ON TAXONOMIC STATUS OF *PSEUDOBERYLMYS MUONGBANGENSIS*, A NEW SPECIES AND GENUS DESCRIBED FROM SON LA PROVINCE, VIETNAM; DOES IT NEW OR PSEUDO-NEW SPECIES?

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Wide scope of investigations in ecology and biodiversity has been held during last century in Indochina and Sunda region, but in terms of fauna of mammals, Southeast Asia is still remains one of underinvestigated region in the World. It can be illustrated by the facts that new species have repeatedly being described from Laos, Northern Vietnam and Thailand during last decade [Averianov *et al.*, 2000; 2,3,5,6,9] and there are good reasons to believe that the aria can present to a zoological science a lot of interesting finds. In this way, new species and genus was described in 2009 [10] in Son La province, Northern Vietnam and was named *Pseudoberylmys muongbangensis.* In spite of detailed morphological description, an illustrative materials did not allow to perform careful comparative taxonomical analysis based on the data presented in paper, unfortunately, authors also did not perform any genetic analysis of specimens obtained, which can be used to appreciate the status of new taxon based on molecular data. The fact forced us to organize an independent expedition to obtain original materials from type locality to perform comparative morphological and molecular genetics analysis to validate a taxonomical status and evolutionary relationships of these animals.

I. MATERIAL AND METHODS

Field materials: Six adult and young adult rats (field numbers MB-1, MB-2, MB-8, MB-9, MB-10 and MB-11) were trapped in course of expedition being held from 1 to 10 of March 2011 in Muong Bang commune, Phu Yen district, Son La province at the mountains forest area in vicinity of Muong Bang (N21,08'22.5"; E104,46'07,8≈ 600m) and Ban Cai (N21,08'10.7"; E104,46'19,2 ≈ 700m) villages. Size, general appearance, fur structure and coloration of the animals obtained was exactly the same as indicated in description of *Pseudoberylmys muongbangensis* presented Tran *et al.* (2009) [10]. Any other species could be theoretically confused to the striped-bellied rats are lacking here, what allow us to conclude that our samples may be treated as belonging to the same new-described species trapped, moreover in closest vicinity to type location.

All individual obtained were weighed and photographed just after being sacrificed. Values (in millimeters, mm) for total length, length of tail (LT), length of hind foot, excluding claw (LHF), and length of ear from intertragal notch to crown (LE) are those we obtained in the field and recorded in our field journals. Values for length of head and body (BL) were determined by subtracting length of tail from total length.. All 6 individuals of the new rat were fixed as whole in 70% ethanol in the field and subsequently transferred to molecular grade purity 96% ethanol for storage. Small peace (0.2-0.3 g) of muscle and liver were taken as a samples for molecular genetic analysis and preserved by molecular grade purity 96% ethanol for storage

Morphological materials: Intact skulls were subsequently removed from 5 of 6 specimens boiled and cleaned manually in laboratory. Cleaned skulls were treated by 3% hydrogen

peroxide. Both cleaned skulls and ethanol preserved carcasses were submitted to Zoological Museum of Moscow State University (ZMMU), catalog numbers are being processed.

The following cranial and dental dimensions were measured according to the schema presented in [5, 7]; Occipitonasal length (ONL= greatest length of skull), zygomatic breadth (ZB), interorbital breadth (IB), length of rostrum (LR), breadth of rostrum (BR), breadth of braincase (BBC), height of braincase (HBC), breadth of zygomatic plate (BZP), length of diastema (LD), length of incisive foramina (LIF), breadth of incisive foramina (BIF), length of bony palate (LBP) (palatal bridge), breadth across bony palate at first molars (BBP), postpalatal length (PPL), breadth of mesopterygoid fossa (BMF), length of bulla (LB), crown length of maxillary molar row (CLM1-3). Values (in millimeters, mm) for total length (TL), length of tail (LT), length of hind foot, excluding claw (LHF), and length of ear from intertragal notch to crown (LE) are those we obtained in the field and recorded in our field journals. Weights were obtained in the field with a electronic balance (600-gram capacity). Values for cranial and dental measurements were obtained by digital calipers or by ocular micrometer under MBS-10 stereomicroscope accurate to the nearest 0.05 mm.

