

Notes and Comments

Note on the phylogenetic position of *Eptesicus* species in the Vespertilionidae family (Chiroptera) using Brazilian bats and RAG2 sequences

G. R. Salomon^a , A. L. Gaglioti^b , L. P. B. Machado^a , A. O. T. Carrasco^c  and R. P. Mateus^{a*} 

^aUniversidade Estadual do Centro-Oeste – UNICENTRO, Departamento de Ciências Biológicas, Programa de Pós-graduação em Biologia Evolutiva, Laboratório de Genética e Evolução – LaGEv, Campus CEDETEG, Guarapuava, PR, Brasil

^bUniversidade Estadual do Centro-Oeste – UNICENTRO, Departamento de Ciências Biológicas, Programa de Pós-graduação em Biologia Evolutiva, Campus CEDETEG, Guarapuava, PR, Brasil

^cUniversidade Estadual do Centro-Oeste – UNICENTRO, Departamento de Medicina Veterinária, Laboratório de Doenças Infecciosas e Parasitárias, Campus CEDETEG, Guarapuava, PR, Brasil

The phylogenetic position of the *Eptesicus* genus in the Vespertilionidae family has been constantly changed after morphology, cytogenetics and molecular studies. For example, the reallocation of some African *Eptesicus* species from a subgenus of the *Pipistrellus* genus to the *Neoromicia* genus (Kearney et al., 2002), and also the reallocation of *E. nasutus* to the *Rhynptesicus* genus (Juste et al., 2013), both indicated uncertainty about the monophyly of this genus. Moreover, New World species of *Eptesicus* seems to be more closely related to species of *Histiotus* than they are to Old World species, with *Histiotus* being considered a subgenus of *Eptesicus* (Roehrs et al., 2010; Amador et al., 2016).

Therefore, it is clear that the Vespertilionidae family still has phylogenetic issues to be cleared out, specially regarding the positioning of *Eptesicus* species within the genus and its relation with the *Histiotus* genus. Hence, the more studies that can be carried out on tribes, subfamilies, genera and related species, the better will be the contributions to shed more light on the understanding of phylogenetic relationships within this family. Thus, in this work we performed a phylogenetic analysis using the DNA obtained from a specimen of *Eptesicus furinalis* collected in Southern Brazil and also sequences from all other Brazilian species that are available in the GenBank plataform, using the sequence of RAG2 nuclear gene.

Fresh tissue samples of liver, spleen, lung and kidney of *Eptesicus furinalis* collected in Guarapuava/PR, Brazil (25° 21' 9.40" S 51° 28' 0.30" W), was submitted to DNA extraction using the DNeasy Blood & Tissue (QIAGEN) kit. The other sequences of eleven Brazilian species of the Vespertilionidae family and of *Molossus molossus* (used as outgroup in the phylogenetic analyses) were obtained from GenBank databank (Table 1). *Eptesicus furinalis* RAG2 sequences (GenBank accession number: KX961304) were obtained via PCR amplifications, using RAG2F1 and RAG2R1 primers (Baker et al., 2000).

Complementary DNA sequences were assembled for each accession using Geneious® 8.0.2 (Biomatters, 2015). Multiple-sequence alignment was performed using Clustal W (Chenna et al., 2003) followed by manual optimization. Preliminary phylogenetic analyses were conducted to explore the distribution of phylogenetic signal in RAG2 data matrix with and without coded gaps. The resolution and support were affected in substantial way by inclusion of gaps, with gaps treated as fifth base (results shown). Phylogenetic analyses of RAG2 were performed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). The consistency index (CI; Kluge and Farris, 1969) and retention index (RI; Farris, 1989) were calculated. Based on the Akaike information criterion (Posada and Buckley, 2004), the dataset was evaluated for the appropriate model of molecular evolution using ModelTest v.3.7 (Posada and Crandall, 1998). Clade support for both MP and ML phylogenies were assessed with a bootstrap analysis using 1,000 replicates with 100 random addition sequence replicates, and tree bisection and reconnection (TBR) branch swapping as implemented in PAUP* 4.01b10 (Swofford, 2002) using the interface of Geneious® 8.0.2. The BI tree was implemented in MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) using the interface of Geneious® 8.0.2. Four Markov Chain Monte Carlo simulations were run simultaneously and sampled every 100 generations for a total of 1,000,000 generations until the average deviation of split frequencies was well below 0.01.

