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A New Species of *Murina* from China (Chiroptera: Vespertilionidae)

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Abstract

Three tube-nosed bats (one male and two female) were captured during Chiroptera investigation at the Maolan National Nature Reserve in Libo County, Guizhou Province in July 2016. It was identified as a new species through the morphological and molecular phylogenetic analysis. The new specie differ morphologically, morphometrically, and genetically from previously described species of Murina. Morphological differences include pelage color, size, skull shape and tooth morphology. Analysis of mitochondrial DNA barcodes of the Cytb gene supports valid species status based on divergent phylogenetic lineages. This species has a higher similarity with *Murina cyclotis* and *Murina peninsularis* in morphology, however, there are obvious difference in skull morphlogy and phylogenetic relationship among these three species. It is a new species of Murina, and named *Murina liboensis sp.nov*.

Keywords: new species, murina, morphometrics, China, Chiroptera

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INTRODUCTION

Murina species number is still considered to be an underestimate, while have high cryptic diversity within this group (Francis et al. 2010), the greater use of harp traps in forest environments (Csorba et al. 2011), coupled with more rigorous taxonomic analysis and genetic studies, support this conclusion. So far, the 39 species of which have been found around the world. Until to Simmons (2005), the 18 tube-nosed species distribute. In the last years there were 21 species of which have been described, include: Murina harrisoni (Csorba and Bates 2005), Murina tiensa (Csorba et al. 2007), *Murina harpioloides* (Kruskop and Eger 2008), *Murina eleryi* (Furey et al. 2009), *Murina bicolor*, *Murina gracilis*, *Murina recondita* (Kuo et al. 2006, 2009), *Murina beelzebub*, *Murina cineracea*, *Murina walstoni* (Csorba et al. 2011), *Murina jaintiana*, *Murina pluviallis* (Ruedi et al. 2012), *Murina chrysochaetes*, *Murina lorelieae*, *Murina shuipuensis* (Eger and Lim 2011), *Murina annamitica* and *Murina fionae* (Francis and Eger 2012), *Murina balaensis* and *Murina guilleni* (Soisook et al. 2013), *Murina fanjingshanensis* (He et al. 2015), *Murina rongjiangensis* (Chen and Liu 2017). The total number of the Murina species recorded the around world is increasing rapidly such that to date. Currently, 18 of the 39 species of Murina currently known to science are from China Asia, However, there are only 6 species of them have been described in guizhou province, respectively: *Murina aurata, Murina leucogaster, Murina eleryi, Murina shuipuensis, Murina fanjingshanensis, Murina rongjiangensis*. Since Guizhou is a typical karst landform, we can assume that species of tube-Nosed Bat has been underestimated.

In 1992, Corbet and Hill first divided the genus Murina into two groups: '*suilla-group*' and '*cyclotis-group*' (Corbet and Hill 1992), based on the relative size of the crown area of canines, the first and second upper premolars and the position of the incisors (Corbet and Hill 1992, Csorba and Bates 2005, Csorba et al. 2007, 2011, Furey et al. 2009, Koopman 1994, Matveev and Csorba 2005). According to these characteristics, Chen and Liu has divided about 12 species into two species group in subsequent studies in 2017 (Chen and Liu 2017). The new specie belongs to '*cyclotis-group*' based on there description of morphologically, morphometrically and genetically.

MATERIALS AND METHODS

Bats were captured in the field using birdnet in combination with several ecotone mist nets. Bats were then measured, photographed, and initially identified following the field guides (Borissenko and Kruskop 2003, Francis 2008). All captured bats were adults as confirmed by the presence of fully ossified

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Table 1. External, cranial, and dental measurements (in mm, except mass in g) among the three M.species

