



## The taxonomic position of *Tonkinomys daovantieni* (Rodentia: Muridae) based on karyological and molecular data

ALEXANDER E. BALAKIREV<sup>1,2</sup>, VLADIMIR V. ANISKIN<sup>2</sup>, TRAN QUANG TIEN<sup>1</sup>  
& VIATCHESLAV V. ROZHNOV<sup>1,2</sup>

<sup>1</sup>Joint Vietnam-Russian Tropical Research and Technological Centre, Nguyen Van Huyen, Nghia Do, Cau Giay, Hanoi, Vietnam.  
E-mail: alexbalakirev@mail.ru

<sup>2</sup>A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow 119071, Russia.  
E-mail: rozhnov.v@gmail.com

### Abstract

*Tonkinomys daovantieni* was recently described from Northern Vietnam, but very sparse information exists for the taxon. We report for the first time the karyotype of this species and investigate its phylogenetic position in the *Dacnomys* division using both mitochondrial and nuclear genetic data. The diploid chromosome number of the species is  $2n=44$ . This chromosomal set consists of one submetacentric pair, one metacentric pair, and nineteen pairs of subtelocentric/acrocentric autosomes progressively decreasing in size. The X chromosome is submetacentric and approximately equal in size to the largest subtelocentric autosome. The Y chromosome is metacentric and equal in size to the smallest pair of autosomes. The phylogenetic reconstruction based on the Cyt *b* COI and GHR genes reveals that *Saxatilomys paulinae*, a species distributed in the karst formations of the Lao PDR, is the closest relative to *T. daovantieni*. These two taxa are similar not only in a number of morphological characters, but also in their major ecological preferences (both are petrophilic species associated with limestone karst formations). Based on our data, we can conclude that the similarities among the ecological adaptations, natural conditions and habitat preferences of these species are a reflection of their phylogenetic relationship.

**Key words:** limestone rats, Southeast Asia, Vietnam, taxonomy, molecular phylogeny, karyotype

### Introduction

The monotypic genus *Tonkinomys* Musser, Lunde & Nguyen 2006, comprising the species *Tonkinomys daovantieni* Musser, Lunde & Nguyen 2006, was first described from the forested tower karst formations of the Huu Lien Nature Reserve of north-eastern Vietnam (Musser *et al.* 2006). Based on its morphological peculiarities, this species was classified as a member of this *Dacnomys* division of the tribe Rattini. According to Musser & Carleton (2005), the division includes four Indo-Sundaic genera (*Dacnomys* Thomas, *Niviventer* Marshall, *Leopoldamys* Ellerman, and *Chiromyscus* Thomas), the Sri Lankan endemic *Srilankamys* Musser, and the Philippine genus *Anonymomys* Musser. The composition of the *Dacnomys* division was recently subjected to a taxonomic revision based on molecular data (Balakirev *et al.* 2011, 2012, 2013) and it was demonstrated that the genus *Saxatilomys* Musser (Musser *et al.* 2005) should be considered an additional member of the *Dacnomys* division. It was also discovered that *Srilankamys* should be excluded from the *Dacnomys* division and placed instead in the *Rattus* division (Buzan *et al.* 2011, Balakirev *et al.* 2012). Until the present study, no molecular or chromosomal data have been available for *Tonkinomys*, and the species was known only from its original morphological description.

Musser *et al.* (2006), when describing the new taxon, stated that the phylogenetic position of *Tonkinomys* with respect to *Leopoldamys*, *Niviventer*, and *Saxatilomys*, the three extant genera with morphologies most similar to that of *Tonkinomys* was unresolved; the differences between *Tonkinomys daovantieni* and the species of *Leopoldamys* and *Niviventer* reflected a combination of primitive and derived features. The pelage coloration, as well as the tail shape and its length relative to the length of head and the body in *Leopoldamys* and *Niviventer*, are in contrast with those of *Tonkinomys*. *Tonkinomys* and *Saxatilomys* display a series of common morphological