All three phylogenetic analyses performed in this study (Figure 1), using only Brazilian species of bats, showed *Histiotus macrotus* as a separated clade from *Eptesicus* species clade, with high supported nodes. These results were not observed by Giménez et al. (2019), which observed a weak support for this separation using one nuclear and one mitochondrial gene, mostly because of the incongruence of ML with the BI and MP analyses. Moreover, Jones et al. (2002) found that *Histiotus* is phylogenetically

*e-mail: rogeriopmateus@gmail.com

Received: November 12, 2021 – Accepted: January 2, 2022



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Table 1. Eleven Vespertilionidae species and *Molossus molossus* (used as outgroup), with GenBank accession number, used in the phylogenetic analysis.

Species	Accession Number	Species	Accession Number	Species	Accession Number
<i>Eptesicus brasiliensis</i>	HM561644	<i>Lasiurus ega</i>	HM561639	<i>Myotis nigricans</i>	GU328088
<i>Eptesicus diminutus</i>	GU328056	<i>Myotis ruber</i>	AM265688	<i>Myotis riparius</i>	GU328089
<i>Histiotus macrotus</i>	HM561648	<i>Myotis albescens</i>	GU328076	<i>Myotis simus</i>	AM265691
<i>Lasiurus cinereus</i>	HM561638	<i>Myotis levis</i>	GU328085	<i>Molossus molossus</i>	AY141017.1