	M.liboensis sp.nov.							*M.cyclotis*				*M. peninsularis*			
	N2016072	N2016072	N2016072	N2016072	N2016072			36♂		40♀		23♂		19♀	
	7021♂	7022♀	7023♂	7024♂	7025♀	range	average	range	average	range	average	range	average	range	average
MASS	4.50	5.00	4.80	5.00	5.50		4.96		5.0, 5.8, 2		6.1, 1	5.5-8.3	7.00	8.5-11.9	9.70
HBL	37.73	42.57	39.52	41.19	44.54	37.73-44.54	41.11	38.7-46.4	42.3±2.3	41.1-50.0	45.10	39.9-50.1	46.60	42.0-55.1	49.90
TL	31.54	36.66	33.02	34.12	38.12	31.54-38.12	34.69	26.2-39.0	34.90	32.0-41.1	37.40	32.4-42.8	38.70	38.5-46.0	42.30
FA	28.16	32.28	28.87	30.98	32.64	28.16-33.64	30.59	29.4-33.0	30.70	31.6-36.8	33.90	33.8-38.1	35.70	34.5-39.4	37.70
TIB	16.14	17.53	16.37	16.75	18.85	16.14-18.85	17.13	14.5-19.3	17.60	17.3-20.3	18.80	18.2-21.6	19.50	17.6-21.0	19.30
HFL	5.14	6.30	5.31	6.16	6.43	5.14-6.43	5.87	6.5-8.8	7.90	7.0-9.7	8.30	5.6-9.1	7.80	7.1-10.0	9.00
EL	11.52	13.84	12.19	12.92	14.37	11.52-14.37	12.97	12.0-17.6	14.00	12.7-16.0	14.50	11.9-18.8	14.20	13.0-17.0	15.10
EW	9.12	10.04	9.75	10.87	11.57	9.12-11.57	10.27								
TRL	6.16	7.92	6.52	7.03	8.39	6.16-8.39	7.38	6.8-9.3	8.10	5.8-9.2	7.90	7.4-10.3	8.40	7.6-9.0	8.30
TRW	1.73	1.85	1.77	1.81	1.96	1.73-1.96	1.83								
PLL	91.56	98.21	95.38	99.62	101.32	91.56-101.32	97.22								
PW	46.37	51.32	48.37	50.69	53.12	46.37-53.12	50.38								
2DM	27.42	30.13	28.64	29.56	31.73	27.42-31.73	29.50								
3DM	28.75	31.71	29.83	30.74	32.54	28.75-32.54	30.26	26.5-32.4	29.20	30.4-34.9	32.00	32.0-37.6	34.00	35.7-37.7	36.50
3D1P	12.56	14.24	13.87	13.91	14.31	12.56-14.31	14.08	12.8-14.5	13.60	13.7-16.2	14.80	14.5-17.6	15.90	15.4-18.2	17.10
3D2P	11.21	13.44	11.90	13.07	14.87	11.21-14.87	12.90	11.1-14.1	13.00	12.7-15.4	14.40	13.8-16.8	15.10	15.5-16.4	15.80
4DM	28.32	30.18	29.07	30.14	31.74	28.32-31.74	29.89	24.7-31.9	28.30	29.7-34.4	31.40	29.9-35.4	32.70	34.0-36.4	35.10
4D1P	8.67	9.95	10.35	9.75	10.38	8.67-10.38	9.82								
4D2P	6.11	7.66	6.81	7.54	8.21	6.81-8.21	7.80								
5DM	25.00	31.59	27.41	29.77	32.15	25.00-32.15	29.04	25.1-31.8	29.00	30.8-35.1	32.30	31.3-36.8	33.30	35.0-36.5	35.60
5D1P	9.43	10.25	8.91	9.53	10.78	9.43-10.78	9.84								
5D2P	6.71	8.36	7.55	8.42	8.82	6.71-8.82	8.53								
GLS	15.54	16.92	16.3	16.52	16.97	15.54-16.97	16.45	15.86-17.08	16.47	16.60-18.18	17.21	17.39-18.52	17.79	17.59-19.33	18.70
CBL	10.16	13.35	10.87	11.54	13.85	10.16-13.85	11.95	14.00-15.67	14.97	14.95-16.86	15.85	15.68-16.91	16.06	16.11-17.69	17.11
CIL	12.17	14.36	13.04	13.76	14.52	12.17-14.52	13.57	13.60-15.12	14.45	14.34-16.17	15.22	14.90-16.41	15.52	15.53-16.89	16.40
PL	5.15	6.60	5.74	6.27	6.78	5.15-6.78	6.35								
ZW	7.75	9.42	8.53	9.32	9.76	7.75-9.76	9.06	8.78-10.05	9.36	9.33-10.43	9.84	9.76-11.31	10.36	10.12-11.22	10.80
MW	5.02	6.73	5.68	6.53	7.12	5.02-7.12	6.22	7.11-8.48	7.90	7.64-8.58	8.20	8.32-9.39	8.74	8.08-9.62	9.02
BCB	6.32	7.67	7.03	7.45	7.78	6.32-7.78	7.20	7.16-8.10	7.64	7.40-8.17	7.71	7.72-8.48	8.12	7.70-8.58	8.22
BCH	5.84	7.51	6.73	7.46	7.75	5.84-7.75	7.06	6.08-7.22	6.49	6.10-7.21	6.50	6.79-8.22	7.32	7.10-8.37	7.48
IOW	3.17	4.07	3.62	3.84	4.82	3.17-4.82	3.62	3.92-4.48	4.17	3.99-4.52	4.25	4.31-4.97	4.57	4.46-4.88	4.68
C-P^4	2.18	2.73	2.45	2.71	2.85	2.18-2.85	2.58	2.21-2.96	2.66	2.21-3.11	2.76	2.73-3.30	2.99	2.14-3.36	2.90
CM^3	4.26	5.39	4.97	5.13	5.58	4.26-5.58	5.07	5.12-5.68	5.41	5.06-6.00	5.61	5.52-6.09	5.76	5.68-6.39	6.07
M^2-M^2	4.54	5.28	4.64	5.36	5.64	4.54-5.64	5.21								
M^3-M^3	4.19	5.41	4.68	5.32	5.75	4.19-5.75	5.17	5.07-5.79	5.39	5.18-6.05	5.57	5.45-6.22	5.72	5.69-6.20	5.94
C^1-C^1	3.04	3.99	3.47	3.78	4.16	3.04-4.16	3.73	3.73-4.27	4.00	4.00-4.68	4.25	4.28-5.28	4.66	4.46-5.26	4.97
ML	9.67	11.63	10.24	11.53	12.28	9.67-12.28	11.28	10.52-11.68	11.17	11.32-12.78	11.86	11.25-12.92	11.92	12.09-13.59	12.75
CM_3	4.84	5.74	5.29	5.53	5.94	4.84-5.94	5.51	5.57-6.18	5.84	5.75-6.49	6.11	5.94-8.02	6.31	6.28-6.94	6.55
POC	3.10	3.66	3.12	3.47	3.71	3.10-3.71	3.41								
HCP	3.74	4.67	3.89	4.15	4.83	3.74-4.83	4.28	3.77-4.60	4.14	4.16-5.30	4.71	4.30-5.33	4.86	4.72-6.08	5.51

