

New records of *Kerivoula titania* (Chiroptera: Vespertilionidae) from Hainan Island and Taiwan

Yi Wu¹, Yuchun Li², Liang-Kong Lin³, Masashi Harada⁴, Zhong Chen⁵
and Masaharu Motokawa^{6,*}

¹ College of Life Science, Guangzhou University, Guangzhou 510006, China

² Marine College, Shandong University at Weihai, Weihai 264209, China

³ Department of Life Science, Tunghai University, Taichung 407, Taiwan

⁴ Graduate School of Medicine, Osaka City University, Osaka 545-8585, Japan

⁵ Department of Biology, Hainan Normal University, Haikou 571158, China

⁶ Kyoto University Museum, Kyoto University, Kyoto 606-8501, Japan

The genus *Kerivoula* includes about 20 species, and 12 species have been recorded in the Indomalayan Region, including recently described species such as *K. kachinensis* from northern Myanmar, *K. krauensis* from Peninsular Malaysia, and *K. titania* from Southeast Asia (Corbet and Hill 1992; Bates et al. 2004, 2007; Simmons 2005; Thong et al. 2006; Francis et al. 2007). Among them, *K. titania* was recently described by Bates et al. (2007) from the Seima Biodiversity Conservation Area, Cambodia, and it is widely distributed in mainland Southeast Asia in 13 localities in five countries: Myanmar, Thailand, Lao PDR, Cambodia, and Vietnam (Bates et al. 2007).

Only two *Kerivoula* bats have been recorded from China, *K. picta* and *K. hardwickii* (Zhang 1997; Wang 2003). Recently, we collected eight specimens identified as *K. titania* for the first time from Hainan Island and Taiwan as reported below.

Materials and methods

Five external and 11 cranial and dental measurements were taken from specimens (Table 1) using digital calipers with accuracies of 0.1 and 0.01 mm, respectively, following the definition of Bates et al. (2007). Chromosomal preparations were made from tail and lung tissue cultures following Harada and Yosida (1978). Differential staining using the G-band and C-band techniques was applied following Seabright (1971) and Sumner (1972), respectively. Cytochrome *b* genes (1,140 bp) from six specimens were sequenced and deposited in GenBank with accession numbers JN112241–JN112246. Polymerase chain reaction (PCR) amplification of cyto-

chrome *b* genes was performed using the primers K1 (L14724: 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' and KCBH747: 5'-TAGGTCAGGGGAGAATA GGAC-3') and K2 (KCBL479: 5'-CTCGTAGAATGATCTGAGG-3' and M1: 5'-TAGAATCTCAGCTTTGG GTGTTG-3) (Khan et al. 2010). The PCR sequence involved 10 min of initial denaturation at 94°C, followed by 38–40 cycles (45 s denaturation at 94°C, 45 s annealing at 45–48°C, and 60 s extension at 72°C) and a 10 min final extension at 72°C. Phylogenetic analyses were performed using our data for *K. titania* and data published by Khan et al. (2010) for *K. kachinensis*, *K. hardwickii*, *K. intermedia*, and *K. minuta*. A neighbor-joining tree created using Kimura's two parameter distances (Kimura 1980) was constructed by MEGA (Kumar et al. 2001).

Results and discussion

From Hainan Island, two *K. titania* specimens (IBHG 08279 and IBHG 08280, both females) were collected in a harp trap that was set across a narrow stream in essentially undisturbed tropical rain forest at the core zone of Diaoluoshan National Forest Park (18°43.56'N, 109°52.01'E, 923 m elevation), Lingshui Xian, Hainan Island, about 217 km southeast of Haikou on 15 September 2008 by Wu, Harada, and Motokawa. Specimens are deposited at the Institute of Biodiversity in Huanan, Guangzhou University, Guangzhou (IBHG).