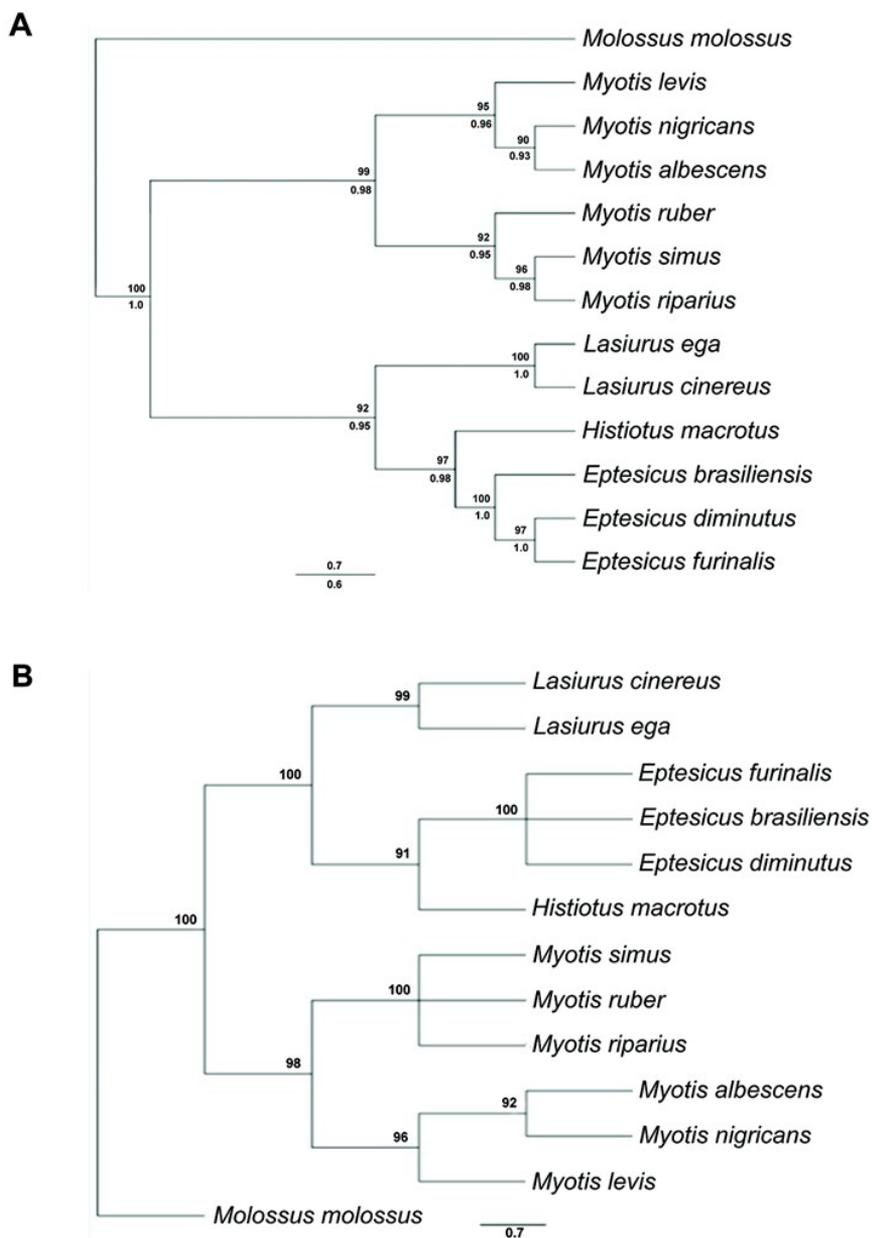


Figure 1. Dendrograms of Brazilian species of Chiroptera generated using: (A) Maximum Likelihood (ML) and Bayesian Method (BM) – above nodes/lines = ML bootstrap supports, below nodes/lines = BM supports, above scale = ML distance, below scale = BM distance; (B) Maximum Parsimony (MP) – above nodes/lines = MP bootstrap supports. *Molossus molossus* was used as outgroup.

distant from *Eptesicus*. Thus, our results demonstrated that this species needs to be included in other molecular works for a better understanding of the phylogenetic relationships within the Vespertilioninae subfamily.

Other important results were the monophyly of the Vespertilioninae subfamily (congregates species of *Lasiurus*, *Histiotus* and *Eptesicus* genera) and of the *Myotis* genus (*Myotinae* subfamily), with high statistical support. These results were demonstrated in other cytogenetic and molecular studies (Ruedi et al., 2013, as an example). However, regarding the *Myotis* genus, *M. albescens* appears as closely related to *M. levis* and *M. nigricans* in some studies (Lack et al., 2010, as an example), a fact that was not observed here. Nonetheless, the close phylogenetic relationship among these three species is always observed in analyzes involving Neotropical species (Jones et al., 2002; Ruedi et al., 2013).

Acknowledgements

This work was supported by CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Master Scholarship for G. R. Salomón), FINEP and UNICENTRO.

