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Multilocus species delimitation in the *Crotalus triseriatus* species group (Serpentes: Viperidae: Crotalinae), with the description of two new species

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Abstract

Members of the *Crotalus triseriatus* species group of montane rattlesnakes are widely distributed across the highlands of Mexico and southwestern USA. Although five species are currently recognized within the group, species limits remain to be tested. Genetic studies suggest that species may be paraphyletic and that at least one cryptic species may be present. We generate 3,346 base pairs of DNA sequence data from seven nuclear loci to test competing models of species delimitation in the *C. triseriatus* group using Bayes factor delimitation. We also examine museum specimens from the Trans-Mexican Volcanic Belt for evidence of cryptic species. We find strong support for a nine-species model and genetic and morphological evidence for recognizing two new species within the group, which we formally describe here. Our results suggest that the current taxonomy of the *C. triseriatus* species group does not reflect evolutionary history. We suggest several conservative taxonomic changes to the group, but future studies are needed to better clarify relationships among species and examine genetic patterns and structure within wide-ranging lineages.

Key words: Bayes factor delimitation, cloud forest, Mexico, Trans-Mexican Volcanic Belt

Resumen

Miembros del grupo *Crotalus triseriatus* se encuentran ampliamente distribuidos en las tierras altas de México y el suroeste de Estados Unidos. Aunque actualmente se reconocen cinco especies dentro del grupo, los límites entre especies no han sido formalmente evaluados. Estudios genéticos sugieren que las especies pueden ser parafiléticas y que al menos una especie críptica puede estar presente. Generamos una secuencia de datos de 3,346 pares de bases de ADN provenientes de siete loci nucleares para evaluar modelos contrastantes de delimitación de especies en el grupo *C. triseriatus* usando el factor de delimitación de Bayes. En la búsqueda de especies crípticas, también examinamos ejemplares de museo provenientes del Eje Neovolcánico. Encontramos fuerte soporte para un modelo de nueve especies y evidencia genética y morfológica para reconocer dos nuevas especies dentro del grupo, las que formalmente describimos aquí. Nuestros resultados sugieren que la taxonomía actual de las especies del grupo *C. triseriatus* no refleja la historia evolutiva. Sugerimos varios cambios taxonómicos conservadores al grupo, requiriéndose de estudios futuros para delinear de manera más fina las relaciones entre especies y para examinar la estructura filogeográfica dentro de linajes de amplia distribución.

Palabras clave: factor de delimitación de Bayes, bosque de niebla, México, Eje Neovolcánico

