Short Note

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Discovery of *Kerivoula kachinensis* and a validity of *K. titania* (Chiroptera: Vespertilionidae) in China

https://doi.org/10.1515/mammalia-2021-0102
Received June 9, 2021; accepted November 10, 2021;

Abstract: In April 2019, 15 (10♂, 5♀) *Kerivoula* bats were collected by harp traps from Xishuangbanna, Yunnan Province, China. External and craniodental examination, multivariate statistical analyses and molecular phylogenetic inference (*CoI*, *Cytb* and *Rag2* gene markers) indicated they are *Kerivoula kachinensis* and *Kerivoula titania*, respectively. Former represents a new chiropteran record from China, while the latter is a valid occurrence of *K. titania* in this region because recent study indicate a misidentification of "*K. titania*" in Guangdong, Guangxi and Hainan, China. All specimens are presently preserved at Key Laboratory of Conservation and Application in Biodiversity of South China in Guangzhou University, Guangzhou, China. Nowadays, four woolly bats occur in China including, *Kerivoula furva*, *K. kachinensis*, *Kerivoula picta* and *K. titania*, whilst there is a risk of underestimation the actual species diversity in China region when comparing those of neighboring region such as Vietnam. Supports for field survey need to be continued in future.

Keywords: China; Chiroptera (bats); first record; *Kerivoula kachinensis*; *Kerivoula titania*; Yunnan.

The genus *Kerivoula* (Chiroptera: Vespertilionidae), that are commonly referred as woolly bats, contains 27 species ranging from Indomalaya-Australasia to Afrotropic ecozone (Bates et al. 2004; 2007; Francis et al. 2007; Kuo et al. 2017; Simmons 2005; Soisook et al. 2014; Tu et al. 2018; Yu et al. 2018; Wilson and Mittermeier 2019). Typically, these species are rare in collections since their apparent ability avoiding mist net (Francis 1989), which have contributed to poor understanding of their true diversity and distribution. Presently, only occurrence of *Kerivoula picta* and *Kerivoula furva* are confirmed in China, whilst the historical records of *Kerivoula hardwickii* [review in Yu et al. (2018)] from China are invalid. Strikingly, 5 of 8 *Kerivoula* species from Vietnam occur in the northern region (Kruskop 2013; Wilson and Mittermeier 2019), which is close to border region of China, such as Guangxi and Yunnan.

In April 2019, 15 woolly bats individuals were collected using two-band harp traps during field survey in Xishuangbanna Tropical Botanical Garden, Yunnan Province, China (101°29′58 E, 21°32′11 N, 1020 m a.s.l.). Sampling site is typical karst tropical humid region with a small stream nearby. All field survey and sample collection protocols complied with the current laws of Yunnan Province. We followed the guidelines of the American Society of Mammalogists (Sikes et al. 2016) for the care and use of animals. All voucher specimens were determined to be adults based on the degree of epiphyseal-diaphyseal fusion (Brunet-Rossinni and Wilkinson 2009). They were preserved in 75% ethanol and deposited at the School of Life Sciences, Guangzhou University, China. After the external and craniodental examination, multivariate statistical analyses and molecular phylogenetic inference (*CoI*, *Cytb* and *Rag2*), they were identified as *Kerivoula kachinensis* and *Kerivoula titania*, respectively (Voucher ID of *K. kachinensis*: GZHU 19123, 19178, 19179, 19195, 19196, 19199, 19309, 19310; Voucher ID of *K. titania*: GZHU 19154, 19161, 19175, 19176, 19177, 19303, 19304). The former represents the first record of China, while the latter is a valid record of *K. titania* in China. Their external, cranial measurements and body mass

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(MASS) were taken using digital calipers (GUOGEN, Serial number: 00000315) and an electronic scale (TANITA, 100 g) following the definitions of Yu et al. (2018) for further morphometric analyses.