The monophyly for the *Saxatilomys*/*Tonkinomys* clade, along with its clear morphological distinctiveness, provide strong support for its phylogenetic relationship, and the intergroup genetic distances of 0.1363–0.1455 for *Cytb* and 0.1130–0.1179 for *COI* are high enough to indicate generic specificity in agreement with the genetic species conception (Breadly & Baker, 2001, Baker & Breadly, 2006). These genera undoubtedly belong to an original phylogenetic clade within the *Dacnomys* division (comprising the *Dacnomys*, *Leopoldamys* and *Niviventer*/*Chiromyscus* genera). Although, the basal tree topology of the *Dacnomys* division remains unresolved, the data reported into present study and in Balakirev *et al.* (2012, 2013) indicate that *Saxatilomys* and *Tonkinomys* genera are more closely related to each other than to other members of the division. Similarly, the *Leopoldamys* and *Dacnomys* genera as well as the *Chiromyscus* and *Niviventer* genera (Balakirev *et al.*, 2012, 2013) compose another pairs of most closely related taxa. It should also be noted also that the position of *Maxomys* clade, which is usually regarded as an outgroup to the *Dacnomys* division (as a member of a separate *Maxomys* division, Musser & Carleton 1993, 2005) may be in need of revision. A special survey is needed to investigate this question in details, because of the complex composition of the genus *Maxomys*.

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## References

- Badenhorst, D., Herbreteau, V., Chaval, Y., Pages, M., Robinson, T.J., Rerkamnuaychoke, W., Morand, S., Hugot, J-P. & Dobigny, G. (2009) New karyotypic data for Asian rodents (Rodentia, Muridae) with the first report of B-chromosomes in the genus *Mus*. *Journal of Zoology*, 279, 44–56.  
<http://dx.doi.org/10.1111/j.1469-7998.2009.00588.x>
- Baker, R.J. & Bradley, R.D. (2006) Speciation in mammals and the genetic species concept. *Journal of Mammalogy*, 87, 643–662.  
<http://dx.doi.org/10.1644/06-mamm-f-038r2.1>
- Balakirev, A.E. & Rozhnov, V.V. (2010) Phylogenic relationships and species composition in the genus *Niviventer* (Rodentia: Muridae) based on studies of the cytochrome *b* gene of mtDNA. *Moscow University Biological Sciences Bulletin*, 65, 171–174.  
<http://dx.doi.org/10.3103/s0096392510040139>
- Balakirev, A.E., Abramov, A.V. & Rozhnov, V.V. (2011) Taxonomic revision of *Niviventer* (Rodentia, Muridae) from Vietnam: a morphological and molecular approach. *Russian Journal of Theriology*, 10, 1–26.
- Balakirev, A.E., Abramov, A.V., Tikhonov, A.N. & Rozhnov, V.V. (2012) Molecular phylogeny of *Dacnomys* division (Rodentia, Muridae): the taxonomic positions of *Saxatilomys* and *Leopoldamys*. *Doklady Biological Sciences*, 445, 251–254.
- Balakirev, A.E., Abramov, A.V. & Rozhnov, V.V. (2013) Revision of the genus *Leopoldamys* (Rodentia, Muridae) as inferred from morphological and molecular data, with a special emphasis on the species composition in continental Indochina. *Zootaxa*, 3640 (4), 521–549.  
<http://dx.doi.org/10.11646/zootaxa.3640.4.2>
- Bradley, R.D. & Baker, R.J. (2001) A test of the genetic species concept: cytochrome *b* sequences and mammals. *Journal of Mammalogy*, 82, 960–973.  
[http://dx.doi.org/10.1644/1545-1542\(2001\)082<0960:atotgs>2.0.co;273](http://dx.doi.org/10.1644/1545-1542(2001)082<0960:atotgs>2.0.co;273)
- Buzan, E.V., Krystufek, B., Hanfling, B. & Hutchinson, W.F. (2008) Mitochondrial phylogeny of Arvicolinae using comprehensive taxonomic sampling yields new insights. *Biological Journal of the Linnean Society*, 94, 825–835.  
<http://dx.doi.org/10.1111/j.1095-8312.2008.01024.x>
- Buzan, E.V., Pages, M., Michaux, J. & Krystufek, B. (2011) Phylogenetic position of the Ohiya rat (*Srilankamys ohiensis*) based on mitochondrial and nuclear gene sequence analysis. *Zoologica Scripta*, 40, 545–553.  
<http://dx.doi.org/10.1111/j.1463-6409.2011.00494.x>