metacarpalphalangeal joints. During Chiroptera investigation at the Maolan National Nature Reserve in Libo County on July 27, 2016. Five unknown Murina specimens were captured in an abandoned cave where the vegetation was mainly evergreen broad-leaved forests and thickets. The specimens ID numbers are GZNU20160727021 (male), GZNU20160727022 (female), GZNU20160727023 (male), GZNU20160727024 (male), GZNU20160727025 (female), respectively. External measurements were taken when captured, and craniodental measurements were taken in the laboratory (Basic measurements of craniodental was cited in Yang et al. (2007)). Detailed analysis of variance was carried out among the similar species. These species differ morphologically, morphometrically, and genetically from previously described species of Murina. Contrast with other species for craniodental, the new species belongs to '*cyclotis-group*'. Holotype specimen is deposited at the animal specimen room of School of Life Sciences, Guizhou Normal University.

MEASUREMENTS

External measurements and Craniodental measurements were both taken with a digital caliper (to the nearest 0.01 mm). The definitions of measurements follow Bates and Harrison (1997), Yang et al. (2007), Csorba et al. (2011), Francis and Eger (2012) unless otherwise stated. All measurements are in mm except for MASS which is in grams.

Abbreviations for External and Craniodental Measurements Data

External and craniodental measurements (**Table 1**) were taken from a living bats or the animal specimen room specimens to the nearest 0.01 mm. Mass-the weight of bat in gram; all the following measurements were based primarily on Eger and Lim (2011) or Chen and Liu (2017).

External Measurements Data and Craniodental Measurements Data

External measurements include: Head and body length (HBL); tail length (TL); forearm length (FA); length of tibia (TIB); hind foot length (HFL); ear length (EL); ear width (EW); length of tragus (TRL); width of tragus (TRW); plagiopatagium length (PLL);

Fig. 1. External morphology of face mask (a), ventral (b), dorsal (c), and hind foot (left) (d) pelage of the holotype of M.liboensis sp.nov (GZNU 201607270222♀)

plagiopatagium width(PW); 2DM, 3DM, 4DM, 5DM, 3D1P, 3D2P, 4D1P, 4D2P, 5D1P, 5D2P. **Cranial measurements include:** greatest length of skull (GLS); condylobasal length (CBL); condyloincisive length (CIL); palatal length(PL); zygomatic width (ZW); mastoid width (MW); braincase width (BBC); interorbital width (IOW)l; length of maxillary toothrow (C-M^3); upper canine-premolar length (C-P^4); width across upper molars (M^2-M^2); greatest width across the upper molars (M^3-M^3); C1-C1; greatest length of mandible (ML); length of mandibular toothrow (C-M_3); postorbital constriction (POC); height of coronoid process (HCP); lacrimal width (LW).