Genetic samples treatment and procedures: DNA was isolated from ethanol fixed liver or muscle tissues by standard phenol-chloroform method (Sambrook *et al*., 1989). A full-sized (1143 bp long) cytochrome *b* gene (Cyt *b*) was amplified using primers H15915R and CytbRglu2L, (Kocher *et al*., 1989; Irvin *et al.*, 1991), and CytbRCb9H (Robins *et al*., 2007). 5'-proximal 680 bp portion of the subunit I cytochrome oxidase gene (COI) was amplified using universal conservative primers BatL 5310 and R6036R (Kocher *et al*., 1989; Irwin *et al.*, 1991). A portion of exon 1 of the IRBP gene (1200-1610 bp long) was amplified with primers IRBP125f, IRBP1435r and IRBP1801r according to the method of Stanhope *et al.* (1992).

The PCR was conducted in a KCl-containing buffer in a volume of 30–50 µl, the mixture contained 50 mM of each dNTP, 2 mM of MgCl2, 10–12 pmol of each primer, 1U Taq of DNA-polymerase, and 20–50 ng of DNA. Amplification for mtDNA genes was conducted according to the following protocol: denaturation 1 min 30 s at 95°, 40 cycles 30 s long at 95°, 1 min at 50°, 30 s at 72°, and terminating elongation, 2 min at 72°. For IRBP gene an annealing temperatures were elevated to 62-64° (Stanhope *et al.*, 1992). PCR products were purified using a DNA Purification Kit (Fermentas) and sequenced with Applied Biosystems 3130 Genetic Analyzers sequencer. The sequences obtained were submitted in GenBank (JN105085-JN105108). All sequences of *Berylmys* species available to the moment in GenBank were bring to study along with some others presented neighbor genera such as: *Rattus tanezumi* (JN105087, -102), *Maxomys moi* (JN105086, -94, -101), *Leopoldamys edwardsi* (JN105088, -95, -103), *Niviventer langbianis* (JN105085, -93, -100; original data) and *Bandicota indica* (HM217628, HM217521, HM217390) which were taken as outer groups. The sequences were aligned using the BioEdit (Hall, 1999). Genetic distances were calculated according to the model of Tamura (T3P) in the MEGA. 4.0 program (Tamura *et al.*, 2007). Trees were built by minimum evolution (ME) and neighbor joining (NJ) methods as realized in MEGA 4.0, and by the maximum likelihood (ML) method of using the DNAMLK program version 3.5c (Felsenstein, 1981) associated with BioEdit. The level of bootstrap support of nodes was calculated by 1000 replications.

II. RESULTS

Morphological traits: Morphological analysis of the samples obtained showed that the morph *Pseudoberilmys muongbangensis* which was described in great details by Tran *et al.* (2009) is actually very similar to *Berylmys* species. Just like as a typical *Berylmys* what was

described by [4] these animals possesses all the characteristics of the genus: (1) dense and crisp iron gray pelage over upperparts, white fur on underparts, dorsum and venter sharply demarcated; (2) either eight mammae (one pectoral pair, one postaxillary pair, and two inguinal pairs); (3) braincase triangular in dorsal view, its dorsolateral margins, as well as those of postorbital and interorbital regions, outlined by weak and low ridges; (4) nasolacrimal canals highly inflated; (5) incisive foramina end before or at anterior margins of upper molar rows; (6) short palatal bridge, its posterior margin anterior to backs of third upper molars; (7) pterygoid fossae complete, not breached by sphenopterygoid vacuities; (8) back of occiput expansive and sloping forward; (9) upper incisors orthodont, enamel of uppers and lowers is cream or pale orange; (10) third upper and lower molars small relative to others in row; (11) labial cusps t3, t6, and t9 on first upper molars and cusps t6 and t9 on second uppers so broadly merged with central cusps they are indistinct or appear absent; (12) cusp t3 occurs infrequently on second upper molars of three species and is absent from third upper molars of three out of the four species;