Samples of *K. kachinensis* from Yunnan, China, are large-sized woolly bat with forearm lengths of 39.87–41.97 mm (Table 1). The dorsal fur is brown with the dark grey bases, grey-brown middle and light brown tips. The bases of the ventral hairs are dark grey, while the tips are slightly white and light brown (Figure 1A–C). The ears are large with a long pointed tragus. Skull is relatively large with a GTL of 17.01–17.84 mm, a GBB of 8.23–8.79 mm, and a BH of 5.19–5.68 mm (Table 1). Its skull is characteristically flattened and broad, with very scarcely concaved frontal profile. In the upper dentition, the second incisor (I^3) is compressed between I^2 and the canine (C) and is equal to I^2 in crown area (Figure 1D and E). The length and width of second premolar (P^3) are about equal. The third molar (M^3) has the metastyle absent. In the lower dentition, the premolars are compressed in the toothrow; the width of the first (P_2) exceeds its length; in the second (P_3) and third (P_4), the length slightly exceeds the width; the crown area of the second premolar (P_3) is about 90% that of P_2.

Individuals of *K. titania* from Yunnan, China, are medium-sized woolly bat with forearm lengths of 32.67–34.79 mm (Table 1). Dorsal fur is generally grey-brown with three banding, being black basally, light grey in middle and dark grey at tips; on the ventral pelage, the roots are light black and the tips whitish (Figure 1F–H). The ears are large with a long, narrow and pointed tragus. The skull has a GTL of 15.44–15.90 mm, GBB of 7.70–8.07 mm, and BH of 5.27–5.83 mm (Table 1). The braincase is broad, smooth and relatively flattened (Figure 1I and J). In the upper dentition, the first incisor (I^2) is unicuspid and with a cingulum on its internal posterior border. The canine (C) and is equal to I^2 in crown area. Transverse diameters (width) of three upper premolars slightly exceed their respective longitudinal diameters (length) (Figure 1). In the lower dentition, their lengths of three lower premolars exceed their widths. The crown area of the talonid of the first lower molar (M_1) slightly exceeds that of the second (M_2).

For morphological analyses, a principal component analysis (PCA) and a discriminant analysis of principal components (DAPC) (Jombart 2008; Jombart et al. 2010) were performed with inclusion of morphometric data from Kuo et al. (2017) and Yu et al. (2018), two the most comprehensive morphological matrices to date of *Kerivoula* taxa. Our matrix contains 163 specimens belonging to six *Kerivoula* species from Cambodia, China, India, Indonesia, Malaysia, Myanmar, Sri Lanka, Thailand, Vietnam (Figure 2). All analyses were conducted in the R base package and the R add-in package psych (Revelle 2013), ade4 (Dray et al. 2007), and adegenet (Jombart 2008). The principal component analysis (PCA) based on nine craniodental measurements revealed 90% of the total variance from first two principal components (PCs) (75% for PC1 and 15% for PC2, respectively. PC1 was positively loaded with all indexes, with high scores of loading scores for CCL, ZB and GBB (Table 1), which mainly reflected skull length and width. For PC2, all measurements had low loadings except for braincase height (BH) (Table 1). Scatter plots from PCA scores indicate *K. kachinensis* occur an explicitly larger skull but a regular braincase height, while *K. titania* is similar to *K. furva* in skull length and width but with higher braincase (Figure 2A). DAPC scatter plot show that the eight *K. kachinensis* individuals from Yunnan and other countries explicitly formed a separated cluster (green triangles in Figure 1B), while seven *K. titania* specimens from Yunnan were grouped with other *K. titania* (purple square in Figure 2B), although some overlapped with scatters from *K. hardwickii* and *K. furva*.

Total genomic DNA was isolated from approximately 20 mg of muscle tissue using the traditional phenol chloroform extraction method. We further sequenced the partial segments of *CoI*, *Cytb* and *Rag2* following protocol and primers from Francis et al. (2010), Khan et al. (2010) and Yu et al. (2018). Gene sequences of *Kerivoula* were downloaded from the NCBI-nt database and used for reconstruction phylogenetic trees with the newly generated sequences (GenBank accession numbers of *K. kachinensis*: *CoI*: MZ438737–MZ438743; *Cytb*: MZ438728–MZ438735; *Rag2*: MZ438744–MZ438750; GenBank accession numbers of *K. titania*: *CoI*: MZ417537–MZ417543; *Cytb*: MZ417524–MZ417530; *Rag2*: MZ417531–MZ417536). After the nucleotides substitution model selected by Modeltest 3.06 (Posada and Crandall 1998), phylogenetic trees were reconstructed with RAxML 7.2.7 (Stamatakis 2014) using 500 bootstrap replicates. Our phylogenetic inferences showed similar and well-supported specific clades, which consistently clustering all our samples with *K. kachinensis* (bootstraps values: 99 for *CoI*, 100 for *Cytb*, 97 for *Rag2*) and *K. titania* (bootstraps values: 96 for *CoI*, 100 for *Cytb*, 92 for *Rag2*), respectively (Figure 2C–E). The intraspecific relationships of *K. kachinensis* and *K. titania*, however, are ambiguous since limitation of sampling (Figure 2C–E).