Isolation of the Cytb Gene and Construct the Phylogenetic Tree

The 0.5g fresh tissues was isolated from muscle and stored in 95% ethanol. Amplification and sequencing of the Cytb molecular marker was carried out in Beijing Jinnuoruijie Gene Technology Co., Ltd. DNA star software was used for stitching and proofreading of the nucleotide sequences. The complete mitochondrial cytochrome b (Cytb), the reaction volume of PCR and the touchdown PCR reaction. ***Construct the phylogenetic tree:*** Download Cytb sequence of Murina from GenBank. Calculated the genetic distance and reconstruct phylogenetic relationships (Bayes tree) through MEGA 6 and MrBayes 3.2.1, respectively (Chen and Liu 2017), with the Myotis muricola as outer group. There methods cited from Chen and Liu (2017).

Principal Component Analysis

The differences in shape were displayed using principal component analyses (PCA) and multivariate regressions along axes (Monteiro 1999) by R computational. The first PCA axes obtained from each of the three datasets were extracted and then combined in a new PCA in order to synthesize our different results.

RESULTS

Type specimens. ***Holotype.*** An adult female, GZNU20160727022 (female), with skin and skull, kept in anhydrous ethanol. Collected in Libo County, Guizhou Province on July 27, 2016 (**Fig. 1**). (686 m, 25°13′13.54″N, 108°01′51.89″E) in an abandoned cave (wenggen). Measurements (in mm) and body mass (in g) including mandible.

Paratypes. GZNU20160727021 (male), GZNU20160727023 (male), GZNU20160727023 (male), GZNU20160727025 (female), were kept in anhydrous ethanol. Collected on July 27, 2016.

Diagnostic characteristic. This is a smaller Murina with a FA, TL, GLS, CBL, CIL. The first upper incisor (I^2) and second upper incisor (I^3) are bicuspidate, with the secondary cusp posterior to the primary. The two tips of the I^2 looks very obvious, the outer tip is almost equal height with the inside tip. The height of the outer tip of the I^3 is equal height with the I^2 of inside tip, and about two times of itself inside tip, the inside tip is slightly dull (**Fig. 2**). Two upper incisor look from the side only the I^3 can be seen, and I^3 is 1/3 of the canine in hight. The braincase is hemispherical and relatively low with analysis of the BCH and BCB. Forehead of the neurocranium has a deep pronounced rostrum which slopes gently and slightly concave (**Fig. 3d**). The snout is wide, recessed into the ditch between the nasal obvious (**Fig. 3b**). Zygomatic arch is slender, have a distinct rise in the middle, followed by the zygomatic arch gradually become thinner. On the palate inverted front end, back-end with 2 notch. The upper tooth column is shorter than the lower tooth column.

Fig. 2. Front view of upper incisors

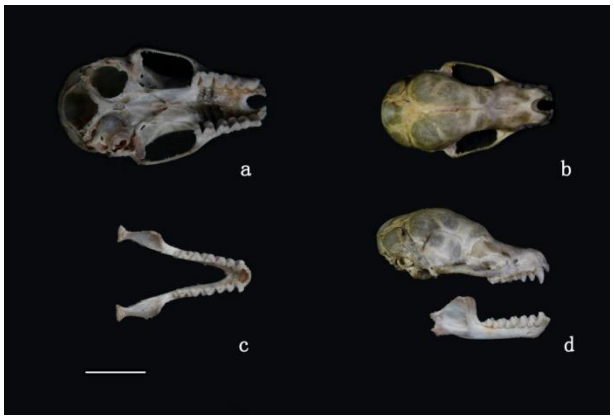

Fig. 3. Skulls of the M.liboensis sp.nov., bventral view of cranium (a), dorsal view of cranium (b), front view of mandible (c) and lateral view of cranium (d), from Guizhou China. Scale = 5 mm

The dorsal pelage is fluffy with brownish grey and the tip golden yellow, the pelage extends onto the tail membrane and the hind feet. The ventral pelage is relatively short, pale grayish black basally and ash grey at the tips but with more brown near the chin and on the side of the abdomen. The ears is ovate-orbicular, the anterior border of the ear is dull circle and without an indentations on the posterior border of the pinna. The insertion point of the plagiopatagium close to the 1/2 of the 1st toe (**Fig. 1**).

