Parahelicops, Pararhabdophis, Paraphyly: Phylogenetic Relationships among Certain Southeast Asian Natricine Snakes (Hebius)

Authors: Kizirian, David, Truong, Nguyen Quang, Ngo, Hanh Thi, and Le, Minh Duc

Source: American Museum Novitates, 2018(3906) : 1-7

Published By: American Museum of Natural History

URL: https://doi.org/10.1206/3906.1

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

AMERICAN MUSEUM NOVITATES

Number 3906, 7 pp. August 21, 2018

Parahelicops, Pararhabdophis, Paraphyly: Phylogenetic Relationships among Certain Southeast Asian Natricine Snakes (*Hebius*)

DAVID KIZIRIAN,[1] NGUYEN QUANG TRUONG,[2,3]
HANH THI NGO,[4] AND MINH DUC LE[1,5,6]

ABSTRACT

We investigate the phylogenetic relationships of two poorly known Natricinae, *Parahelicops* and *Pararhabdophis*, for which we obtained nucleotide sequence data from one mitochondrial gene (cytochrome *b*) and three nuclear genes (CMOS, NT3, and RAG1). Maximum parsimony, maximum likelihood, and combined and partitioned Bayesian analyses suggest that both *Parahelicops* and *Pararhabdophis* are embedded within the genus *Hebius*. To align classification with phylogeny, we synonymize *Parahelicops* and *Pararhabdophis* with *Hebius*.

INTRODUCTION

Parahelicops annamensis Bourret, 1934, has a history of entanglement with *Amphiesma* (e.g., Stuart, 2006; Teynie et al., 2013; David et al., 2015) and *Opisthotropis* (e.g., Bourret, 1934b; Smith, 1943; Stuart, 2006; Stuart and Chuaynkern, 2007; Murphy et al., 2008; Teynie et al., 2013; David et al., 2015). Citing unpublished data, Teynie et al. (2013) thought *Parahelicops annamensis*

---

[1] Division of Vertebrate Zoology, American Museum of Natural History.
[2] Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.
[3] Graduate University of Science and Technology, Vietnam Academy of Science and Technology.
[4] Faculty of Biology, Hanoi University of Science, Vietnam National University.
[5] Faculty of Environmental Sciences, Hanoi University of Science, Vietnam National University.
[6] Central Institute for Natural Resources and Environmental Studies, Hanoi National University.

Copyright © American Museum of Natural History 2018 ISSN 0003-0082

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

seemed “referable to the genus *Amphiesma*” but ultimately decided to retain the original name (despite using “*Amphiesma annamense*” in their key to species). David et al. (2015) cited unpublished molecular data that supported the monophyly of *Amphiesma* with respect to *Parahelicops* and, hence, retained the latter. At the same time, however, they listed specific morphological character states in *Parahelicops* also found in the *A. venningi* complex. Stuart (2006) recognized the validity of the genus *Parahelicops*, at least for *P. annamensis,* and suggested a close relationship of *Parahelicops* with *Opisthotropis*. The poorly known *Pararhabdophis chapaensis* Bourret (1934a) also exhibits morphological similarity to *Parahelicops annamensis* (David et al., 2015).

More inclusive phylogenetic studies have resulted in rearrangements of taxa relevant to the positions of *Parahelicops* and *Pararhabdophis*. After finding *Amphiesma* to be polyphyletic, Guo et al. (2014) resurrected the genus *Hebius* Thompson, 1913, for all species except *Amphiesma stolatum*. Guo et al. (2012) and Figueroa et al. (2016) found *Opisthotropis* to be outside the clade that includes *Hebius* and *Amphiesma* and closer to *Sinonatrix* and New World Natricinae. None of the previous molecular studies, however, addressed *Parahelicops* or *Pararhabdophis*. Herein, we reevaluate the phylogenetic relationships of *Parahelicops* and *Pararhabdophis* with respect to other natricines using nucleotide sequence data.