According to our morphological examination, morphometric analysis and phylogenetic inferences, *Kerivoula* samples from Yunnan were confidently identified as *K. kachinensis* and *K. titania*, respectively. The craniodental variations within *K. kachinensis* were also noticed in this study (e.g., GBB, PC and ML; Table 1), our phylogenetic relationship, however, reveals an intertwined phylogenetic

Table 1: List of external and skull measurements of *Kerivoula kachinensis* and *Kerivoula titania* (weight: g, length: mm), and tables of variable loadings on principal components (PCA) and contribution of original variables in discriminant functions (DFs).

Measurements	***Kerivoula kachinensis***			***Kerivoula titania***			**PC1**	**PC2**	**LD1**	**LD2**
	Yunnan, China (this study; *n* = 8) Mean ± SD (range)	**Southeast Asia[a] (Kuo et al. 2017; *n* = 11) Mean ± SD (range)**	***t***	**Yunnan, China (this study; *n* = 7) Mean ± SD (range)**	**Southeast Asia[a] (Kuo et al. 2017; *n* = 11) Mean ± SD (range)**	***t***				
Wt	6.83 ± 0.18 (6.10–7.60)	–	–	4.90 ± 0.26 (4.00–5.70)	–	–	–	–	–	–
HB	44.93 ± 0.93 (40.92–48.45)	–	–	38.68 ± 0.62 (35.92–40.80)	–	–	–	–	–	–
TL	55.78 ± 0.84 (53.04–59.88)	–	–	49.96 ± 0.93 (46.60–53.75)	–	–	–	–	–	–
EL	13.50 ± 0.27 (12.68–15.17)	–	–	13.91 ± 0.32 (12.68–14.75)	–	–	–	–	–	–
HF	9.89 ± 0.28 (8.16–10.79)	–	–	7.85 ± 0.36 (6.64–9.12)	–	–	–	–	–	–
FA	40.77 ± 0.27 (39.87–41.97)	42.43 ± 0.38 (40.40–44.70)	3.36*	34.15 ± 0.28 (32.67–34.79)	33.75 ± 0.22 (32.40–34.70)	-1.01^{NS}	–	–	–	–
TIB	22.21 ± 0.16 (21.62–23.07)	22.80 ± 0.21 (21.80–23.50)	2.25^{NS}	19.19 ± 0.18 (18.38–19.70)	18.87 ± 0.19 (18.40–19.70)	-0.64^{NS}	–	–	–	–
GTL	17.47 ± 0.11 (17.01–17.84)	17.83 ± 0.13 (17.14–18.39)	2.02^{NS}	15.61 ± 0.06 (15.44–15.90)	15.54 ± 0.07 (15.18–15.83)	-0.94^{NS}	–	–	–	–
CCL	15.81 ± 0.14 (15.36–16.60)	15.99 ± 0.11 (15.48–16.53)	1.05^{NS}	13.79 ± 0.06 (13.54–14.05)	13.57 ± 0.07 (13.12–13.87)	-1.78^{NS}	0.95	0.23	0.21	0.03
GBB	8.53 ± 0.08 (8.23–8.79)	8.36 ± 0.04 (8.13–8.54)	-2.00^{NS}	7.85 ± 0.05 (7.70–8.07)	7.75 ± 0.06 (7.47–8.17)	-0.98^{NS}	0.89	0.3.0	0.04	–
ZB	10.21 ± 0.09 (9.87–10.60)	10.59 ± 0.09 (10.16–10.95)	2.95*	9.09 ± 0.04 (8.90–9.23)	8.89 ± 0.07 (8.50–9.13)	-2.16^{NS}	0.96	0.21	0.10	–
PC	3.70 ± 0.05 (3.51–3.88)	3.61 ± 0.04 (3.45–3.89)	-1.34^{NS}	3.45 ± 0.05 (3.23–3.67)	3.33 ± 0.04 (3.17–3.58)	-1.49^{NS}	0.82	–	0.03	0.04
BH	5.43 ± 0.06 (5.19–5.68)	5.60 ± 0.08 (5.11–5.89)	1.59^{NS}	5.59 ± 0.08 (5.27–5.83)	5.54 ± 0.07 (5.17–5.98)	-0.27^{NS}	0.19	0.97	0.29	0.85
CBL	16.28 ± 0.08 (15.99–16.63)	16.47 ± 0.15 (15.64–17.31)	1.00^{NS}	14.34 ± 0.07 (14.02–14.58)	14.02 ± 0.09 (13.51–14.49)	−2.37*	–	–	–	–
C^1-M^3	6.71 ± 0.06 (6.44–6.99)	6.93 ± 0.06 (6.64–7.16)	2.63^{NS}	5.90 ± 0.04 (5.72–6.02)	6.07 ± 0.04 (5.80–6.24)	3.65*	0.91	0.34	0.08	–
C^1-C^1	4.28 ± 0.03 (4.10–4.36)	–	–	3.54 ± 0.04 (3.40–3.69)	–	–	–	–	–	–
M^3-M^3	6.29 ± 0.06 (6.06–6.47)	6.43 ± 0.02 (6.31–6.59)	2.38*	5.59 ± 0.05 (5.38–5.78)	5.50 ± 0.06 (5.05–5.81)	-0.88^{NS}	0.94	0.18	0.02	0.01
C_1-M_3	7.23 ± 0.05 (6.97–7.41)	7.44 ± 0.05 (7.14–7.58)	2.48*	6.32 ± 0.05 (6.11–6.50)	6.33 ± 0.04 (5.98–6.51)	0.17^{NS}	0.93	0.29	0.1.0	0.01
MDL	11.97 ± 0.09 (11.57–12.35)	12.76 ± 0.08 (12.15–13.04)	6.55*	10.41 ± 0.04 (10.31–10.60)	10.69 ± 0.10 (10.26–11.27)	2.76*	0.93	0.21	0.12	0.05