In Taiwan, six specimens were collected by Lin from Dapu, Chiayi County (THU B990008, male, on 21 May 1999; 23°18.14'N, 120°35.49'E, 350 m elevation), Baolai, Kaohsiung County (THU B000079, female, on 27 August 2002; 23°06.20'N, 120°42.16'E, 470 m eleva-

*To whom correspondence should be addressed. E-mail: motokawa@inet.museum.kyoto-u.ac.jp

Table 1. External, cranial and dental measurements (in mm) of *Kerivoula titania* from Hainan Island and Taiwan (mean with range and sample size in parentheses)

Character	Hainan Island	Taiwan
Forearm length (FA)	31.7, 35.5	32.0, 35.0 (2)
Tail length (TAIL)	44.2, 42.2	40.0, 45.0 (2)
Foot (HP)	7.2, 9.3	8.0, 7.0 (2)
Length of tibia (TIBIA)	17.14, —	17.0, 18.0 (2)
Ear length (EAR)	13.3, 14.0	—
Body mass (MASS, in gram)	4.1, 5.2	5.5, 5.3 (2)
Greatest length of skull (GTL)	14.66, 15.29	14.43 (14.14–14.90, 5)
Condylar length (CCL)	13.22, 13.78	12.60 (12.01–13.03, 5)
Zygomatic breadth (ZB)	8.75, 9.03	8.69 (8.60–8.80, 5)
Breadth of braincase (BB)	6.82, 6.92	7.11 (6.95–7.18, 5)
Greatest width of the braincase (GBB)	7.32, 7.42	7.36 (7.21–7.51, 5)
Braincase height (BH)	5.21, 5.27	5.70 (5.27–6.19, 4)
BI/CCL × 100	38.2, 39.4	45.5 (43.5–48.3, 4)
BI/GBB × 100	71.0, 71.2	76.9 (71.2–82.4, 4)
Post-orbital constriction (PC)	3.44, 3.43	3.40 (3.19–3.69, 5)
Maxillary tooth row length (C–M3)	5.60, 5.93	5.49 (5.40–5.56, 5)
External palatal width (M2–M2)	5.18, 5.65	5.44 (5.24–5.68, 5)
Mandibular tooth row length (C–M3)	6.15, 6.54	6.06 (5.95–6.25, 5)
Mandible length (MDL)	10.19, 10.61	10.40 (9.85–10.77, 5)

tion), Meilan Trail, Kaohsiung County (THU B030028, male, and B030041, female, on 10 July 2003; 23°17.24'N, 120°49.46'E, 920 m elevation), and Meilong Trail, Kaohsiung County (THU B030025, female, on 9 July 2003 and B040007, male, on 16 March 2004; 23°05.28'N, 120°43.27'E, 850 m elevation). The vegetation in these areas was predominantly secondary evergreen forests mixed with sparse bamboo plantations. These specimens were deposited at Tunghai University, Taichung (THU).

Specimens from Hainan Island and Taiwan had similar values in external and cranial measurements and non-metric characteristics (Fig. 1) to those of *K. titania* from Southeast Asia reported by Bates et al. (2007). The overall size of the specimens, however, was slightly smaller than the Southeast Asian specimens, such as in FA (31.7–35.5 versus 32.4–35.9), CCL (12.01–13.78 versus 13.4–13.9), and CM3 (5.31–5.56 versus 6.0–6.5). Although *K. titania* is said to be larger than but morphologically similar to *K. hardwickii* (see Bates et al. 2007), measurements from *K. titania* specimens from Hainan Island and Taiwan overlapped more with *K. hardwickii*. Despite the presence of size variation, the specimens examined in this study were identified as *K. titania* from morphological characteristics that were different from those of *K. hardwickii*, such as having a larger narial pit

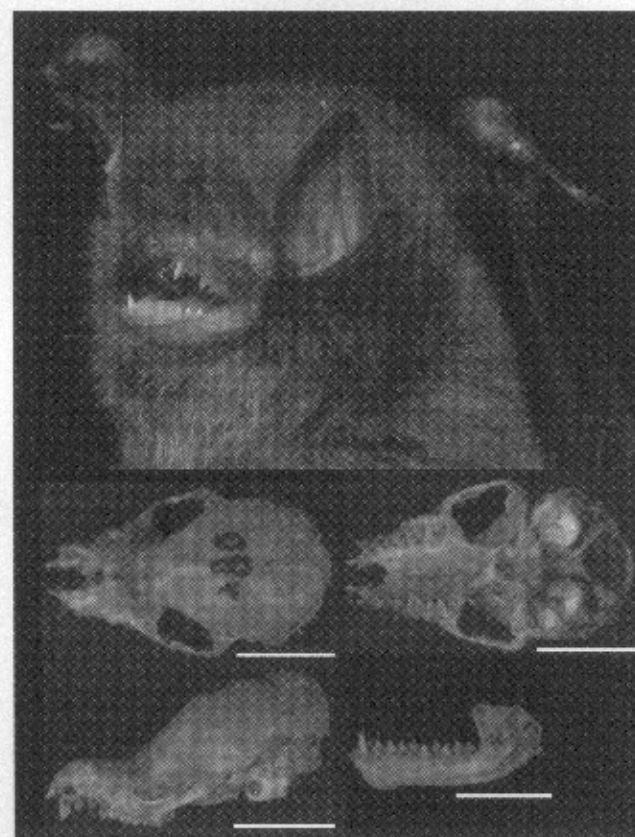
**Fig. 1.** External features and skull of *Kerivoula titania* from Hainan Island (IBHG 8280). Bars indicate 5 mm.