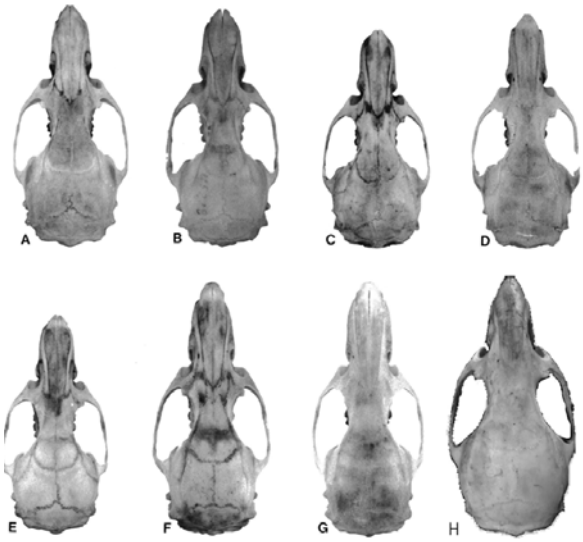

Figure 1: **Dorsal view of crania from adult *Berylmys bowersi* (from Musser and Newcomb, 1983) and original sample**

Legends: A. Northeastern India (USNM 543092); B. Northern Burma (AMNH 115238); C. Northern Laos (USNM 355544); D. Sapa, Northern Vietnam (USNM 259497); E. Chiengmai Province, Northern Thailand (AMNH 167928); F. Narathiwat Province, Peninsular Thailand (USNM 533375); G. Selangor State, Malaya (USNM 357890); H. Original sample, young adult. Son La Province, Muong Bang village (MB-8).

(13) small and narrow lamina at front of each first lower molar; (14) anterolabial cusps absent from second lower molars in most specimens, anterolabial cusps absent from third lower molars. From four currently accepted species of *Berylmys*, *Berylmys bowersi* is apparently closest relatives to Muong Bang samples judging by large general size, number of mammilla (2+2 pairs), comparative length of tail and its coloration and comparative size of bulla. The only most prominent external feature of these animals was well-shaped glandular band on the belly which prompted the authors to name it as stripe-bellied rats. Detailed investigation of skull dimensions and occlusive pattern of teeth proved the *Berylmys* species is morphologically closest to morph *muongbangensis.*

Morphological variability for different population of *Berylmys bowersi* is illustrated in Figure 1 and Table 1. As it can be seen, the population has a number of peculiar traits as compared with other ones distributed in different of Indochina and Malay Peninsula. The most spectacular is generally shorter and narrower rostral part of the skull what are prominent in smaller values of such measurements as LR, BR, LD, LID and BIF. Bullas are on the contrary generally larger than in any other population but much smaller than in different species, for example *B. berdmorei*. It should be noted the very great difference in breadth of rostrum (BR) between our samples (8.5-10.83 mm) and type series (12.8-14.7 mm) presented by [10] which may be caused by different mode of measurement or even be typos. All the other the skulls measurements of *Pseudoberylmys muongbangensis* both type series and our paratypic materials are in general range of variation *Berylmys bowersii* from different geographic populations inhabits Indochina and Malay Peninsula.

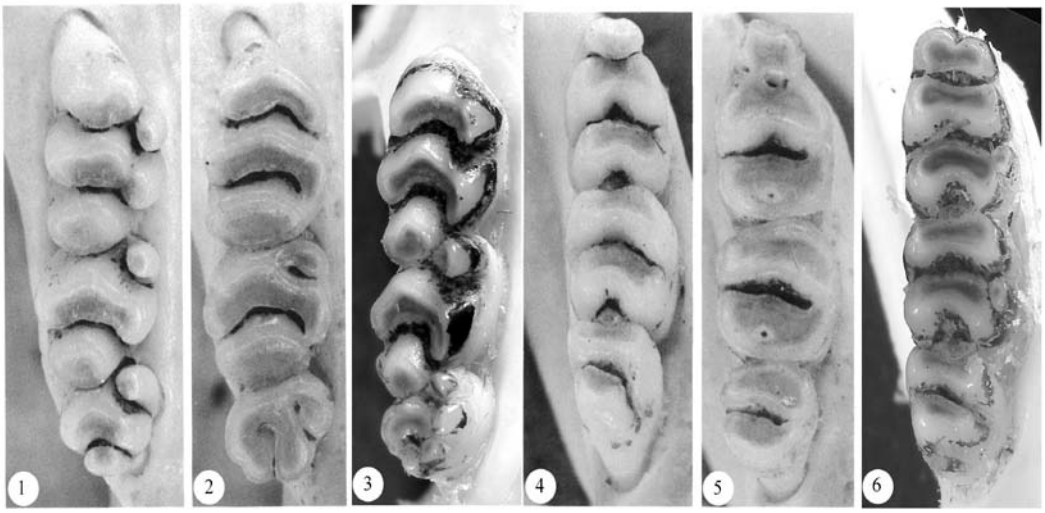

Figure 2: **Occlusal views of right upper (1-3) and lower (4-6) molars in *Berylmys bowersi* as compared with Muong Bang population specimen (from Musser and Newcomb, 1983) and original samples**