References

- Anderson, C.N., Liu, L., Pearl, D. & Edwards, S.V. (2012) Tangled trees: the challenge of inferring species trees from coalescent and noncoalescent genes. *Methods in Molecular Biology*, 856, 3–28.
http://dx.doi.org/10.1007/978-1-61779-585-5_1
- Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A. & Alekseyenko, A.V. (2012) Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution*, 29, 2157–2167.
<http://dx.doi.org/10.1093/molbev/mss084>
- Baele, G., Li, W.L.S., Drummond, A.J., Suchard, M.A. & Lemey, P. (2013) Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. *Molecular Biology and Evolution*, 30, 239–243.
<http://dx.doi.org/10.1093/molbev/mss243>
- Bossu, C.M. & Near, T.J. (2009) Gene trees reveal repeated instances of mitochondrial DNA introgression in Orangethroat Darters (Percidae: *Etheostoma*). *Systematic Biology*, 58, 114–129.
<http://dx.doi.org/10.1093/sysbio/syp014>
- Bouckaert, R.R. (2010) DensiTree: making sense of sets of phylogenetic trees. *Bioinformatics*, 26, 1372–1373.
<http://dx.doi.org/10.1093/bioinformatics/btq110>
- Boulenger, G.A. (1896) *Catalogue of the Snakes in the British Museum (Natural History)*. Vol. III. Trustees of the British Museum, London, xiv + 727 pp., 15 plates.
- Brattstrom, B.H. (1964) Evolution of the pit vipers. *Transactions of the San Diego Society of Natural History*, 13, 185–268.
- Bryson, R.W., Nieto-Montes de Oca, A., Jaeger, J.R. & Riddle, B.R. (2010) Elucidation of cryptic diversity in a widespread Nearctic treefrog reveals episodes of mitochondrial gene capture as frogs diversified across a dynamic landscape. *Evolution*, 64, 2315–2330.
<http://dx.doi.org/10.1111/j.1558-5646.2010.01014.x>
- Bryson, R.W., Murphy, R.W., Lathrop, A. & Lazcano-Villareal, D. (2011) Evolutionary drivers of phylogeographical diversity in the highlands of Mexico: a case study of the *Crotalus triseriatus* species group of montane rattlesnakes. *Journal of Biogeography*, 38, 697–710.
<http://dx.doi.org/10.1111/j.1365-2699.2010.02431.x>
- Campbell, J.A. (1979) A new rattlesnake (Reptilia, Serpentes, Viperidae) from Jalisco, Mexico. *Transactions of the Kansas Academy of Science*, 81 (4), 365–370.
- Campbell, J.A. & Lamar, W.W. (2004) *Venomous Reptiles of the Western Hemisphere*. Cornell University Press, Ithaca, New York, 976 pp.
- Castoe, T.A. & Parkinson, C.L. (2006) Bayesian mixed models and the phylogeny of pitvipers (Serpentes: Viperidae). *Molecular Phylogenetics and Evolution*, 39, 91–110.
<http://dx.doi.org/10.1016/j.ympev.2005.12.014>
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
<http://dx.doi.org/10.1038/nmeth.2109>
- de Queiroz, K. (1998) The general lineage concept of species, species criteria, and the process of speciation. In: Howard, D.J. & Berlocher, S.H. (Eds.), *Endless Form: Species and Speciation*. Oxford University Press, New York, pp. 57–75.
- de Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56, 879–886.
<http://dx.doi.org/10.1080/10635150701701083>
- Degnan, J.H. & Rosenberg, N.A. (2006) Discordance of species trees with their most likely gene trees. *PLoS Genetics*, 2 (5), e68.
<http://dx.doi.org/10.1371/journal.pgen.0020068>
- Dolman, G. & Phillips, B. (2004) Single copy nuclear DNA markers characterized for comparative phylogeography in Australian wet tropics rainforest skinks. *Molecular Ecology*, 4, 185–187.
<http://dx.doi.org/10.1111/j.1471-8286.2004.00609.x>
- Dorcas, M.E. (1992) Relationships among montane populations of *Crotalus lepidus* and *Crotalus triseriatus*. In: Campbell, J.A. & Brodie Jr., E.D. (Eds.), *Biology of the Pitvipers*. Selva, Tyler, Texas, pp. 71–88.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, 7, 214.
<http://dx.doi.org/10.1186/1471-2148-7-214>
- Figuerola-Rangel, B.L., Willis, K.J. & Olvera-Vargas, M. (2010) Cloud forest dynamics in the Mexican Neotropics during the last 1300 years. *Global Change Biology*, 16, 1689–1704.
<http://dx.doi.org/10.1111/j.1365-2486.2009.02024.x>
- Flores-Villela, O. & Hernandez-Garcia, E. (1989) New state records from northern Guerrero, Mexico. *Herpetological Review*, 20, 15–16.
- Flores-Villela, O. & Sánchez-H, O. (2003) A new species of *Abronia* (Squamata: Anguillidae) from the Sierra Madre del Sur of Guerrero, Mexico, with comments on *Abronia deppii*. *Herpetologica*, 59, 524–531.
<http://dx.doi.org/10.1655/02-39>