Description. The dorsum is fluffy by underfur and guard hair, the color of whole body is brownish grey (**Fig. 1c**). The underfur is short and gray. The base of guard hairs is french gray to gray in the middle place, 1/3 the tip golden yellow, thus the whole back is brownish grey. Ventral fur short than dorsum, the base of hairs is dark gray to gray in the middle place, the tip ash grey, thus the whole back is smoky gray. The nostrils of the new species looks like short tubular, open out to the side, and the areas of from nose to forehead has significantly raised. The TRL is slightly exceed the 1/2 of ear length. The 3 metacarpal are almost in the same length: 3DM>5DM>4DM>2DM. Each plagiopatagium is black brown in colour, forearm and the base of the plagiopatagiumbase covered with densely yellow-brown fluff on both of the dorsal and ventral surfaces. The back of the interfemoral membrane is covered with hair, the tip of hair is golden yellow, in accordance with the same color of the back, and obvious hair edge at the distal part. The last coccygeal vertebra exposes the interfemoral membrane. The dorsal of foot and toe are also covered by golden yellow hair, consistent with the back coat color (**Fig. 1**).

The external measurements data was shown in **Table 1**.

In the Skull

The skull is robust with the domed shape, the sagittal crest and the lambdoid crest exists but is not significant. In lateral view, the profile from the posterior part of the rostrum to the anterior part of the braincase exhibits a deep pronounced concavity (**Fig. 3**). The M^3-M^3 and C^1-C^1, which is 72.55% and 72.35% of the M^3-M^3. The C-M^3 and the C-P^4, which is 45.04% and 47.88% of the C-M^3. The 1st upper incisor (I^2) and 2nd upper incisor (I^3) are bicuspidate, with the secondary cusp posterior to the primary. In front view, the two tips of the I^2 looks very obvious, the outer tip is almost equal height with the inside tip, however only the outer tip can be seen from lateral view (**Fig. 2**). The height of the outer tip of the I^3 is equal height with the I^2 of inside tip is 1/3 of the canine in hight, and about two times of inside tip, the inside tip is slightly dull. Both of the upper canine and the twos upper premolars are stout almost vertical down, the hight of canine exceed the tows upper premolars, the size is double than the latter. The height of the I^2 and I^3 is almost equal height, nearly flush with the canine tooth. The size of the 1st upper molar (M^1) is almost same size to the 2nd upper molar (M^2). The 3rd upper molar (M^3) is less than 1/2 of the M^1 in size, only a row of sharp teeth were left.

In mandible, Analysis of the ML and C-M_3, the length of the lower teeth row is longer than the upper teeth row, each incisor crown trifoliate, the 1st to the 3rd incisors increased in size slightly. The lower canine is higher than the 1nd lower premolar, both of the 1nd lower premolar and 2nd lower premolar are stout. The size of the 1st lower premolar size is slightly smaller than the 2nd lower premolar, but the 1st lower premolar is higher from lateral view (**Fig. 3**). The lower