References

- AMADOR, L.I., MOYERS ARÉVALO, R.L., ALMEIDA, F.C., CATALANO, S.A. and GIANNINI, N.P., 2016. Bat systematics in the light of unconstrained analyses of a comprehensive molecular supermatrix. *Journal of Mammalian Evolution*, vol. 23, pp. 1-34. <http://dx.doi.org/10.1007/s10914-016-9363-8>.
- BAKER, R.J., PORTER, C.A., PATTON, J.C. and VAN DEN BUSSCHE, R., 2000. Systematic of bats of the family Phyllostomidae based on RAG2 DNA sequences. *Occasional Papers of the Museum of Texas Tech University*, vol. 202, pp. 1-20.
- BIOMATTERS, 2015 [viewed 12 November 2021]. *Geneious® 8.0.2* [online]. Biomatters Development Team. Available from: <http://www.geneious.com>
- CHENNA, R., SUGAWARA, H., KOIKE, T., LOPEZ, R., GIBSON, T.J., HIGGINS, D.G. and THOMPSON, J.D., 2003. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research*, vol. 31, no. 13, pp. 3497-3500. <http://dx.doi.org/10.1093/nar/gkg500>. PMID:12824352.
- FARRIS, J.S., 1989. The retention index and the rescaled consistency index. *Cladistics*, vol. 5, no. 4, pp. 417-419. <http://dx.doi.org/10.1111/j.1096-0031.1989.tb00573.x>. PMID:34933481.
- GIMÉNEZ, A.L., GIANNINI, N.P. and ALMEIDA, F.C., 2019. Mitochondrial genetic differentiation and phylogenetic relationships of three *Eptesicus* (*Histiotus*) species in a contact zone in Patagonia. *Mastozoología Neotropical*, vol. 26, no. 2, pp. 349-358. <http://dx.doi.org/10.31687/saremMN.19.26.2.0.10>.
- JONES, K.E., PURVIS, A., MACLARNON, A., BININDA-EMONDS, O.R.P. and SIMMONS, N.B., 2002. A phylogenetic supertree of the bats (Mammalia: chiroptera). *Biological Reviews of the Cambridge Philosophical Society*, vol. 77, no. 2, pp. 223-259. <http://dx.doi.org/10.1017/S1464793101005899>. PMID:12056748.
- JUSTE, J., BENDA, P., GARCIA-MUDARRA, J.J. and IBÁÑEZ, C., 2013. Phylogeny and systematics of Old World serotine bats (genus *Eptesicus*, Vespertilionidae, Chiroptera): an integrative approach. *Zoologica Scripta*, vol. 42, no. 5, pp. 441-457. <http://dx.doi.org/10.1111/zsc.12020>.
- KEARNEY, T.C., VOLLETH, M., CONTRAFATTO, G. and TAYLOR, P.J., 2002. Systematic implications of chromosome GTG-band and bacula morphology for Southern African *Eptesicus* and *Pipistrellus* and several other species of Vespertilioninae (Chiroptera: vespertilionidae). *Acta Chiropterologica*, vol. 4, no. 1, pp. 55-76. <http://dx.doi.org/10.3161/001.004.0107>.
- KLUGE, A.G. and FARRIS, J.S., 1969. Quantitative phyletics and the evolution of anurans. *Systematic Biology*, vol. 18, no. 1, pp. 1-32. <http://dx.doi.org/10.1093/sysbio/18.1.1>.
- LACK, J.B., ROEHRS, Z.P., STANLEY, C.E. Jr., RUEDI, M. and VAN DEN BUSSCHE, R.A., 2010. Molecular phylogenetics of *Myotis* indicate familial-level divergence for the genus *Cistugo* (Chiroptera). *Journal of Mammalogy*, vol. 91, no. 4, pp. 976-992. <http://dx.doi.org/10.1644/09-MAMM-A-192.1>.
- POSADA, D. and CRANDALL, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, vol. 14, no. 9, pp. 817-818. <http://dx.doi.org/10.1093/bioinformatics/14.9.817>. PMID:9918953.
- POSADA, D. and BUCKLEY, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology*, vol. 53, no. 5, pp. 793-808. <http://dx.doi.org/10.1080/10635150490522304>. PMID:15545256.
- ROEHRS, Z.P., LACK, J.B. and VAN DEN BUSSCHE, R.A., 2010. Tribal phylogenetic relationships within Vespertilioninae (Chiroptera: Vespertilionidae) based on mitochondrial and nuclear sequence data. *Journal of Mammalogy*, vol. 91, no. 5, pp. 1073-1092. <http://dx.doi.org/10.1644/09-MAMM-A-325.1>.
- RONQUIST, F. and HUELSENBECK, J.P., 2003. MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)*, vol. 19, no. 12, pp. 1572-1574. <http://dx.doi.org/10.1093/bioinformatics/btg180>. PMID:12912839.
- RUEDI, M., STADELMANN, B., GAGER, Y., DOUZERY, E.J.P., FRANCIS, C.M., LIN, L.K., GUILLÉN-SERVENT, A. and CIBOIS, A., 2013. Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera). *Molecular Phylogenetics and Evolution*, vol. 69, no. 3, pp. 437-449. <http://dx.doi.org/10.1016/j.ympev.2013.08.011>. PMID:23988307.
- SWOFFORD, D.L., 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sunderland: Sinauer Associates.