- Duncan, J.F., van Peenen, P.F.D. & Ryan, P.F. (1970) Somatic chromosomes of eight mammals from Con Son Island, Southern Vietnam. *Caryologia*, 23, 173–181.
- Duncan, J.F. & van Peenen, P.F.D. (1971) Karyotypes of ten rats (Rodentia: Muridae) from Southeast Asia. *Caryologia*, 24, 331–346.
- Ford, C.E. & Hamerton, J.L. (1956) A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. *Stain Technology*, 31, 247–251.
- Gorog, A.J., Sinaga, M.H. & Engstrom, M.D. (2004) Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*). *Biological Journal of the Linnean Society*, 81, 91–109.  
<http://dx.doi.org/10.1111/j.1095-8312.2004.00281.x>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclear Acids Symposium Series*, 41, 95–98.
- Heaney, L.R., Balete, D.S., Rickart, E.A., Veluz, M.J. & Jansa, S.A. (2009) A new genus and species of small “tree-mouse” (Rodentia, Muridae) related to the Philippine giant cloud rats. *Bulletin of American Museum of Natural History*, 331, 205–229.  
<http://dx.doi.org/10.1206/582-7.1>
- Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.  
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Irwin, D., Kocher, T.D. & Wilson, A.S. (1991) Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution*, 32, 128–144.  
<http://dx.doi.org/10.1007/bf02515385>
- Jaeger, J.J., Tong, H. & Denys, C. (1986) Age de la divergence *Mus-Rattus*: comparaison des donnees paleontologiques et moleculaires. *Comptes Rendus de l'Academie des Sciences, Serie II*, 302, 917–922.
- Jansa, S.A., Barker, F.K. & Heaney, L.R. (2006) The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences. *Systematic Biology*, 55, 73–88.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F. & Wilson, A. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of National Academy of Sciences of USA*, 86, 6196–6200.  
<http://dx.doi.org/10.1073/pnas.86.16.6196>
- Latinne, A., Waengsothorn, S., Herbreteau, V. & Michaux, J.R. (2011) Evidence of a complex phylogeographic structure for the threatened rodent *Leopoldamys neilli*, in Southeast Asia. *Conservation Genetics Resources*, 12, 1495–1511.  
<http://dx.doi.org/10.1007/s10592-011-0248-3>
- Lecompte, E., Aplin, K., Denys, C., Catzeflis, F., Chades, M. & Chevret, P. (2008) Phylogeny and biogeography of African Murinae based on mitochondrial and nuclear gene sequences, with a new tribal classification of the subfamily. *BMC Evolutionary Biology*, 8, 199–220.  
<http://dx.doi.org/10.1186/1471-2148-8-199>
- Li, Yu., Wu, Yi., Harada, M., Lin, L-K. & Motokawa, M. (2008) Karyotypes of three rat species (Mammalia: Rodentia: Muridae) from Hainan Island, China, and the valid specific status of *Niviventer lotipes*. *Zoological Science*, 25, 686–692.  
<http://dx.doi.org/10.2108/zsj.25.686>
- Lunde, D. & Musser, G. (2008) *Saxatilomys paulinae*. In: *IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2*. Available from: <http://www.iucnredlist.org> (Accessed on 20 May 2013)
- Michaux, J.R., Chevret, P., Filippucci, M.G. & Macholán, M. (2002) Phylogeny of the genus *Apodemus* with a special emphasis to the subgenus *Sylvaemus* using the nuclear IRBP gene and two mitochondrial markers: cytochrome *b* and 12S rRNA. *Molecular Phylogenetics and Evolution*, 23, 123–136.
- Michaux, J.R., Magnanou, E., Paradis, E., Nieberding, C. & Libois, R. (2003) Mitochondrial phylogeography of the woodmouse (*Apodemus sylvaticus*) in the western Palaearctic region. *Molecular Ecology*, 12, 685–697.  
<http://dx.doi.org/10.1046/j.1365-294x.2003.01752.x>
- Musser, G.G. & Carleton, M.D. (2005) Family Muridae. In: Wilson, D.E. & Reeder, D.M. (Eds.), *Mammal Species of the World. A Taxonomic and Geographic Reference*. 3rd edition. The John Hopkins University Press, Baltimore, pp. 894–1531.
- Musser, G.G., Smith A.L., Robinson, M.F. & Lunde, D.P. (2005) Description of a new genus of rodent (Murinae, Muridae, Rodentia) from the Khammouan Limestone National Biodiversity Conservation Area in Lao PDR. *American Museum Novitates*, 3497, 1–31.  
[http://dx.doi.org/10.1206/0003-0082\(2005\)497\[0001:doanga\]2.0.co;2](http://dx.doi.org/10.1206/0003-0082(2005)497[0001:doanga]2.0.co;2)
- Musser, G.G., Lunde, D.P. & Nguyen, T.S. (2006) Description of a new genus and species of rodent (Murinae, Muridae, Rodentia) from the Tower Karst Region of Northeastern Vietnam. *American Museum Novitates*, 3517, 1–41.  
[http://dx.doi.org/10.1206/0003-0082\(2005\)497\[0001:doanga\]2.0.co;2](http://dx.doi.org/10.1206/0003-0082(2005)497[0001:doanga]2.0.co;2)
- Page, R.D.M. (1996) Tree View: An application to display phylogenetic trees on personal computers. *Computers and Applied Bioscience*, 12, 357–358.  
<http://dx.doi.org/10.1093/bioinformatics/12.4.357>