Materials and Methods

Molecular Data: Tissue samples (appendix) were obtained from the American Museum of Natural History, New York (AMNH), the Field Museum, Chicago (FMNH), and the North Carolina Museum of Natural Sciences, Raleigh (NCSM). In total, we included six new samples (fig. 1), from two genera *Pararhabdophis* and *Parahelicops*, in the matrix published by Guo et al. (2014). In addition, we included *Opisthotropis cheni* and *O. lateralis* in phylogenetic analyses to root phylogenetic trees. Extracted DNA from the fresh tissue was amplified by PCR Master Mix (Fermentas, Burlington, ON, Canada) using the same primers and conditions employed by Guo et al. 2014. PCR products were subjected to electrophoresis through a 1% agarose gel (UltraPure™, Invitrogen, La Jolla, CA). Gels were stained for 10 min in 1 X TBE buffer with 2 pg/ml ethidium-bromide, and visualized under UV light. Successful amplifications were purified to eliminate PCR components using a GeneJET™ PCR Purification kit (Fermentas). Purified PCR products were sent to FirstBase Malaysia for sequencing.

Phylogenetic Analyses: The sequences were aligned in Clustal X v2 (Thompson et al., 1997) with default settings. Data were analyzed using maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP 4.0b10 (Swofford, 2001), and Bayesian analysis in MrBayes 3.2 (Ronquist et al., 2012). For MP analysis, heuristic analysis was conducted with 100 random taxon-addition replicates using tree-bisection and reconnection (TBR) branch-swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) (Felsenstein, 1985) was calculated using 1,000 pseudoreplicates and 100 random taxon-addition replicates. All characters were equally weighted and unordered. For ML analysis, we used the optimal evolution model as selected by ModelTest v3.7 (Posada and Crandall, 1998). To estimate BP in the ML analysis, a simple taxon-addition option and 100