Legends: 1. Young adult *B. bowersi*, Northern Burma (AMNH 115232); 2. Old adult *B. bowersi*, Northern Burma (AMNH 115238); 3. Young adult MB-8, Son La province, Muong Bang; 4. Young adult *B. bowersi*, Northern Burma (AMNH 115232); 5. Old adult *B. bowersi*, Northern Burma (AMNH 115238); 6. Young adult MB-8, Son La province, Muong Bang.

Thus, by comparison of whole set of dimensions of study with a measurements presented by [1, 4] we can conclude that *Berylmys bowersii* is closest relative to our samples from Muong Bang. The fact is become most obvious if we compare an occlusive pattern of teeth presented in Figure 2. As it can be seen, both general molar and maxillar rows structure and pattern of every tooth are typical for *Berylmys* [4] and where are no any sharply demarcated peculiarities which would separated it from *B. bowersi.* Thus, we can conclude at the stage that there are not any reasons to separate new genus based on morphological traits of the morph. Nevertheless, the questions still arrive about taxonomical status of the morph within genus *Berylmys*. The problem can be explicitly resolved by genetic analysis.

Table 1

Measurements (in millimeters) of Adult *Berylmys bowersi* from different population of Indochina and Malay Peninsula

Measurement	NE India, Assam, and Northern Burma*	Northern Thailand*	Northern Laos*	Northern Vietnam***	Peninsular Thailand and Malaya*	Ps.muong bangensis Muong Bang Type series***	Ps.muong bangensis Muong Bang (our data)
Length of head and body (TL)	249.7±12.6 (16) 236-285	234.3±1.2 (3) 233-235	245.3±15.4 (4) 228-261	263.6±15.2 (7) 240-285	264.2±18.6 (24) 230-300	? (7) 220-260	253.7±19.9 (6) 230-278
Length of tail (LT)	269.3±11.4 (16) 249-292	248.3±7.6 (3) 240-255	271.3±14.2 (4) 250-280	284.8±18.0 (6) 260-310	283.3±11.5 (24) 262-300	? (7) 240-290	267.8±20.8 (6) 245-295
Length of hind foot (LHF)	57.0±2.3 (16) 52-61	52.7±1.2 (3) 52-53	54.3±1.5 (4) 53-56	55.6±1.1 (8) 54-57	55.0±1.8 (24) 52-59	? (7) 50-55	51.7±1.1 (6) 50-54
Length of ear (LE)	33.8±1.1 (11) 32-36	28.0±1.0 (3) 27-29	32.3±1.3 (4) 31-34	34.0±2.3 (7) 30-37	30.5±2.2 (24) 28-36	? (7) 29-34	30.5±1.52 (6) 28-32
Weight	–	–	–	–	427.7±103.9 (23) 270-650	–	363.7±52.45 (6) 269-410
Greatest length of skull (ONL)	56.3±1.9 (14) 52.1-58.5	52.1±1.3 (2) 51.2-53.0	53.3±2.0 (4) 50.9-55.7	55.8±.8 (6) 54.7-56.7	57.7±2.5 (27) 52.0-63.1	? (7) 51.8-56.4	55.8±2.99 (5) 50.36-57.43
Zygomatic breadth (ZB)	27.4±0.8 (16) 25.4-28.3	26.1±0.6 (3) 25.4-26.5	25.8±1.1 (4) 24.8-27.0	26.7±1.3 (7) 24.6-28.2	27.0±1.2 (27) 24.4-28.4	? (7) 25.6-26.9	26.8±1.05 (5) 26.01-28.63
Interorbital breadth (IB)	8.3±0.3 (16) 7.9-8.9	7.7±0.1 (3) 7.6-7.8	7.9±0.5 (4) 7.4-8.3	8.3±0.1 (7) 7.8-8.9	8.5±0.4 (27) 7.9-9.3	? (7) 7.5-8.0	8.39±0.37 (5) 7.97-8.99
Length of nasals	23.4 ±1.1 (15) 20.7-24.9	21.2±1.1 (3) 20.2-22.3	22.0±1.4 (4) 20.5-23.8	22.4±.5 (7) 21.6-23.0	24.9±1.4 (27) 22.0-28.7	? (27) 22.0-23.5	
Length of rostrum (LR)	19.3±0.8 (15) 17.5-20.7	17.1±0.8 (3) 16.3-17.8	18.1±0.3 (4) 17.8-18.5	18.4±0.6 (7) 17.8-19.2	20.6±1.1 (27) 18.5-22.9	? (7) 17.8-20.5	17.19±2.15 (5) 15.0-19.67
Breadth of rostrum (BR)	10.3±0.6 (16) 9.2-11.5	9.8±0.4 (3) 9.4-10.2	9.4±0.4 (4) 8.9-9.9	10.4±0.6 (7) 9.7-11.7	10.9±0.7 (27) 9.5-12.0	? (7) 12.8-14.7	9.2±1.04 (5) 8.5-10.83
Breadth of braincase (BBC)	20.8±0.6 (16) 19.9-22.5	19.8±0.6 (3) 19.3-20.4	20.7±0.9 (4) 19.6-21.5	20.9±0.3 (7) 20.4-21.2	20.4±0.5 (27) 19.4-21.2	? (7) 19.5-21.2	21.43±0.52 (5) 20.77-22.04
Height of braincase (HBC)	15.2±0.5 (16) 14.5-16.4	14.8±0.0 (2) -	14.8±0.6 (4) 14.2-15.6	15.8±0.4 (6) 15.4-16.4	15.2±0.5 (27) 14.3-16.2		15.49±0.46 (5) 14.96-16.04

