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A taxonomic revision of the Asian keelback snakes, genus *Amphiesma* (Serpentes: Colubridae: Natricinae), with description of a new species

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Abstract

The Asian keelback snakes (genus *Amphiesma*) are a widely distributed group of Old World natricines, inhabiting a variety of niches and exhibiting significant morphological variation. Recent molecular phylogenies suggest that this genus is not monophyletic, and that additional cryptic diversity is also likely present. We conducted a phylogenetic analysis of the group based on 3162 bp of one mitochondrial gene (*Cyt. b*) and three nuclear genes (*C-mos*, *Rag1*, *NT3*), sampling 18 species in addition to those sequenced in previous works. All analyses consistently show that *Amphiesma* consists of three distinct, monophyletic lineages with strong support. We divide *Amphiesma* into three genera, *Amphiesma*, *Hebius*, and *Herpetoreas*. The genus *Amphiesma* is monotypic, *Herpetoreas* contains three species, and *Hebius* comprises the remaining 39 species. On the basis of a combination of molecular analyses and external morphological comparisons, we describe a new species in the *Herpetoreas* group from China as *H. burbrinki* sp. nov. Several other species are shown to be non-monophyletic or contain significant levels of intraspecific genetic diversity. Another Old World natricine genera, *Xenochrophis* is also found to be non-monophyletic. Our results indicate that further taxonomic revisions are needed in Natricinae, at multiple levels.

Key words: Natricinae, *Amphiesma*, *Hebius*, *Herpetoreas*, Snakes, new species, Southeastern Asia, systematics

Introduction

The genus *Amphiesma* sensu lato is one of the largest and most diverse groups in Natricine, with at least 42 species (Pyron *et al.* 2011; Guo *et al.* 2012; Uetz 2013). The members of this group are generally small- or medium-sized, with total lengths not exceeding one meter (Zhao 2006). They are terrestrial to semiaquatic, oviparous, and generally considered harmless (non-venomous). Species in this group also have a wide distribution throughout southern, eastern, and southeastern Asia, ranging from Pakistan and India to eastern China, north into southernmost Russia and Japan, and southwards to Sumatra and Sulawesi (Fig. 1; see Uetz 2013).

Based on morphological characters including hemipenial morphology, dentition, and external scalation, Malnate (1960) divided the genus *Natrix* sensu lato into several genera, revalidating the genus *Amphiesma*, which had been erected by Duméril, Bibron, and Duméril (1854) with the type species *A. stolatum*. The diagnostic characters of *Amphiesma* are defined as: hemipenes and sulci spermaticus simple; maxillary teeth in continuous series, gradually becoming larger posteriorly in the series or the last two teeth abruptly enlarged; terrestrial; internasals broad anteriorly, nostrils lateral; apical pits present or absent (Malnate 1960).

Due to their wide distribution, secretive habits, rarity of many species, and cryptic diversity, it is very difficult to collect the samples needed for a comprehensive systematic study on this group. A recent molecular study of Natricinae found that this genus is paraphyletic (Guo *et al.* 2012). A similar result was also found in subsequent works (Pyron *et al.* 2013a, b). However, incomplete sampling in these works precluded a taxonomic

H. concinarum, ***H. craspedogaster***, ***H. deschauenseei***, *H. flavifrons*, *H. frenatum*, *H. groundwateri*, *H. inas*, *H. ishigakiense*, ***H. johannis***, *H. kerinciense*, ***H. khasiense***, *H. leucomystax*, ***H. metustum***, *H. miyajimae*, ***H. modestum***, *H. monticola*, *H. nicobariense*, ***H. octolineatum***, ***H. optatum***, ***H. parallelum***, *H. pealii*, *H. petersii*, ***H. popei***, *H. pryeri*, *H. sanguineum*, *H. sarasinorum*, *H. sarawacense*, ***H. sauteri***, ***H. venningi***, ***H. vibakari***, *H. viperinum*, and *H. xenura*.

Notes. Species in bold are sampled in the molecular phylogeny, and confidently placed in *Hebius*. The remaining 23 species are placed tentatively, as this group contains the majority of morphological variation and geographic coverage of the former *Amphiesma*, and thus it seems likely that most of these species are allied with *Hebius*. However, it is possible that future studies will show that some are actually placed in *Amphiesma* sensu stricto or *Herpetoreas*. Gender of name is masculine. Several of these taxa are not monophyletic, and thus likely contain multiple cryptic species.

Conclusion

This study is the first presenting an overview of the relationships of Asian keelback snakes of *Amphiesma* sensu lato in the framework of a multi-locus molecular phylogeny. Although not all species were included and analyzed, the data generated and results presented here will benefit subsequent work on the systematics and taxonomy of this group. Future studies should focus on addressing the specific boundaries and distribution, and resolving the genus-level taxonomy of *Amphiesma* sensu stricto, *Atretium*, *Rhabdophis*, and *Xenochrophis*.