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

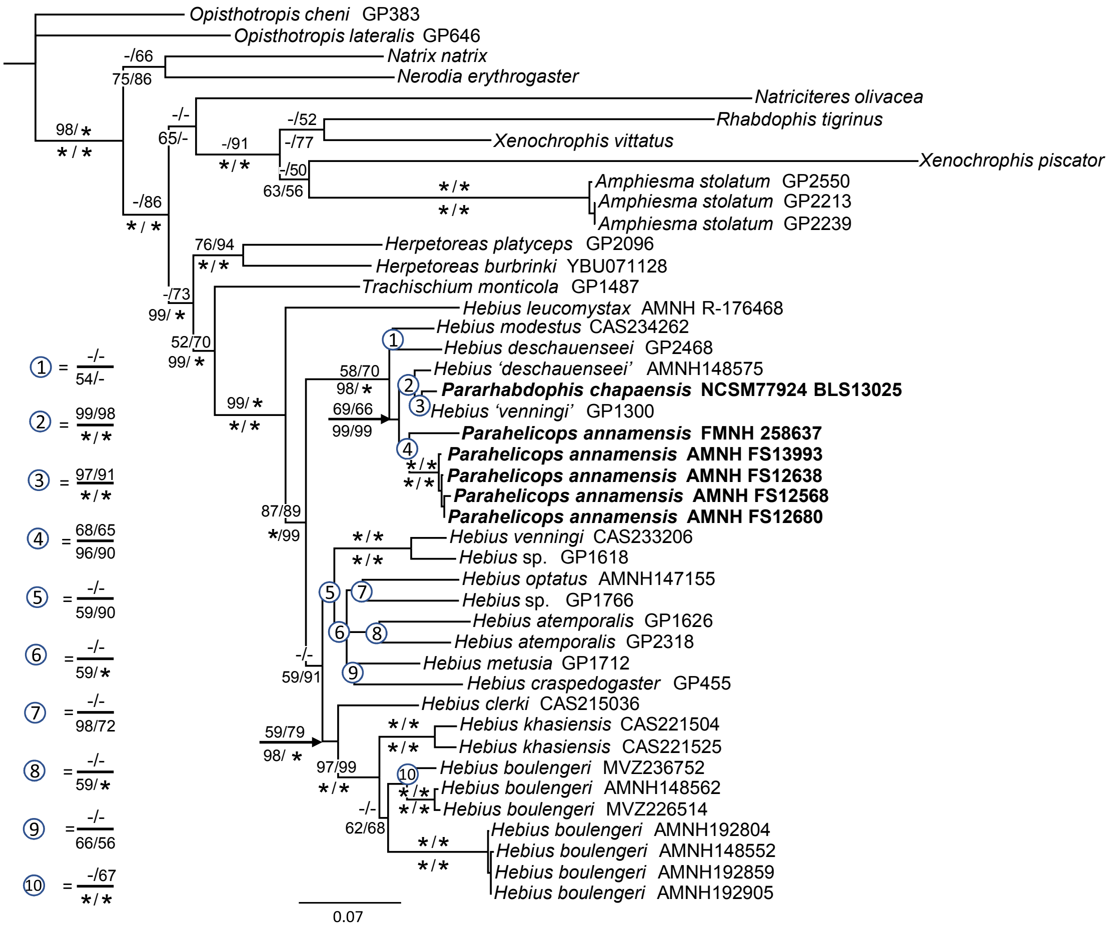

FIGURE 1. Combined Bayesian phylogram based on all concatenated data. Numbers above and below branches are MP/ML bootstrap values and combined/partitioned Bayesian posterior probabilities (>50%), respectively. Hyphen and asterisk denote <50% and 100% values, respectively. Bold text indicates samples sequenced for this study.

pseudoreplicates were employed. We arbitrarily assumed bootstrap values of ≥70 % to represent strong support and values of <70 % as weak support (Hillis and Bull, 1993).

For Bayesian analyses, we used the optimal model determined by Modeltest with parameters estimated by MrBayes 3.2.1. Two simultaneous analyses with four Markov chains (one cold and three heated) were run for 10 million generations with a random starting tree and sampled every 1000 generations. Log-likelihood scores of sample points were plotted against generation time to determine stationarity of Markov chains. Trees generated before log-likelihood scores reached stationarity were discarded from the final analyses using the burn-in function. The posterior probability (PP) values for all clades in the final majority-rule consensus tree are provided. We ran analyses using both combined and partitioned datasets to examine the robustness of the tree topology (Nylander et al., 2004; Brandley et al., 2005). In the mixed-model analysis, we partitioned the data into 12 sets based on gene codon positions (first,

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

Table 1. Models used in Bayesian analyses

Data analysis	**Model determined by Modeltest**
Combined Bayesian analysis	
Concatenated matrix	TIM2+I+R
Partitioned Bayesian analysis	
Cytochrome *b* 1st position	TrN+I+G
Cytochrome *b* 2nd position	TIM+I+G
Cytochrome *b* 3rd position	TVM+I+G
Cmos 1st position	K80
Cmos 2nd position	JC
Cmos 3rd position	HKY
NT3 1st position	JC
NT3 2nd position	K80+G
NT3 3rd position	K80+I
Rag1 1st position	HKY
Rag1 2nd position	TrNef+I
Rag1 3rd position	HKY

second, and third) of cytochrome *b*, CMOS, NT3, and RAG1. Optimal models of molecular evolution for the partitions were calculated using Modeltest, and then assigned to these partitions in MrBayes 3.2 using the command APPLYTO. Model parameters were inferred independently for each data partition using the UNLINK command. All models employed in Bayesian analyses are shown in table 1.

RESULTS

The final matrix consisted of 3162 aligned characters, of which 614 were parsimony informative. The alignment contained no gap. MP analysis of the dataset recovered nine most parsimonious trees with 3259 steps (CI = 0.38; RI = 0.59). In the ML analysis, the -Ln likelihood score of the single best tree found was 18,729.82. The cutoff point for the burn-in function was set to 20 and 21 in combined and partitioned Bayesian analyses as -ln*L* scores reached stationarity after 20,000 and 21,000 generations, respectively. The topologies derived from our study are similar to those in Guo et al. (2014). Most relevant here, we found *Parahelicops* and *Pararhabdophis* nested within *Hebius* with strong support in all analyses, and within the smallest clade including *H. deschauenseei, H. modestus,* and some *H. venningi* with high statistical values from all, but the MP analysis (fig. 1).