Measurement	NE India, Assam, and Northern Burma*	Northern Thailand*	Northern Laos*	Northern Vietnam***	Peninsular Thailand and Malaya*	Ps.muong bangensis Muong Bang Type series***	Ps.muong bangensis Muong Bang (our data)
Breath of zygomatic plate (ZBP)	5.8±0.5 (16) 5.0-6.5	5.2±0.2 (3) 5.0-5.4	5.4±0.4 (4) 5.0-5.9	5.6±0.4 (7) 5.0-6.0	5.5±0.4 (27) 4.9-6.4		5.46±0.23 (5) 5.25-5.83
Length of diastema (LD)	16.0±0.9 (16) 15.4-18.2	16.3±0.6 (3) 15.6-16.7	16.1±0.9 (4) 15.1-17.2	16.8±0.7 (7) 16.0-17.8	17.6±1.1 (27) 15.4-19.9	? (7) 15.2-16.7	14.23±1.34 (5) 13.0-16.7
Postpalatal length (PPL)	21.2±0.7 (6) 20.0-21.9	19.1±0.1 (2) 19.0-19.1	19.3±0.7 (3) 18.7-20.1	22.0± 0.0 (1) _	21.2±1.1 (27) 19.2-23.7	21.2±1.1 (27) 19.2-23.7	18.06± 1.22 (5) 16.5-19.5
Length of incisive foramina (LIF)	10.9±0.5 (16) 10.2-11.8	9.5± 1.1 (3) 8.5-10.6	9.9±0.3 (4) 9.5-10.1	10.6±0.5 (7) 9.7-11.2	10.4±0.5 (27) 9.6-11.3	? (7) 10.5-11.2	10.03 ± 0.53 (5) 9.33-10.67
Breadth of incisive foramina (BIF)	4.0±0.3 (16) 3.5-4.5	3.3±0.2 (3) 3.1-3.5	3.4±0.2 (4) 3.2-3.6	3.7±0.3 (7) 3.4-4.2	3.8±0.3 (27) 3.3-4.4	? (7) 3.4-3.9	3.13±0.42 (5) 2.83-3.67
Length of palatal bridge (LBP)	9.8±0.4 (16) 9.3-10.9	9.9±0.6 (3) 9.6-10.6	10.1±0.7 (4) 9.3-10.7	10.2±0.6 (7) 9.5-11.2	11.1±0.8 (27) 9.6-12.4		8.77±0.54 (5) 8.17-9.33
Breadth of palatal bridge at M1 (BBP)	5.0±0.4 (16) 4.3-5.5	4.9±0.6 (3) 4.4-5.5	4.5±0.2 (4) 4.2-4.5	4.8±0.2 (7) 4.5-5.1	5.1±0.3 (27) 4.5-5.7		4.6±0.40 (5) 4.17-5.17
Breadth of mesopterygoid fossa (BMF)	3.7±0.2 (16) 3.4-4.1	3.6±0.3 (3) 3.2-3.8	3.3±0.3 (3) 3.0-3.5	3.8±0.2 (7) 3.5-4.2	3.7±0.4 (27) 3.0-4.5		3.6±0.25 (5) 3.33-3.83
Length of bulla (LB)	7.8±0.3 (15) 7.2-8.2	7.1±0.6 (3) 6.5-7.6	7.6±0.2 (4) 7.4-7.7	7.5±0.3 (7) 7.3-8.0	7.0±0.3 (27) 6.2-7.3	? (7) 8.0-8.7	8.41±0.29 (5) 8.0-8.67
Alveolar length of M 1-3 (CLM1-3)	9.9±0.3 (16) 9.4-10.6	9.0±0.6 (3) 8.8-9.2	9.6±0.4 (4) 9.1-10.1	9.8±0.4 (7) 9.2-10.6	9.8±0.4 (27) 9.1-10.5	? (7) 9.5-10.2	9.38±0.19 (5) 9.06-9.55
Breadth of M1	2.8±0.1 (15) 2.7-3.0	2.6±0.2 (3) 2.5-2.9	2.8±0.1 (4) 2.7-3.0	2.8±0.1 (7) 2.7-2.9	2.7±0.1 (25) 2.5-3.0	? (7) 2.6-2.8	