[a] Includes vouchers from Cambodia, Myanmar, Thailand, Vietnam. *Indicates significant result.

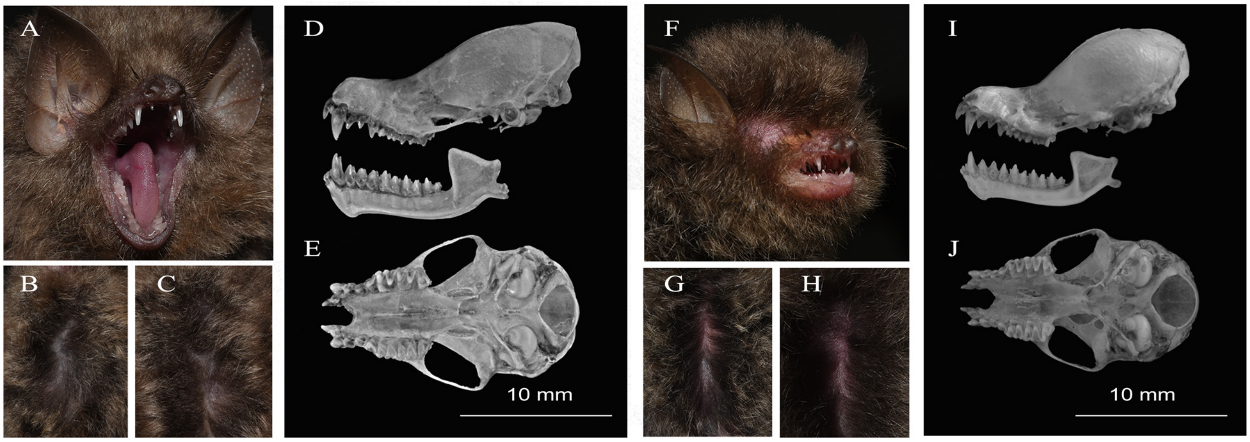

Figure 1: External and craniodental characteristic of *Kerivoula kachinensis* and *Kerivoula titania* from Yunnan, China.
(A–C) External characteristic of *K. kachinensis* (GZHU 19199, ♂); (F–H) external characteristic of *K. titania* (GZHU 19154, ♀); (A and F) head; (B and G) venter pelage; (C and H) dorsal pelage; (D–E) craniodental characteristic of *K. kachinensis* (GZHU 19123, ♂); (I–J) craniodental characteristic of *K. titania* (GZHU 19154, ♀); (D and I) lateral view of maxilla and mandibula; (E and J) ventral view of maxilla.