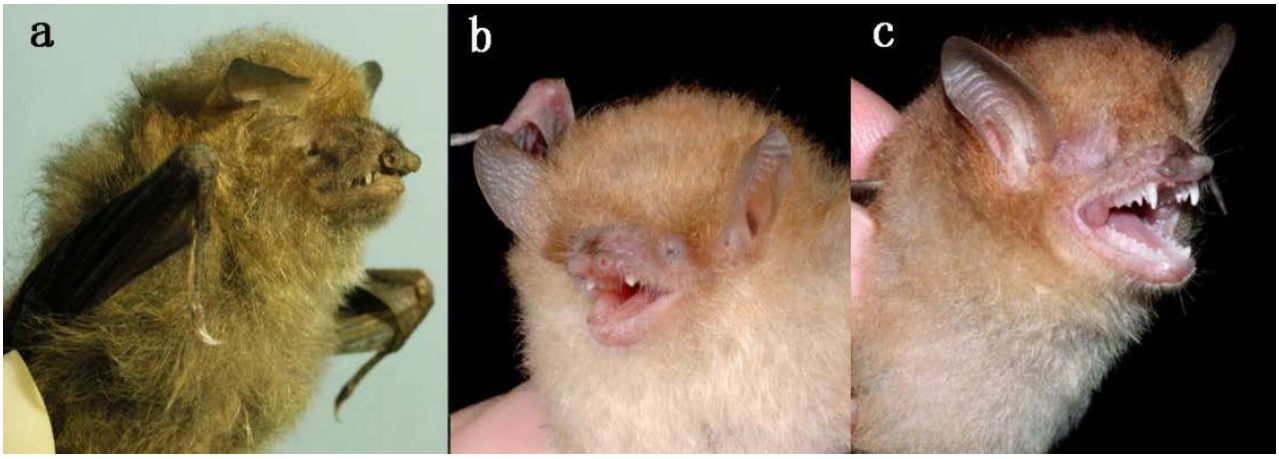

Fig. 4. Three Murina species: (a) M.liboensis sp.nov(GZNU 201607270229) (paratype), from Guizhou China, (b) M. cyclotis, Y, PSUZC-MM 2006.179, (c) M. peninsularis, Y, PSUZC-MM 2012.12. Cited in Soisook et al. (2013) (b and c). Not to scale

coronoid of mandible is develpment, the lower canine is $M_1 > M_2 > M_3$.

Tooth type: 2.1.2.3/3.1.2.3=34 (**Fig. 3**).

Etymology: Because the species was collected from Libo county, hence we name the new species as libo tube-nosed bat (Murina liboensis sp. nov.). English name is Libo Tube-nosed bat.

Molecular Phylogenetic Analysis

Through Mage6 analysis, in *M. liboensis sp. nov.*, the Cytb sequence was at 1041 bp, in which T = 31.4%, C = 25.3%, A = 29.3%, and G = 14.0%. Between the new species and *M. cyclotis* the genetic distances was 0.0770 in Cytb gene, which were smaller than the genetic distance among species of other categories. The genetic distance among Murina ranged from 0.0088 to 0.2244 in Cytb, the minimum genetic distance between *Murina leucogaster* and *Murina sp. GGJ-2006*, was 0.0088.

According to Bayesian consensus tree, the *M. liboensis sp. nov.* is clearly placed sister to *M. cyclotis* with 100% bootstrap or posterior probability, then is placed sister to *M. peninsularis* with 100% bootstrap or posterior probability, however *M. peninsularis* just one separate clade from other two species, *M. peninsularis* and *M. cyclotis* were clustered as one clade with posterior probability 100% (**Fig. 6**). The genetic distances between *M. liboensis sp. nov.* and *M. cyclotis*, *M. peninsularis* were greater than the minimum inter-specific distance of Murina.

Principal Component Analysis

Fig. 7 shows the PCA constructed using the first PCA axes obtained from each of the three datasets corresponding to lateral and occlusal views of mandible, and lateral and ventral views of cranium (**Fig. 5**). The first PCA axis tends to separate *Murina cyclotis* from *Murina peninsularis*. The differences in shape variation are mainly explained by the lateral view of the cranium (correlation: 0.39) and the front views of the mandible (correlations of 0.39). The second PCA axis tends to separate *M. liboensis sp. nov.* and other two Murina. The differences in shape variation are mainly explained by the ventral view of cranium (correlation: -0.86) and the lateral view of the cranium (correlation: 0.47). The results show therefore that the new species can be distinguished from *Murina cyclotis* and *Murina peninsularis* on the basis of their skull shape.