Legends: Mean and standard deviation, number of specimens in parentheses, and observed range are listed for each measurement. Most peculiar skull measurements are stressed by gray.

* From Musser and Newcomb (1983).

** From Lunde and Nguyen Truong Son (2001).

*** From Tran *et al.* (2009).

Phylogenetic trees constructed by NJ and ME algorithm based on both individual genes (Cyt*b* and COI mtDNA genes and IRBP gene) and generalized sequences comprising all three genes had the same topology being different only in bootstrap values for some nodes (Figure 3). Can be seen on that samples originated from Muong Bang village clustered together with *Berylmys bowersi* ones descended from Northern Thailand (see Page *et al.*, 2010 for exact samples locations) forming entire pool. It should be noted also, that our samples, which were collected from very closely situated localities are not even construct monophyletic clade. Instead of grouping together they proved to be form very homogenous united clade (intragroup *d*'s are 0.011, 0.010. 0.007 for Cyt *b*, COI and IRBP genes respectively) combining all the samples. Means interspecies genetic distances *d*'s between samples of "*Pseudoberylmys*" and *B. bowersi* was proved to be equal only 0.035, 0.018, 0.009 for Cyt *b*, COI and IRBP genes respectively with interspecies ones between *Berylmys* species being 0.135, 0.082, 0.059 respectively. The facts allows us to decide that the population is not merit to have not only different genus or species taxonomical status, but even subspecies cannot be separated. Instead of more or less evident geographic subdivision, which may well be expected for morphologically divergent populations of *Berylmys bowersi*, we can only admit rather great intraspecific genetic unity within the species.