- Pages, M., Chaval, Y., Herbreteau, V., Waengsothorn, S., Cosson, J.F., Hugot, J.P., Morand, S. & Michaux, J. (2010) Revisiting the taxonomy of the Rattini tribe: a phylogeny-based delimitation of species boundaries. *BMC Evolutionary Biology*, 10, 184.  
<http://dx.doi.org/10.1186/1471-2148-10-184>
- Rambaud, A. & Drummond, A.J. (2007) Tracer, version 1.4. Available from: <http://tree.bio.ed.ac.uk/software/tracer> (accessed 20 May 2013)
- Robins, J.H., Hingston, M., Matisoo-Smith, E. & Ross, H.A. (2007) Identifying *Rattus* species using mitochondrial DNA. *Molecular Ecology Notes*, 7, 717–729.  
<http://dx.doi.org/10.1111/j.1471-8286.2007.01752.x>
- Rowe, K.C., Reno, M.L., Richmond, D.M., Adkins, R.M. & Stepan, S.J. (2008) Pliocene colonization and adaptive radiations in Australia and New Guinea (Sahul): multilocus systematics of the old endemic rodents (Muroidea: Murinae). *Molecular Phylogenetics and Evolution*, 47, 84–101.  
<http://dx.doi.org/10.1016/j.ympev.2008.01.001>
- Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Lab, Cold Spring Harbor, 256 pp.
- Serizawa, K., Suzuki, H. & Tsuchiya, K. (2000) A phylogenetic view on species radiation in *Apodemus* inferred from variation of nuclear and mitochondrial genes. *Biochemical Genetics*, 38, 27–40.
- Suzuki, H., Sato, J.J., Tsuchiya, K., Luo, J., Zhang, Y.-P., Wang, Y.-X. & Jiang, X.-L. (2003) Molecular phylogeny of wood mice (*Apodemus*, Muridae) in East Asia. *Biological Journal of the Linnean Society*, 80, 469–481.  
<http://dx.doi.org/10.1046/j.1095-8312.2003.00253.x>
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28, 2731–2739.  
<http://dx.doi.org/10.1093/molbev/msr121>
- Thomas, O. (1916) A new Rat from Tenasserim. *The Annals and Magazine of Natural History (Zoology, Botany and Geology)*, 17, 425.
- Thomas, O. (1925) The mammals obtained by Mr. Herbert Stevans on the Sladen-Godman Expedition to Tonkin. *Proceedings of the Zoological Society of London*, 95 (2), 495–506.  
<http://dx.doi.org/10.1111/j.1096-3642.1925.tb01524.x>
- Wang, J.-X., Zhao, X.-F., Koh, H. S., Deng, Y., & Qi, H.-Y. (2003) Chromosomal polymorphisms due to heterochromatin growth and pericentric inversions in white-bellied rat, *Niviventer confucianus*, from China. *Hereditas*, 138, 59–64.  
<http://dx.doi.org/10.1034/j.1601-5223.2003.01686.x>
- Yosida, T.H. (1973) Evolution of karyotypes and differentiation in 13 *Rattus* species. *Chromosoma*, 4, 285–297.  
<http://dx.doi.org/10.1007/bf00326182>
- Yong, H.-S. (1968) Karyotypes of three species of rats Hong Kong and Thailand (Muridae, genus *Rattus* Fischer). *Cytologia*, 34, 394–398.
- Yong, H.-S. (1969) Karyotypes of Malay rats (Rodentia: Muridae, genus *Rattus* Fischer). *Chromosoma*, 27, 245–267.

