

Simoselaps

0.77/49

Oxyuranus (MN)

0.98/83

Pseudonaja (MN)

0.88/87

Vermicella

0.96/60

Furina (MN)

0.56/25

Pseudechis (MN)

0.87/48

Denisonia devisi

0.96/70

Elapognathus coronatus

74

Rhinoplocephalus bicolor

99

Cryptophis nigrescens (Genus MN)

90

Suta/Parasuta

Acanthophis (MN)

Echiopsis curta

0.99/57

Drysdalia

Austrelaps

98

Paroplocephalus atriceps

0.76/68

0.99/86

Hoplocephalus bitorquatus

0.99/78

Notechis scutatus

90

Tropidechis carinatus

Hemisaspis

0.67/68

Sea Snakes (MN)

0.2 substitutions/site

A

- Narrow ventrals (long/thin habitus)
- Wide ventrals (normal habitus)

- *T. stanleyanus*
- *T. preussi*
- *T. holopelturus*
- *T. misimae*
- *T. longissimus*
- *T. loriae* Clade 1
- *T. loriae* Clade 2
- *T. loriae* Clade 3
- *T. loriae* Clade 4
- *T. pachysomus*
- *T. loriae* Clade 5
- *T. mintoni*
- *T. loriae* Clade 6

B

- Preocular fused to prefrontal
- Preocular unfused to prefrontal

- *T. stanleyanus*
- *T. preussi*
- *T. holopelturus*
- *T. misimae*
- *T. longissimus*
- *T. loriae* Clade 1
- *T. loriae* Clade 2
- *T. loriae* Clade 3
- *T. loriae* Clade 4
- *T. pachysomus*
- *T. loriae* Clade 5
- *T. mintoni*
- *T. loriae* Clade 6

C

- Anal plate divided
- Anal plate undivided

- *T. stanleyanus*
- *T. preussi*
- *T. holopelturus*
- *T. misimae*
- *T. longissimus*
- *T. loriae* Clade 1
- *T. loriae* Clade 2
- *T. loriae* Clade 3
- *T. loriae* Clade 4
- *T. pachysomus*
- *T. loriae* Clade 5
- *T. mintoni*
- *T. loriae* Clade 6

D

- Internasal fused to prefrontal
- Subcaudal undivided

- *T. stanleyanus*
- *T. preussi*
- *T. holopelturus*
- *T. misimae*
- *T. longissimus*
- *T. loriae* Clade 1
- *T. loriae* Clade 2
- *T. loriae* Clade 3
- *T. loriae* Clade 4
- *T. pachysomus*
- *T. loriae* Clade 5
- *T. mintoni*
- *T. loriae* Clade 6

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