Fig.
Hain
K. ti

in th
than
ilar
dom
of th
Yosh
chara
reco
by Ye
misid
titania
from
from t
focus
morph
This
based o
08280)
was 2n
submeta
acrocent
medium
submeta
covered
one spec

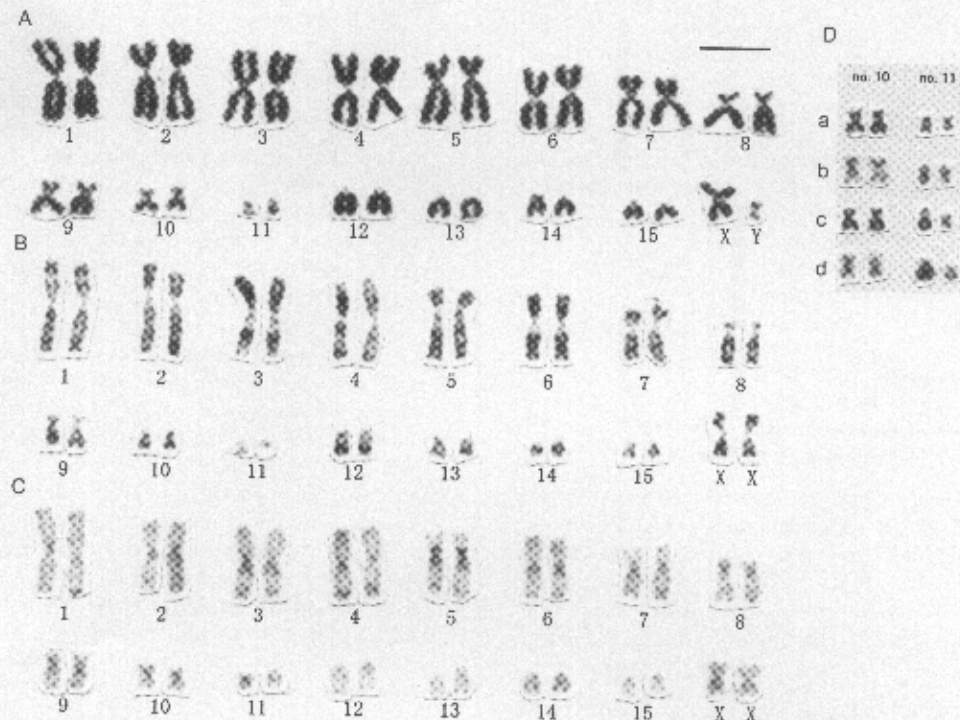


Fig. 2. Conventional (A, THU B990008), G-banded (B, IBHG 08279), and C-banded (C, IBHG 08280) karyotypes of *Kerivoula titania* from Hainan Island and Taiwan. The bar indicates 10 μ m. Individual differences in chromosome no. 11 (conventional and C-banded chromosomes) of *K. titania* from Hainan Island are shown; IBHG 08279 (a and b) and IBHG 08280 (c and d).

in the rostrum, a postorbital constriction that is narrower than the posterior part of the rostrum (versus having similar widths), and a flattened braincase (versus a large domed one), as described by Bates et al. (2007). Pictures of the skull of a specimen that are presented in Yoshiyuki et al. (2010) also share these morphological characteristics of *K. titania*. Therefore, the recent new record of *K. hardwickii* from Taiwan that was reported by Yoshiyuki et al. (2010) might have been based on a misidentification and actually represents a record of *K. titania*. The smaller overall size of *K. titania* specimens from Hainan Island and Taiwan compared to samples from the southeast Asia is interesting and should be the focus of a future comprehensive study on geographic morphological variation in *K. titania*.