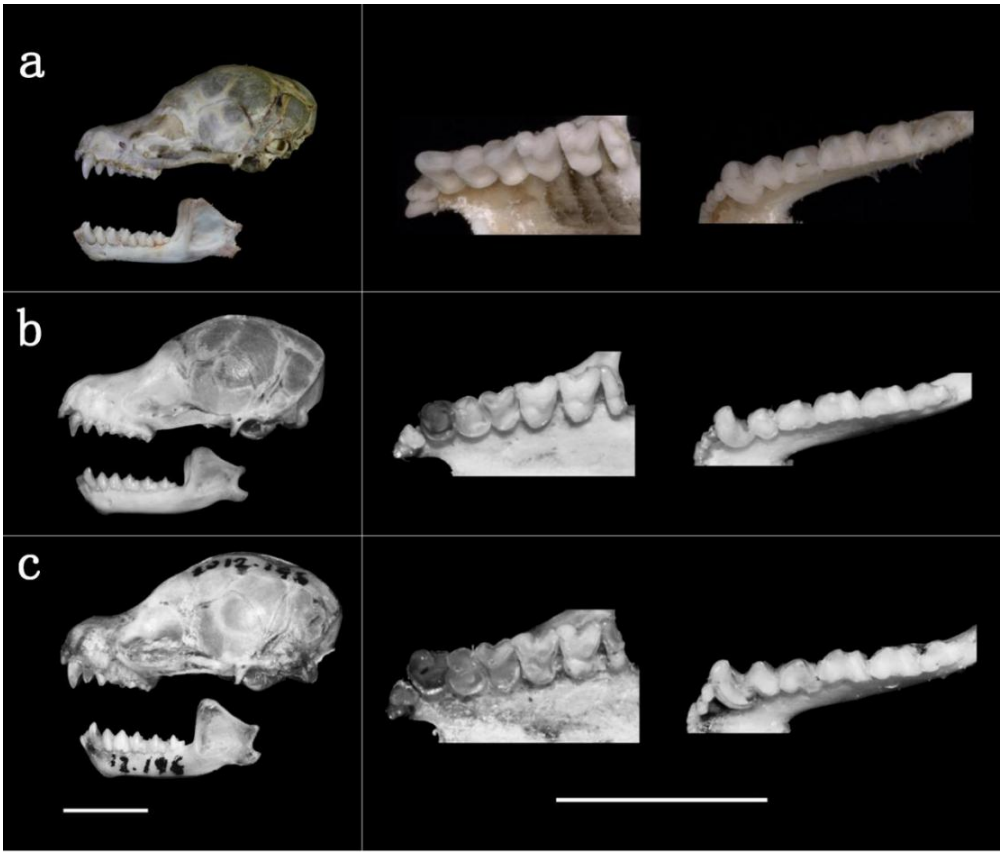

Fig. 5. Lateral view of the skulls of three Murina species (left), occlusal view of center upper (left of each pair) and right lower dentition (right of each pair) of three species of Murina: (a) M. liboensis sp. nov.,from libo China., (b) M. cyclotis, (c) M. peninsularis, Cited in Soisook et al. (2013) (b and c). Scale = 5 mm

DISCUSSION

The data on the morphological measurement showed that the size of *M. liboensis sp. nov.* is smaller than *M. cyclotis* (Dobson 1872) and *M. peninsularis* (Hill, 1964), likewise, existed significant differences in morphological data and molecular measurement data. *M. liboensis sp. nov.* is the smallest among these three species in body size, which dorsal and ventral color is different from other two murina species. The color of ventral of *M. liboensis sp. nov*. becomes darker gradually from chest to throat, abdominal. Like this hair color change is similar to *M. peninsularis* (**Fig. 4**). Furthermore, the craniodental measurements data also show that *M. liboensis sp. nov.* is smaller than *M. peninsularis.*

In the skull, The new species of the profile from the posterior part of the rostrum to the anterior part of the braincase exhibits a deep pronounced concavity from lateral view and the snout is wide, recessed into the ditch between the nasal obvious , but *M. cyclotis* and *M. peninsularis* is inapparent. The two tips of the *M. liboensis sp. nov.* first upper incisor looks very obvious, the outer tip is almost equal height with the inside tip. Both of the upper canine and the twos upper premolars are stout almost vertical down, the size is double than the latter. The height of the I^2 and I^3 is almost equal height, nearly flush with the canine tooth. The size of the 1st upper molar (M^1) is almost same size to the 2nd upper molar (M^2). All of the characteristics have described is obvious differences with *M. cyclotis* and *M. peninsularis* (**Fig. 5**).

The genetic distances of the gene marker (Cytb) indicate that the *M. liboensis sp. nov.* having a closest relationship with the *M. cyclotis* and *M. peninsularis*, however the genetic distance between the *M. liboensis sp. nov.* and other two species (*M. cyclotis* and *M. peninsularis*) were significantly larger than interspecific genetic distance of Murina. Therefore, the M. liboensis sp. nov. was identified as a new species through the morphology and the molecular evidence. Based on gene maker of Cytb, the phylogenetic tree show that the new species, *M. liboensis sp. nov.*, *M. cyclotis*, *M. peninsularis* are clustered into one clade (**Fig. 6**).