- Flores-Villela, O. & Hernández-García, E. (2006) Herpetofauna de la Sierra de Taxco, Guerrero. *Publicaciones de la Sociedad Herpetológica Mexicana*, 3, 266–282.
- Gallcia, L. & García-Romero, A. (2007) Land use and land cover change in highland temperate forests in the Izta-Popo National Park, central Mexico. *Mountain Research and Development*, 27, 48–57.
[http://dx.doi.org/10.1659/0276-4741\(2007\)27\[48:lualcc\]2.0.co;2](http://dx.doi.org/10.1659/0276-4741(2007)27[48:lualcc]2.0.co;2)
- Gamble, T., Bauer, A.M., Greenbaum, E. & Jackman, T.R. (2007) Evidence for Gondwanan vicariance in an ancient clade of gecko lizards. *Journal of Biogeography*, 35, 88–104.
<http://dx.doi.org/10.1111/j.1365-2699.2007.01770.x>
- Gibbs, H.L. & Diaz, J. (2010) Identification of single copy nuclear DNA markers for North American pit vipers. *Molecular Ecology Resources*, 10, 177–180.
<http://dx.doi.org/10.1111/j.1755-0998.2009.02707.x>
- Gloyd, H.K. (1940) The rattlesnakes, genera *Sistrurus* and *Crotalus*. A study in zoogeography and evolution. *Special Publication of the Chicago Academy of Sciences*, 4, 1–270.
- Gómez-Tuena, A., Orozco-Esquivel, Ma T. & Ferrari, L. (2007) Igneous petrogenesis of the Trans-Mexican Volcanic Belt. *Geological Society of America, Special Paper*, 422, 129–181.
- Grummer, J.A., Bryson, R.W. & Reeder, T.W. (2014) Species delimitation using Bayes factors: simulations and application to the *Sceloporus scalaris* species group (Squamata: Phrynosomatidae). *Systematic Biology*, 63, 119–133.
<http://dx.doi.org/10.1093/sysbio/syt069>
- Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology*, 52, 696–704.
<http://dx.doi.org/10.1080/10635150390235520>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, 27, 570–580.
<http://dx.doi.org/10.1093/molbev/msp274>
- Kass, R.E. & Raftery, A.E. (1995) Bayes factors. *Journal of the American Statistics Association*, 90 (430), 773–795.
<http://dx.doi.org/10.1080/01621459.1995.10476572>
- Klauber, L.M. (1952) Taxonomic studies of the rattlesnakes of mainland Mexico. *Bulletin of the Zoological Society of San Diego*, 26, 1–143.
- Klauber, L.M. (1972) *Rattlesnakes: Their Habits, Life Histories and Influence on Mankind*. 2nd Edition. University of California Press, Berkeley and Los Angeles, California, 400 pp.
- Knowles, L.L. (2009) Estimating species trees: methods of phylogenetic analysis when there is incongruence across genes. *Systematic Biology*, 58, 463–467.
<http://dx.doi.org/10.1093/sysbio/syp061>
- Kubatko, L.S., Gibbs, H.L. & Bloomquist, E.W. (2011) Inferring species-level phylogenies and taxonomic distinctiveness using multilocus data in *Sistrurus* rattlesnakes. *Systematic Biology*, 60, 393–409.
<http://dx.doi.org/10.1093/sysbio/syr011>
- Lartillot N. & Philippe, H. (2006) Computing Bayes factors using thermodynamic integration. *Systematic Biology*, 55, 195–207.
<http://dx.doi.org/10.1080/10635150500433722>
- Leaché A.D. (2010) Species trees for spiny lizards (genus *Sceloporus*): Identifying points of concordance and conflict between nuclear and mitochondrial data. *Molecular Phylogenetics and Evolution*, 54, 162–171.
<http://dx.doi.org/10.1016/j.ympev.2009.09.006>
- Leaché, A.D., Fujita, M.K., Minin, V. & Bouckaert R. (2014) Species delimitation using genome-wide SNP data. *Systematic Biology*, 63, 534–542.
<http://dx.doi.org/10.1093/sysbio/syu018>
- Murphy, R.W., Fu, J., Lathrop, A., Feltham, J.V. & Kovak, V. (2002) Phylogeny of the rattlesnakes (*Crotalus* and *Sistrurus*) inferred from sequences of five mitochondrial DNA genes. In: Schuett, G.W., Höggren, M., Douglas, M.E. & Greene, H.W. (Eds.), *Biology of the Vipers*. Eagle Mountain Publishing, Eagle Mountain, UT, USA, pp. 69–92.
- Myers, E.A., Rodríguez-Robles, J.A., DeNardo, D.F., Staub, R.E., Stropoli, A., Ruane, S. & Burbrink, F.T. (2013) Multilocus phylogeographic assessment of the California Mountain Kingsnake (*Lampropeltis zonata*) suggests alternative patterns of diversification for the California Floristic Province. *Molecular Ecology*, 22, 5418–5429.
<http://dx.doi.org/10.1111/mec.12478>
- Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics*, 24, 581–583.
<http://dx.doi.org/10.1093/bioinformatics/btm388>
- Pérez-Ramos, E., Saldaña de la Riva, L. & Uribe-Peña, Z. (2000) A checklist of the reptiles and amphibians of Guerrero, México. *Anales del Instituto de Biología Universidad Nacional Autónoma de México, Serie Zoología*, 71, 21–40.
- Ponce-Reyes, R., Reynoso-Rosales, V.-H., Watson, J.E.M., VanDerWal, J., Fuller, R.A., Pressey, R.L. & Possingham, H.P. (2012) Vulnerability of cloud forest reserves in Mexico to climate change. *Nature Climate Change*, 2, 448–452.