This is the first report on the karyotype of *K. titania* based on specimens from Hainan Island (IBHG 08279, 08280) and Taiwan (THU B030028). The karyotype was $2n = 32$, including 10 large-to-small metacentric or submetacentric pairs (nos. 1–10) and four medium-sized acrocentric pairs (nos. 12–15) among the autosomes, a medium-sized metacentric X chromosome, and a small submetacentric Y chromosome. A difference was discovered in chromosome pair no. 11 among specimens: one specimen (IBHG 08279, Fig. 2D, a and b) from

Hainan Island and a specimen from Taiwan (THU B030028, Fig. 2A) had small-sized submetacentric pair, while a specimen from Hainan Island (IBHG 08280, Fig. 2D, c and d) had a medium-sized acrocentric chromosome and a small-sized submetacentric chromosome. Fundamental numbers become $FN = 52$ for the former specimens and $FN = 51$ for the latter. We considered the anisomeric chromosome pair to have occurred by an increase in constitutive C-heterochromatin based on a C-band comparison (Fig. 2D, b and d). Six species of the genus *Kerivoula* have been karyotyped: *K. lanosa* from South Africa with $2n = 28$ (Rautenbach et al. 1993), *K. papillosa* from Thailand with $2n = 38$ (McBee et al. 1986; Khan et al. 2010), *K. minuta* with $2n = 28$, *K. intermedia* with $2n = 28$, *K. hardwickii* with $2n = 26$, and *K. lenis* with $2n = 38$ from Southeast Asia (Khan et al. 2010). The karyotype of *K. titania* differs from the karyotypes of congeneric species in chromosome number.

In the neighbor-joining tree created using cytochrome *b* genes with 10,000 bootstraps (Fig. 3), *K. titania* from Hainan Island and Taiwan formed a monophyletic clade. *Kerivoula titania* clustered with *K. kachinensis* and then with *K. hardwickii*. Sequence differences (K2P distance) between *K. titania* and *K. kachinensis*, and

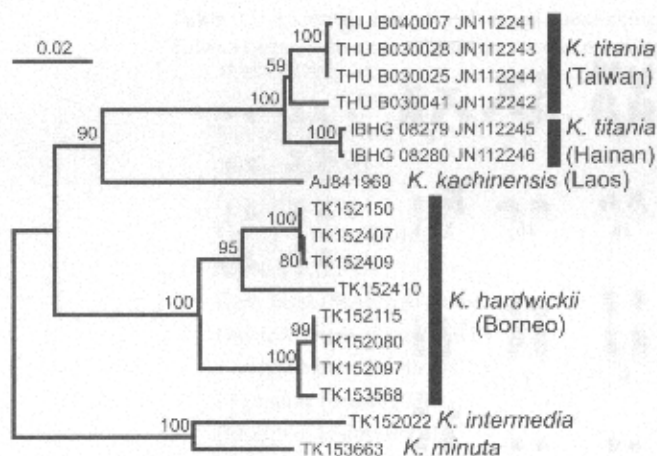


Fig. 3. Neighbor-joining tree of the 1,140 bp cytochrome *b* gene sequence of *Kerivoula titania* from Hainan Island and Taiwan and closely related *Kerivoula* species using the data of Khan et al. (2010).

between *K. titania* and *K. hardwickii*, were 0.11 and 0.13, respectively.

In the present study, *K. titania* was determined to be a distinct species from the other *Kerivoula* species based on analyses of morphology, karyotype, and the cytochrome *b* gene. From a biogeographic viewpoint, *K. titania* is distributed in Southeast Asian countries as well as Hainan Island and Taiwan. Similar distribution patterns in bats have been reported for *Coelops frithii*, *Scotophilus kuhlii*, and *Miniopterus schreibersii* (Lin et al. 2004; Smith and Xie 2008). Future molecular phylogeographic studies from Southeast Asian populations are needed to clarify the evolutionary history of *K. titania*.