#### APPENDIX 1. List of *Tonkinomys daovantieni* samples and its localities.

- ZMMU S-190817 (LD3), Ad. M. 21°41'26.95"N; 106°19'52.70"E (GeneBank IDs; KC209558, KC209569, KF154058)
- ZMMU S-191155 (HL17), Ad. M. 21°40'N; 106°22'22.32"E (GeneBank IDs; KC209559, KC209564, KF154059)
- ZMMU S-191155 (HL22), Ad. F. 21°40'29"N; 106°22'51"E (GeneBank IDs; KC209560, KC209565, KF154060)
- ZMMU, S-191156 (HL23), Ad. F. 21°39'37"N; 106°23'15"E
- ZMMU S-191157 (HL24), Ad. M. 21°40'N; 106°22'22.32"E (GeneBank IDs; KC209561, KC209566, KF154061)
- ZMMU S-191158 (HL25), Sad. M. 21°40'N; 106°22'22.32"E (GeneBank IDs; KC209562, KC209567, KF154062)
- ZMMU S-191159 (HL26), Sad. M. 21°40'N; 106°22'22.32"E (GeneBank IDs; KC209563, KC209568, KF154063)
- ZMMU S-191160 (HL27), Ad. M. 21°37'32"N; 106°20'49"E (GeneBank IDs; KC209558)
- ZMMU S-191161 (HL28), Ad. M. 21°37'32"N; 106°20'49"E, **karyotyped**. (GeneBank IDs; KC209558)
- ZMMU S-191162 (HL30), near 21°40'N; 106°22'22E, no precise locality available (GeneBank IDs; KC209558)
- ZMMU S-191163 (HL31), near 21°40'N; 106°22'22E, no precise locality available (GeneBank IDs; KC209558)
- ZMMU S-191164 (HL32), near 21°40'N; 106°22'22E, no precise locality available (GeneBank IDs; KC209558)
- ZMMU S-191165 (HL33), near 21°40'N; 106°22'22E, no precise locality available (GeneBank IDs; KC209558)
- ZMMU S-191166 (HL34), near 21°40'N; 106°22'22E, no precise locality available (GeneBank IDs; KC209558)