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

DISCUSSION

Our analysis provides a phylogenetic explanation for the reported similarity between *Parahelicops*, *Hebius venningi,* and *H. deschauenseei* noted by David et al. (2015: 216). Specifically, we find *Parahelicops* and *Pararhabdophis* to be imbedded within *Hebius,* in a clade including *H. deschauenseei*, *H. modestus,* and some of the specimens identified as *H. venningi*. To align taxonomy with the recovered phylogeny of this group, we synonymize *Parahelicops* Bourret, 1934, and *Pararhabdophis* Bourret, 1934, with *Hebius* Thompson (1913), yielding the new combinations, *Hebius chapaensis* (Bourret, 1934) and *Hebius annamensis* (Bourret, 1934). We corroborated (not presented) Guo et al. (2012) and Figueroa et al. (2016) who found *Opisthotropis* to be outside *Hebius* and, therefore, not closely related to *Parahelicops* (contra Stuart, 2006).

Sequence divergence (approximately 6%) between *Hebius annamensis* from the Ca (Vietnam samples) and Mekong (Lao samples) drainages suggests that multiple species might exist under that binomial. Divergence between northern and southern *H. annamensis* might reflect isolation by low-elevation habitats characterized by a mixture of evergreen, semievergreen, and dry forest types between northern and central Annamite ranges discussed by Bain and Hurley (2011).

We also corroborate the findings of David et al. (2013) and Guo et al. (2014), who reported variation suggestive of additional unrecognized species diversity under the names *H. boulengeri* and *H. venningi*, the latter of which is polyphyletic in Guo et al. (2014) and herein. Stuart et al. (2006) suggested that there are no "geographically widespread, forest-dwelling frog species in Southeast Asia." Such a pattern may also exist in snakes such as *Hebius*, some of which are known to be connected to anurans through trophic relations (e.g., Moriguchi and Naito, 1982; David et al., 2007).

ACKNOWLEDGMENTS

We thank P. Guo for sharing the data matrix used in Guo et al. (2014). Patrick David provided updated identifications for several specimens in Guo et al. (2014). M.D. Le was supported by the National Foundation for Science and Technology Development (NAFOSTED, grant no. 106-NN.06-2016.59) and the National Geographic Society (grant no. 230151).

REFERENCES

Bain, R.H., and M.M. Hurley. 2011. A biogeographic synthesis of the amphibians and reptiles of Indochina. Bulletin of the American Museum of Natural History 360: 1–138.

Bourret, R. 1934a. Notes herpétologiques sur l'Indochine française. I. Ophidiens de Chapa. Bulletin Général de l'Instruction Publique, 13^{e} année (7, mars): 129–138. [separate reprint: 1–10]

Bourret, R. 1934b. Notes herpétologiques sur l'Indochine française. III. Ophidiens d'Annam et du Moyen Laos. Bulletin Général de l'Instruction Publique, 13^{e} Année (9, mai), 167–176. [separate reprint: 3–12].

Brandley, M.C., A. Schmitz, and T.W. Reeder. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Systematic Biology 54: 373–390.

David, P., et al. 2007. A new species of the natricine snake genus *Amphiesma* from the Indochinese region (Squamata: Colubridae: Natricinae). Zootaxa 1462: 41–60.

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

David, P., O.S.G. Pauwels, T.Q. Nguyen, and G. Vogel. 2015. On the taxonomic status of the Thai endemic freshwater snake *Parahelicops boonsongi,* with the erection of a new genus (Squamata: Natricidae). Zootaxa 3948: 203–217.

David, P., G. Vogel, and J. Van Rooijen. 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.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Figueroa, A., A.D. McKelvy, L.L. Grismer, C.D. Bell, and S.P. Lailvaux. 2016. A species-level phylogeny of extant snakes with description of a new colubrid subfamily and genus. PLoS One 11(9): e0161070. [https://doi.org/10.1371/journal.pone.0161070]

Guo, P., et al. 2012. Out of Asia: natricine snakes support the Cenozoic Beringian Dispersal Hypothesis. Molecular Phylogenetics and Evolution 63: 825–833.

Guo, P., et al. 2014. A taxonomic revision of the Asian keelback snakes, genus *Amphiesma* (Serpentes: Colubridae: Natricinae), with description of a new species. Zootaxa 3873: 425–440.

Hillis, D.M., and J.J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192.