Acknowledgments: This study was supported by the National Natural Science Foundation of China (NSFC, No. 30670277, 31172045), a NSFC Major International (Regional) Joint Research Project Grant (No. 31110103910), a NSFC-JSPS (Japan Society for the Promotion of Science) Joint Research Project Grant (No. 30811140092), the JSPS AA Science Platform Program, and the Heiwa Nakajima Foundation. We thank Messrs. C. D. Wang, Z. L. Jiang, L. Guo, and W. Lei for help with fieldwork on Hainan Island.

References

Bates, P. J. J., Struebig, M. J., Rossiter, S. J., Kingston, T., Oo, S. S. L. and Myamya, K. 2004. A new species of *Kerivoula* (Chiroptera: Vespertilionidae) from Myanmar (Burma). *Acta Chiropterologica* 6: 219–226.

Bates, P. J. J., Struebig, M. J., Hayes, B. D., Furey, N. M., Mya, K. M., Thong, V. D., Tien, P. D., Nguyen, S. T., Harrison, D. L., Francis,

C. M. and Csorba, G. 2007. A new species of *Kerivoula* (Chiroptera: Vespertilionidae) from Southeast Asia. *Acta Chiropterologica* 9: 323–337.

Corbet, G. B. and Hill, J. E. 1992. *The Mammals of the Indomalayan Region: A Systematic Review*. Natural History Museum and Oxford University Press, Oxford, 488 pp.

Francis, C. M., Kingston, T. and Zubaid, A. 2007. A new species of *Kerivoula* (Chiroptera: Vespertilionidae) from Peninsular Malaysia. *Acta Chiropterologica* 9: 1–12.

Harada, M. and Yosida, T. H. 1978. Karyological study of four Japanese *Myotis* bats (Chiroptera, Mammalia). *Chromosoma* 5: 283–291.

Khan, F. A. A., Solari, S., Swier, V. J., Larsen, P. A., Abdullah, M. T. and Baker, R. J. 2010. Systematics of Malaysian woolly bats (Vespertilionidae: *Kerivoula*) inferred from mitochondrial, nuclear, karyotypic, and morphological data. *Journal of Mammalogy* 91: 1058–1072.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120.

Kumar, S., Tamura, K., Jakobsen, I. B. and Nei, M. 2001. MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* 17: 1244–1245.

Lin, L.-K., Lee, L.-L. and Cheng, H.-C. 2004. *Bats of Taiwan*. National Museum of Natural Science, Taichung, 177 pp. (in Chinese).

McBee, K., Bickham, J. W., Yenbutra, S., Nabhitabhata, J. and Schlitter, D. A. 1986. Standard karyology of nine species of vespertilionid bats (Chiroptera: Vespertilionidae) from Thailand. *Annals of Carnegie Museum* 55: 95–116.

Rautenbach, I. L., Bronner, G. N. and Schlitter, D. A. 1993. Karyotypic data and attendant systematic implications for the bats of southern Africa. *Koedoe* 36: 87–104.

Seabright, M. 1971. A rapid banding technique for human chromosomes. *Lancet* 2: 971–972.

Simmons, N. B. 2005. Order Chiroptera. In (D. E. Wilson and D. M. Reeder, eds.) *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd ed, pp. 352–519. Johns Hopkins University Press, Baltimore.

Smith, A. T. and Xie, Y. 2008. *A Guide to the Mammals of China*. Princeton University Press, Princeton, 544 pp.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research* 75: 304–306.

Thong, V. D., Bumrungsri, S., Harrison, D. L., Pearch, M. J., Helgen, K. M. and Bates, P. J. J. 2006. New records of Microchiroptera (Rhinolophidae and Kerivoulineae) from Vietnam and Thailand. *Acta Chiropterologica* 8: 83–93.

Wang, Y. X. 2003. *A Complete Checklist of Mammal Species and Subspecies in China: A Taxonomic and Geographic Reference*. China Forestry Publishing House, Beijing, 394 pp.

Yoshiyuki, M., Lin, L.-K., Mizusawa, T. and Honda, N. 2010. New record of Hardwicke's forest bat, *Kerivoula hardwickii* (Horsfield, 1824) (Chiroptera, Vespertilionidae, Kerivoulineae) from Taiwan. *Animat* 8: 1–10.

Zhang, R. Z. 1997. *Distribution of Mammalian Species in China*. China Forestry Publishing House, Beijing, 280 pp.

Received 29 June 2011. Accepted 4 November 2011.