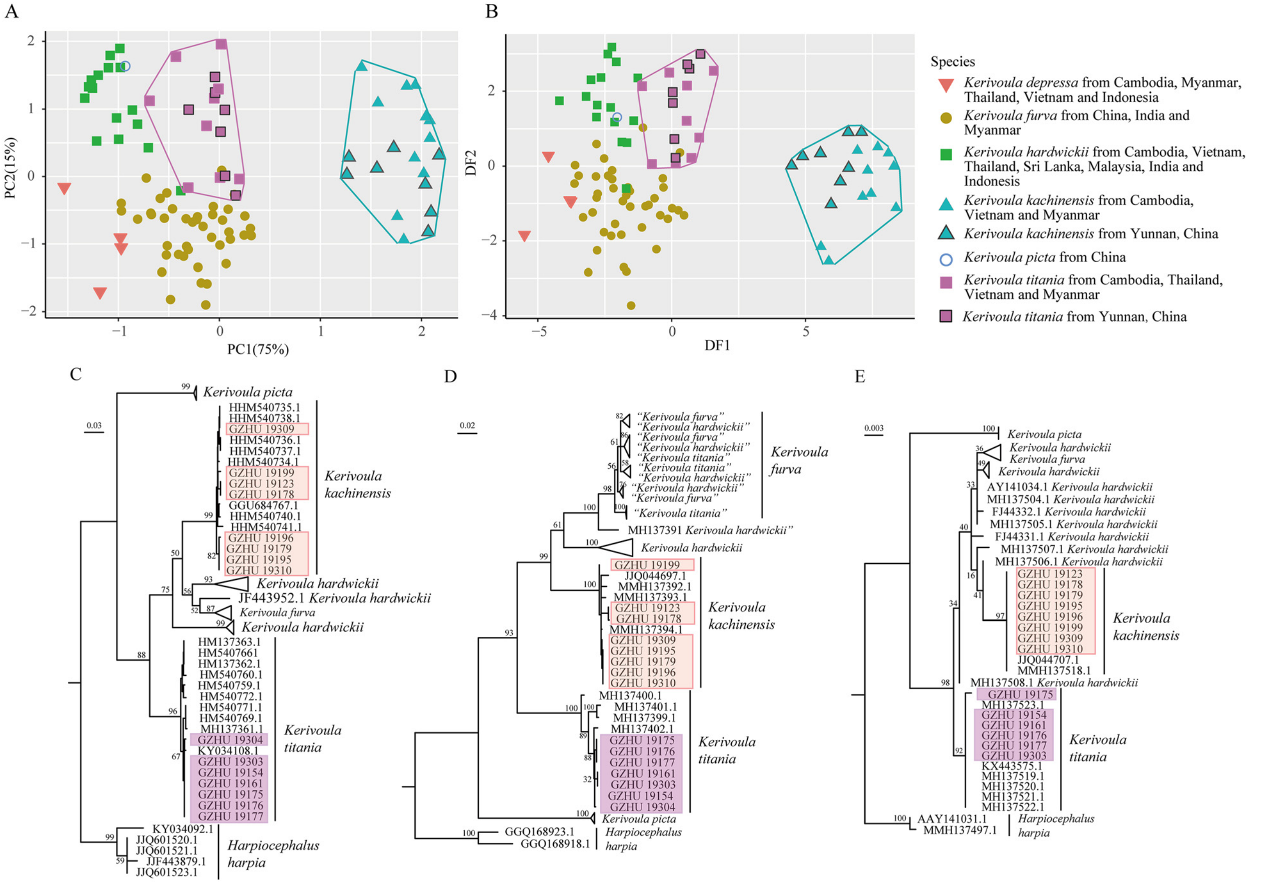

Figure 2: Two-dimensional PCA and DAPC plots of *Kerivoula* species based on nine craniodental measurements and phylogenetic trees of *Kerivoula* species based on (C) *CoI*, (D) *Cytb* and (E) *Rag2* sequences.
(A) PCA plots for *Kerivoula depressa*, *Kerivoula furva*, *Kerivoula hardwickii*, *Kerivoula kachinensis*, *Kerivoula picta* and *Kerivoula titania* showing projections of individual specimens and variable loadings on first two principal components. (B) Projections of 99 specimens and variable loadings on two DFs obtained from external and craniodental measurements. (C–E) Pink and purple rectangles indicate newly generated sequences of *K. kachinensis* and *K. titania* in this study.

relationship (Figure 2C–E). Since limitation in our molecular markers and sampling, in-depth genomics and ecological studies should be conducted for clarification of the variations. The occurrence of *K. kachinensis* in Yunnan Province is thus a new record of chiropteran species in China, while the discovery of *K. titania* represents the first valid record in the region since the misidentifications from Hainan and Taiwan (Wu et al. 2012) and the revision of Yu et al. (2018). Nowadays, four woolly bats occur in China including, *K. furva*, *K. picta*, *K. titania* and *K. kachinensis*, while *K. hardwickii sensu stricto* is only restricted to the southern part of the Indomalayan Region. It is worth noting that there is a risk of underestimation the actual species diversity of woolly bat in China compared to that of neighboring region such as Vietnam (eight distinct species: *Kerivoula depressa*, *Kerivoula dongduongana*, *Kerivoula papillosa*, *K. picta*, *K. titania*, *K. hardwickii*, *K. furva* and *K. kachinensis*) (Kruskop 2013; Tu et al. 2018; Wilson and Mittermeier 2019). Such heterogeneity implies a risk of underestimation in *Kerivoula* diversity in China, supports for field survey should be continued toward true diversity of small vertebrates in China.