Moriguchi, H., and S. Naito. 1982. Activities and food habits of *Amphiesma vibakari* (Boie) and *Rhabdophis tigrinus* (Boie). Snake 14: 136–142.

Murphy, J.C., T. Chan-Ard, S. Mekchai, M. Cota, and H.K. Voris. 2008. The rediscovery of Angel's stream snake, *Paratapinophis praemaxillaris* Angel, 1929 (Reptilia: Serpentes: Natricidae). Natural History Journal of Chulalongkorn University 8: 169–183.

Nylander, J., F. Ronquist, J.P. Huelsenbeck, and J.-L. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. Systematic Biology 53: 47–67.

Posada, D., and K.A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Ronquist, F., et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Smith, M.A. 1943. The fauna of British India, Ceylon and Burma, including the whole of the Indo-Chinese subregion. Reptilia and Amphibia. Vol. 3, Serpentes. London: Taylor & Francis, xii + 583 pp.

Stuart, B.L. 2006. A second specimen of *Parahelicops annamensis* Bourret, 1934 (Serpentes: Colubridae: Natricinae). Hamadryad 30: 167–171.

Stuart, B.L., and Y. Chuaynkern. 2007. A New *Opisthotropis* (Serpentes: Colubridae: Natricinae) from northeastern Thailand. Current Herpetology 26: 35–40.

Stuart, B.L., R.F. Inger, and H.K. Voris. 2006. High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. Biology Letters 2: 470–474. [doi: 10.1098/rsbl.2006.0505]

Swofford, D.L. 2001. PAUP*. Phylogenetic analysis using parsimony (* and other methods), version 4. Sunderland, MA: Sinauer Associates.

Teynie, A., A. Lottier, P. David, T.Q. Nguyen, and G. Vogel. 2013. A new species of the genus *Opisthotropis* Günther, 1872 from northern Laos (Squamata: Natricidae).

Thompson, J.C. 1913. Contributions to the anatomy of the Ophidia. Proceedings of the Zoological Society of London 83: 414–425.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, and D.G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

APPENDIX

Material Examined

Pararhabdophis chapaensis: LAO PDR: Houaphan Province: Phou Louey National Protected Area, Viengthong District, near Tad Loi Waterfall (20.23253°N, 103.2108°E), 1186 m (NCSM 77924).

Parahelicops anammensis: VIETNAM: Ha Tinh: Huong Son District, Huong Son Reserve, Rao An region, near top of Po-mu Mountain (18° 20′ 26″ N, 105° 14′ 13″ E), 870 m (AMNH-R 147129 [corpus], AMNH-FS 13993 [field series], AMCC 106598 [tissue]). Nghe An Province: Pu Mat National Park: Anh Son District (near N 18.8177, E 104.9609), 170 m (AMNH-R 176469 [corpus], AMNH-FS 12568 [field series]), AMCC 192504–06 [tissue]). Nghe An Province: Pu Mat National Park: Anh Son District (N 18.8092, E 104.9499), 357 m (AMNH-R 176470 [corpus], AMNH-FS 12638 [field series], AMCC 192596–97 [tissue]). Nghe An Province: Pu Mat National Park: Anh Son District, Khe Suc River (N 18.8171, E 104.9484), 237 m (AMNH-R 176471 [corpus], AMNH-FS 12680 [field series], AMCC 192626–27 [tissue]). LAO PDR: Xekong Province: Kaleum District: Xe Sap National Biodiversity Conservation Area (16° 04′ 10″ N, 106° 58′ 45″ E), 1280–1500 m (FMNH 258637).

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use

All issues of *Novitates* and *Bulletin* are available on the web (http://digitallibrary.amnh.org/dspace). Order printed copies on the web from:
http://shop.amnh.org/a701/shop-by-category/books/scientific-publications.html

or via standard mail from:
American Museum of Natural History—Scientific Publications
Central Park West at 79th Street
New York, NY 10024

♾ This paper meets the requirements of ANSI/NISO Z39.48-1992 (permanence of paper).

Downloaded From: https://bioone.org/journals/American-Museum-Novitates on 02 Aug 2024
Terms of Use: https://bioone.org/terms-of-use