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References

- Burbrink, F.T., Lawson, R. & Slowinski, J.B. (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): A critique of the subspecies concept. *Evolution*, 54, 2107–2118.
[http://dx.doi.org/10.1554/0014-3820\(2000\)054\[2107:MDPOTP\]2.0.CO;2](http://dx.doi.org/10.1554/0014-3820(2000)054[2107:MDPOTP]2.0.CO;2)
- David, P., Bain, R.H., Nguyen, Q.T., Orlov, N.L., Vogel, G., Vu, N.T. & Ziegler, T. (2007) A new species of the natricine snake genus *Amphiesma* from the Indochinese region (Squamata: Colubridae: Natricinae). *Zootaxa*, 1462, 41–60.
- David, P., Vogel, G. & Van Rooijen, J. (2013) On some taxonomically confused species of the genus *Amphiesma* Duméril, Bibron and Duméril, 1854 related to *Amphiesma khasiense* (Boulenger, 1890) (Squamata, Natricidae). *Zootaxa*, 3694 (4), 301–335.
<http://dx.doi.org/10.11646/zootaxa.3694.4.1>
- Dowling, H.G. (1951) A proposed system for counting ventrals in snakes. *British Journal of Herpetology*, 1, 97–99. Duméril, A.M.C., Bibron, G. & Duméril, A.H.A. (1854) Erpétologie générale ou Histoire Naturelle Complète des Reptiles. *Librairie Encyclopédique de Roret*, 7 (1), 1–780. [Paris]
- Felsenstein, J. (2004) *Inferring Phylogenies*. Sinauer Associates, Sunderland, Mass.
- Flot, J.F. (2010) SEQPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Molecular Ecology Resources*, 10, 162–166.
<http://dx.doi.org/10.1111/j.1755-0998.2009.02732.x>
- Groth, J.G. & Barrowclough, G.F. (1999) Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. *Molecular Phylogenetics and Evolution*, 12, 115–123.

- http://dx.doi.org/10.1006/mpev.1998.0603
- Günther, A. (1860) Contributions to a knowledge of the reptiles of the Himalaya mountains. -I. Descriptions of the new species. II. List of Himalayan reptiles, with remarks on their horizontal distribution. *Proceedings of the Zoological Society of London*, 148–175
- Guo, P., Malhotra, A., Li, P.P., Creer, S. & Pook, C.E. (2007) New evidence on the phylogenetic position of the poorly known Asian pitviper *Protobothrops kaulbacki* (Serpentes: Viperidae: Crotalinae) with a redescription of the species and a revision of the genus *Protobothrops*. *Herpetological Journal*, 17, 237–246.
- Guo, P., Huang, S., Hu, J.R. & Liu, S.Y. (2008) Two snakes new to Xizang Autonomous Region. *Sichuan Journal of Zoology*, 27, 658–659.
- Guo, P., Liu, Q., Xu, Y., Jiang, K., Hou, M., Ding, L., Pyron, R.A. & Burbrink, F.T. (2012) Out of Asia: natricine snakes support the Cenozoic Beringian Dispersal Hypothesis. *Molecular Phylogenetics and Evolution*, 63, 825–833.
http://dx.doi.org/10.1016/j.ympev.2012.02.021
- Guo, P., Liu, S.Y., Huang, S., He, M., Sun, Z.Y., Feng, J.C. & Zhao, E.M. (2009) Morphological variation in *Thermophis Malnate* (Serpentes: Colubridae), with an expanded description of the type species *T. zhaoermii*. *Zootaxa*, 1973, 51–60.
- Guo, P., Zhang, L., Liu, Q., Li, C., Pyron, R.A., Jiang, K. & Burbrink, F.T. (2013) Lycodon and Dinodon: one genus or two? Evidence from molecular phylogenetics and morphological comparisons. *Molecular Phylogenetics and Evolution*, 68, 144–149.
http://dx.doi.org/10.1016/j.ympev.2013.03.008
- Herrmann, H.W., Ziegler, T., Malhotra, A., Thorpe, R.S. & Parkinson, C.L. (2004) Redescription and systematics of *Trimeresurus cornutus* (Serpentes: Viperidae) based on morphology and molecular data. *Herpetologica*, 60, 211–221.
http://dx.doi.org/10.1655/03-37
- Hillis, D.M. & Bull, J.J. (1993) An empirical-test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, 42, 182–192.
http://dx.doi.org/10.1093/sysbio/42.2.182
- Huang, S., Liu, S.Y., Guo, P., Zhang, Y.P. & Zhao, E.M. (2009) What are the closest relatives of the hot-spring snakes (Colubridae, *Thermophis*), the relict species endemic to the Tibetan Plateau? *Molecular Phylogenetics and Evolution*, 51, 438–446.
http://dx.doi.org/10.1016/j.ympev.2009.02.013
- Kass, R.E. & Raftery, A.E. (1995) Bayes factors. *Journal of the American Statistical Association*, 90, 773–795.
http://dx.doi.org/10.1080/01621459.1995.10476572
- Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29, 1695–1701.
http://dx.doi.org/10.1093/molbev/mss020
- Lawson, R., Slowinski, J.B., Crother, B.I. & Burbrink, F.T. (2005) Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution*, 37, 581–601.
http://dx.doi.org/10.1016/j.ympev.2005.07.016
- Mahendra, B.C. (1984) Handbook of the snakes of India, Ceylon, Burma, Bangladesh and Pakistan. *The Annals of Zoology (Agra)*, 22 (b), i–xvi, 1–412
- Malnate, E.V. (1960) Systematic division and evolution of the colubrid snake genus *Natrix*, with comments on the subfamily Natricinae. *Proceedings of the National Academy of Sciences of the United States of America*, 47, 41–71.
- Malnate, E.V. (1966) *Amphiesma platyceps* (Blyth) and *Amphiesma sieboldii* (Günther): sibling species (Reptilia: Serpentes). *Journal of the Bombay Natural History Society*, 63, 1–17.
- Noonan, B., Paul, P. & Chippindale, T. (2006) Dispersal and vicariance: The complex evolutionary history of boid snakes. *Molecular Phylogenetics and Evolution*, 40 (2), 347–358.
http://dx.doi.org/10.1016/j.ympev.2006.03.010
- Pope, C.H. (1935) *The reptiles of China. Turtles, crocodilians, snakes, lizards. Natural History of Central Asia, X.* American Museum of Natural History, New York, xlvi + 604 pp.
- Pyron, R.A. & Burbrink, F.T. (2009) Systematics of the Common Kingsnake (*Lampropeltis getula*; Serpentes: Colubridae) and the burden of heritage in taxonomy. *Zootaxa*, 2241, 22–32.
- Pyron, R.A., Burbrink, F.T., Colli, G.R., de Oca, A.N.M., Vitt, L.J., Kuczynski, C.A. & Wiens, J.J. (2011) The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. *Molecular Phylogenetics and Evolution*, 58, 329–342.
http://dx.doi.org/10.1016/j.ympev.2010.11.006
- Pyron, R.A., Burbrink, F.T. & Wiens, J.J. (2013a) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, 13, 93.
http://dx.doi.org/10.1186/1471-2148-13-93
- Pyron, R.A., Kandambi, H.K.D., Hendry, C.R., Pushpamal, V., Burbrink, F.T. & Somaweera, R. (2013b) Genus-level phylogeny of snakes reveals the origins of species richness in Sri Lanka. *Molecular Phylogenetics and Evolution*, 66, 969–978.
http://dx.doi.org/10.1016/j.ympev.2012.12.004
- Rambaut, A. & Drummond, A.J. (2007) Tracer v1.4. Available from: <http://beast.bio.ed.ac.uk/Tracer> (accessed 23 September 2013).