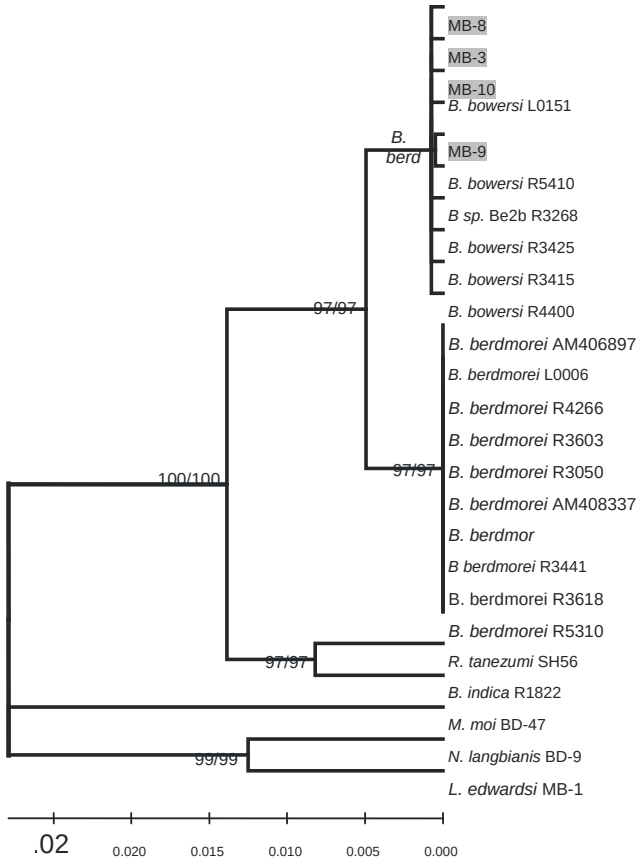

Figure 3: **Phylogenetic position of the samples**

Legends: Generalized (IRBP, Cyt b, COI genes) phylogenetic tree (ME/NJ methods) for currently genotyped *Berylmys* species and relative genera. Bootstrap values for ME/NJ algorithms respectively are given in nodes, value less than 50 are ignored. Samples identifiers are indicated in the names, see GenBank numbers information in Material and Methods for details of samples location. Original samples are stressed by gray.

Discussions

Thus, based on both traditional morphological and genetic data we can conclude that there is not any reason to elevate taxonomical status of strip-bellied rats from Muong Bang commune, Son La province not even to new genus but also to subspecies. Subsequently, the taxon name *Pseudoberylmys muongbangensis*, cannot be treated as valid and have to be discarded. Nevertheless, the questions still exist to be clean out about the only peculiar mark of the morph, namely, well-shaped glandular band on the belly existing onto every trapped individual. It should to drawn attention that already Musser and Newcomb [4] have indicated that abdominal glands are prominent in all sexually adult males in all four species of *Berylmys*, each have a cutaneous glandular area along the midline of the stomach and inguinal region. The gland is conspicuous and nearby fur is stained by its sebaceous secretions. Rudd (1966b, p. 332) [8] noted similar glands on adult *B. bowersi* from Malaya and wrote that one male "with very large testes had the darkest stained area observed in any species the area measured 5 x 67 mm, which is in range of our data, 3-5 x 43-72 mm. We can only add to this notice that the glandular strip (without prominent sebaceous secretions) also can be seen on young adult individuals in Muong Bang population, but being less prominent. We think that it may be caused by special physiological status of animals during the season the study being held. It is needles to stresses here ones more that any morphological feature caused by physiological status of the animal may not be considered as taxonomically relevant. It should be bear it in mind when any strange or unusual morpha are being described as a new or better to say pseudo-new taxa. Detailed comparative investigation of craniological materials and taxonomical descriptions of former established species along with genetic sampling are strongly recommended when any new morpha are being discovered in the nature before these findings are being described as new taxa.

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THỰC TRẠNG VỊ TRÍ PHÂN LOẠI LOÀI VÀ GIỐNG CHUỘT MỚI *PSEUDOBERYLMYS MUONGBANGENSIS* THU THẬP Ở SƠN LA, VIỆT NAM; CÓ THỰC SỰ LÀ MỚI KHÔNG?

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TÓM TẮT

Những bàn luận về đặc điểm phân loại giống và loài mới của của loài chuột *Pseudoberylmys muongbangensis* đã được mô tả dựa trên cơ sở nghiên cứu đặc điểm hình thái và di truyền học phân tử của các gen ty thể và gen nhân các mẫu thu được tại khu vực nghiên cứu. Thực tế cho thấy đơn vị phân loại sinh vật là con người đặt ra và đương nhiên không thể thoả mãn cả điều kiện về đặc điểm hình thái và di truyền của sinh vật. Đặc điểm hình thái loài chuột thu được là một trong các kiểu hình của các quần thể chuột *Berylmys bowersi* ở các phân vùng địa lý khác nhau, đôi khi còn không được coi là phụ loài độc lập. Đặc điểm duy nhất nổi bật ở loài này có thể thấy là có vạch ở phần bụng không những ở các cá thể đực và cái trưởng thành (giống như những loài *Berylmys* khác) mà còn ở cả những cá thể chuột chưa trưởng thành. Tuy nhiên, đặc điểm hình thái này có thể liên quan đến tình trạng sinh lý của động vật và không nên coi là có giá trị trong phân loại.