- Rambaut, A. & Drummond, A.J. (2007) Tracer v1.5. Available from: <http://beast.bio.ed.ac.uk/Tracer> (Accessed 17 October 2012)
- Reyes-Velasco, J., Hermsillo-Lopez, I.A., Grünwald, C.I. & Avila-Lopez, O.A. (2009) New state records for amphibians and reptiles from Colima, Mexico. *Herpetological Review*, 40, 117–120.
- Reyes-Velasco, J., Meik, J.M., Smith, E.N. & Castoe, T.A. (2013) Phylogenetic relationships of the enigmatic longtailed rattlesnakes (*Crotalus ericsmithi*, *C. lannomi*, and *C. stejnegeri*). *Molecular Phylogenetics and Evolution*, 69, 524–534. <http://dx.doi.org/10.1016/j.ympev.2013.07.025>
- Ruane, S., Bryson, R.W., Pyron, R.A. & Burbrink, F.T. (2014) Coalescent species delimitation in milksnakes (genus *Lampropeltis*) and impacts on phylogenetic comparative analyses. *Systematic Biology*, 63, 231–250. <http://dx.doi.org/10.1093/sysbio/syt099>
- Sabaj Pérez, M.H. (Ed.) (2010) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 4.0. American Society of Ichthyologists and Herpetologists, Washington, DC. Available from: <http://www.asih.org/> (accessed 7 May 2014)
- Smith, H.M. (1946) Preliminary notes and speculations on the *Triseriatus* group of rattlesnakes in Mexico. *University of Kansas Science Bulletin*, 31, 75–101.
- 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. & Donnelly, P. (2003) A comparison of Bayesian methods for haplotype reconstruction. *American Journal of Human Genetics*, 73, 1162–1169. <http://dx.doi.org/10.1086/379378>
- 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>
- Townsend, T.M., Alegre, R.E., Kelley, S.T., Wiens, J.J. & Reeder, T.W. (2008) Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. *Molecular Phylogenetics and Evolution*, 47, 129–142. <http://dx.doi.org/10.1016/j.ympev.2008.01.008>
- Vargas-Rodriguez, Y.L., Platt, W.J., Vázquez-García, J.A. & Boqiun, G. (2010) Selecting relict montane cloud forests for conservation priorities: the case of western Mexico. *Natural Areas Journal*, 30, 156–174. <http://dx.doi.org/10.3375/043.030.0204>
- Vázquez, L., Bustamante-Rodríguez, C. & Bahena-Arce, D.G. (2009) Area selection for conservation of Mexican mammals. *Animal Biodiversity and Conservation*, 32, 29–39.
- Wiley, E.O. (1978) The evolutionary species concept reconsidered. *Systematic Zoology*, 27, 17–26. <http://dx.doi.org/10.2307/2412809>
- Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2004) AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. *Bioinformatics*, 24 (4), 681–583. <http://dx.doi.org/10.1093/bioinformatics/btm388>
- Xie, W.G., Lewis, P.O., Fan, Y., Kuo, L. & Chen, M.H. (2011) Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology*, 60, 150–160. <http://dx.doi.org/10.1093/sysbio/syq085>
- Zaldívar-Riverón, A. & Nieto-Montes de Oca, A. (2001) Natural history and distribution of the lizard *Barisia rudicollis* (Anguidae). *The Southwestern Naturalist*, 46, 391–396. <http://dx.doi.org/10.1111/j.1439-0469.2005.00308.x>
- Zaldívar-Riverón, A. & Nieto-Montes de Oca, A. (2002) Variation in the rare lizard *Barisia rudicollis* (Anguidae) (Wiegmann) with the description of a new species from Central Mexico. *Herpetologica*, 58, 313–326. [http://dx.doi.org/10.1655/0018-0831\(2002\)058\[0313:VITRLB\]2.0.CO;2](http://dx.doi.org/10.1655/0018-0831(2002)058[0313:VITRLB]2.0.CO;2)