2014)

- Ride, W.D.L. (Ed.) (1999) *International code of Zoological Nomenclature*. The Natural History Museum, London, 306 pp.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Ronquist, F., Teslenko, M., Mark, P.V. D., Ayres, D., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61 (3), 539–542.
<http://dx.doi.org/10.1093/sysbio/sys029>
- Sambrook, J. & Russell, D.W. (2002) *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, 2100 pp.
- Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, 16, 1114–1116.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026201>
- Smith, M.A. (1943) *The Fauna of British India, Ceylon and Burma, Reptilia and Amphibia. III, Serpentes*. Taylor and Francis, London.
- Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.
<http://dx.doi.org/10.1093/bioinformatics/btl446>
- Stephens, M., Smith, N.J. & Donnelly, P. (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, 68, 978–989.
<http://dx.doi.org/10.1086/319501>
- Suchard, M.A., Weiss, R.E. & Sinsheimer, J.S. (2001) Bayesian selection of continuous time markov chain evolutionary models. *Molecular Biology and Evolution*, 18, 1001–1013.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a003872>
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
<http://dx.doi.org/10.1093/molbev/mst197>
- Thompson, J.C. (1913) Contributions to the anatomy of the Ophidia. *Proceedings of the Zoological Society of London*, 414–426.
- Uetz, P. (2014) The Reptile Database. <http://www.reptile-database.org> (accessed 18 December 2013)
- Zhang, D.X. & Hewitt, G.M. (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution*, 11, 247–251.
[http://dx.doi.org/10.1016/0169-5347\(96\)10031-8](http://dx.doi.org/10.1016/0169-5347(96)10031-8)
- Zhao, E.M. (2006) *Snakes of China*. Anhui Sciences and Technology Press, Hefei, China, 501 pp.
- Zhao, E.M., Huang, M.H. & Zong, Y. (1998) *Fauna Sinica: Reptilia. Vol. 3. Squamata Serpentes*. Science Press, Beijing, xvii + 522 pp.
- Ziegler, T. & Quyet, L.K. (2006) A new natricine snake of the genus *Amphiesma* (Squamata: Colubridae: Natricinae) from the central Truong Son, Vietnam. *Zootaxa*, 1225, 39–56.