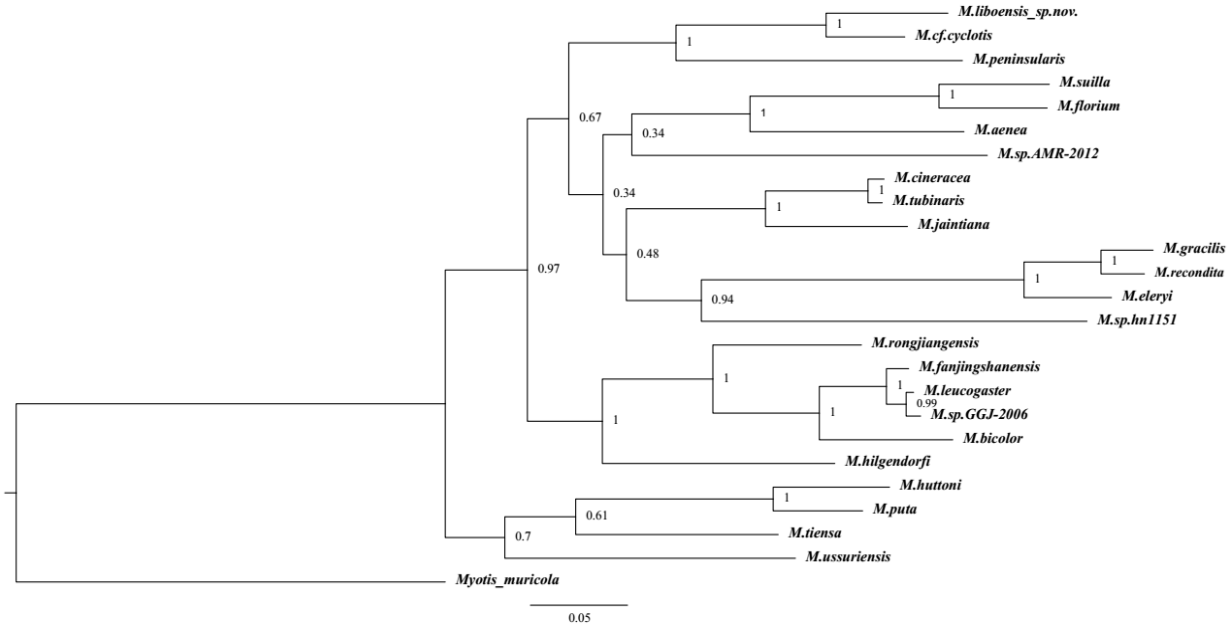

Fig. 6. Phylogenetic tree based on sequences on Cytb gene (A) gene among Murina, using the Bayesian methods. Number indicate posterior probability

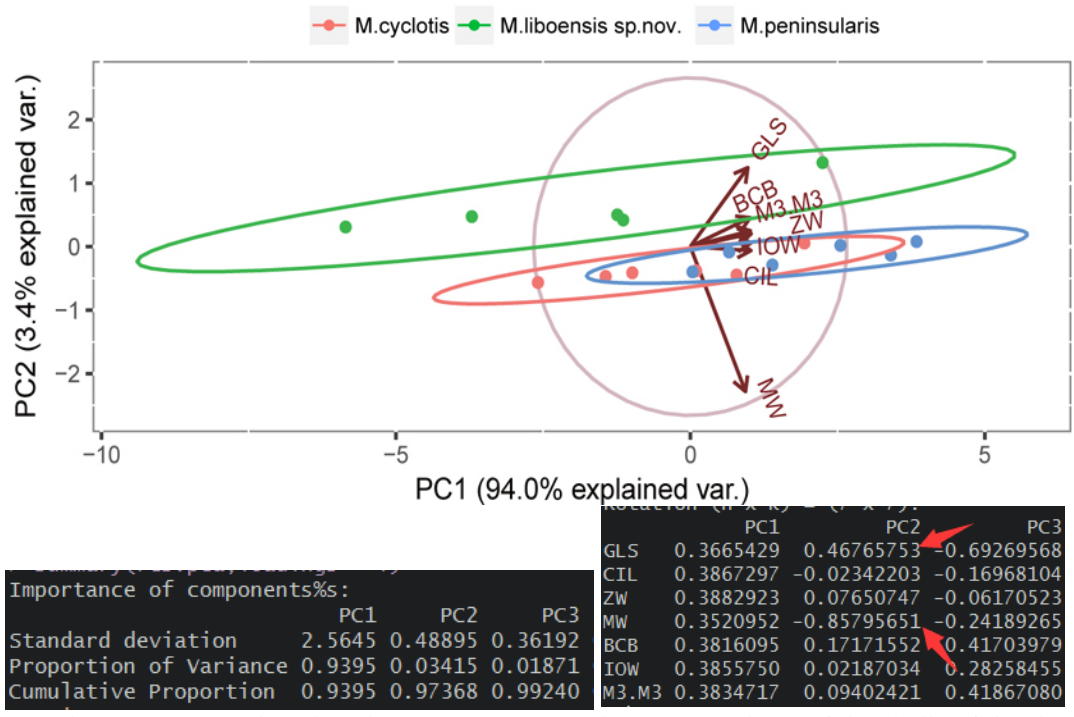

Fig. 7. Principal component analysis based on geometric morphometric analyses of the skulls of the three Murina species collected in 'cyclotis-group'

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