Among four *Kerivoula* species that currently distributed in China, *K. picta* is of strikingly colored pattern in fur and wing, which could be identified unmistakably, while *K. kachinensis* is a diagnostically larger-sized species compared to the rest two species. When comparing *K. titania* and *K. furva*, the latter has a proportionally shorter tibia and a proportionally wider postorbital constriction (Table 1). In coloration of pelage, *K. furva* has a darkest dorsal pelage among the Chinese *Kerivoula* species, with dorsal hairs that are broadly uniformly coloured throughout their length. The dorsal hairs of *K. kachinensis* have darker grey bases and paler grey-brown upper segments (Bates et al. 2004; Soisook et al. 2014), those of *K. titania* have light grey middle segments above black bases (Bates et al. 2007).

Research ethics: Permission for field surveys and samples collection in Xishuangbanna, Yunnan Province, China were granted by the Xishuangbanna Nature Reserve Administration. Research ethics permits were provided by Scientific Research Local Ethics Committee of Guangzhou University.

Acknowledgments: The authors thank National Nature Reserve Administration of Xishuangbanna Dai Autonomous Prefecture for their guides and help during the field survey.

Author contributions: Wenhua Yu and Yi Wu designed the study; Zhenglanyi Huang, Shuo Liu, Song Li, and Yi Wu collected materials for the study; Wenhua Yu, Chuyan Lin, Zhenglanyi Huang, and Yi Wu performed morphometric and phylogenetic analyses; Wenhua Yu, Chuyan Lin, and Yi Wu interpreted the results and prepared the manuscript, photographs, and figures for the study. All authors read and approved the final version of the manuscript.

Research funding: This study was financially supported by the National Natural Science Foundation of China (31970394, 32192421), the Special Foundation for National Science and Technology Basic research program of China [2021FY100303], the Biodiversity Investigation in Xishuangbanna National Nature Reserve, Biodiversity Investigation, Observation, and Assessment Program (2019–2023) of the Ministry of Ecology and Environment of China (8-2-3-4-5), the Science-Technology Basic Condition Platform from the Ministry of Science and Technology of the People's Republic of China (2005DKA21402), and Lancang-Mekong Cooperation Special Fund (Biodiversity Monitoring and Network Construction Along Lancang-Mekong River Basin Project).

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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