 Cytologia **79**(4): 445–456

Chromosome Analysis and Morphometric of Intermediate Roundleaf Bat, *Hipposideros larvatus* (Chiroptera, Hipposideridae) by Conventional, GTG-banding and Ag-NOR Banding Techniques

Sitthisak Jantarat[1], Praween Supanuam[2], Alongklod Tanomtong[3]*, Sumpars Khunsook[3], Narumon Prakrongrak[4] and Sarawut Kaewsri[4]

[1] Biology Program, Department of Science, Faculty of Science and Technology, Prince of Songkla University (Pattani), Muang, Pattani 94000, Thailand
[2] Biology Program, Faculty of Science, Ubon Ratchathani Rajabhat University, Muang, Ubon Ratchathani 34000, Thailand
[3] Applied Taxonomic Research Center (ATRC), Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen 40002, Thailand
[4] Biology Program, Faculty of Science, Buriram Rajabhat University, Muang, Buriram 31000, Thailand

Received June 21, 2013; accepted October 30, 2013

Summary Chromosome analysis and morphometrics of the intermediate roundleaf bat (*Hipposideros larvatus*) from Northeast Thailand were studied. Bat chromosome preparations were conducted by squash technique from bone marrow and testis. Conventional staining, GTG-banding and Ag-NOR banding techniques were applied to stain the chromosomes. The results showed that the diploid chromosome number of *H. larvatus* was 2*n*=32, and the fundamental number (NF) was 66 in both males and females. The types of autosomes observed were 14 large metacentric, 2 large submetacentric, 4 medium metacentric, 4 medium submetacentric, 2 small metacentric, 2 small submetacentric, and 2 small telocentric chromosomes. The X-chromosome was a large submetacentric chromosome and the Y-chromosome was a small submetacentric chromosome. From the GTG-banding technique, the number of bands in *H. larvatus* was 159, and the location of each chromosome pair could be clearly differentiated. NORs are located at the secondary constriction near the centromere on the long arm of the large metacentric chromosome pair 8. We found that during metaphase I the homologous chromosomes showed synapsis, which can be defined as the 16 bivalents. Six external morphological characters were measured as well as 13 cranial and dental measurements. The karyotype formula of *H. larvatus* was as follows:

$2n\ (44)=L_{14}^{m}+L_{2}^{sm}+M_{4}^{m}+M_{4}^{sm}+S_{2}^{m}+S_{2}^{sm}+S_{2}^{t}$+sex-chromosomes

Key words *Hipposideros larvatus*, Karyotype, Chromosome, Morphometric.

Bats are mammals with special morphological and physiological features that enable them to perform true flight. The nocturnal orientation of most bats is provided by a phenomenon known as echolocation which has permitted many species to exploit a variety of sheltered roosts that offer such advantages as microclimate stability and small risk of predation (Varella-Garcia *et al.* 1989). The order Chiroptera is divided into the suborder Megachiroptera, comprising the single family Pteropodidae, and the suborder Microchiroptera, which includes 16 families. There are approximately 1,057 species currently recognized within 192 genera (Schober and Grimmberger

* Corresponding author, e-mail: tanomtong@hotmail.com
DOI: 10.1508/cytologia.79.445

1993, Wilson and Cole 2000, Kleiman *et al.* 2004).

The bat fauna of Thailand includes 10 families, 40 genera and approximately 120 species, ranking it as one of the world's most diverse chiropteran faunas. Nine families of the suborder Microchiroptera have been recorded in Thailand: Emballonuridae, Rhinopomatidae, Craseonycteridae, Nycteridae, Megadermatidae, Rhinolophidae, Hipposideridae, Vespertilionidae, and Molossidae (Lekagul and McNeely 1988, Bumrungsri *et al.* 2006, Francis 2008). Hipposiderid bats are distributed in the tropics and subtropics of the Old World and the family includes nine genera and 81 species. The genus *Hipposideros* is the largest within the family with 67 species (Bogdanowicz and Owen 1998, Simmons 2005).

The systematics of bats is based primarily on classical morphological characteristics such as shoulder articulation, dentition, and other cranial features. More recently, cytogenetic data have shown considerable promise as a valuable tool for systematics studies. The order Chiroptera is an interesting group with chromosome evolution ranging from considerably conservative to quite divergent (Varella-Garcia *et al.* 1989). Chromosomal studies of bats from Thailand have reported only 36 species representing seven families (Harada *et al.* 1982, Hood and Baker 1986, Harada *et al.* 1985a, 1985b, Bickham *et al.* 1986, Hood *et al.* 1988, Wu *et al.* 2009, Supanuam *et al.* 2012).

There have been many previous literature reviews about chromosome analysis of bats in family Hipposideridae (Ray-Chaudhuri and Pathak 1966, Ray-Chaudhuri *et al.* 1971, Dulić and Mutere 1974, 1977, Peterson and Nagorsen 1975, Ando *et al.* 1980, Handa and Kaur 1980, Harada *et al.* 1982, 1985a, Hood *et al.* 1988, Zhang and Wan 1992, Choudhury and Patro 1993, Rautenbach *et al.* 1993, Sreepada *et al.* 1993, Rickart *et al.* 1999, Gu 2002a, 2002b, 2006, Volleth *et al.* 2002, Mao *et al.* 2007, Yi and Harada 2006, Koubínová *et al.* 2010). In the present study, we report the karyotype analysis and chromosomal characteristics of the nucleolar organizer regions (NORs) of the intermediate roundleaf bat (*Hipposideros larvatus*) from Northeast Thailand. In the future, basic knowledge and cytogenetics of *H. larvatus* could be applied to several research areas, especially to assist in protection from extinction.

Materials and methods

The *H. larvatus* samples were obtained from Nongbualamphu Province, Northeast Thailand. The bats, 10 males and 10 females, were transferred to the laboratory and were kept under standard

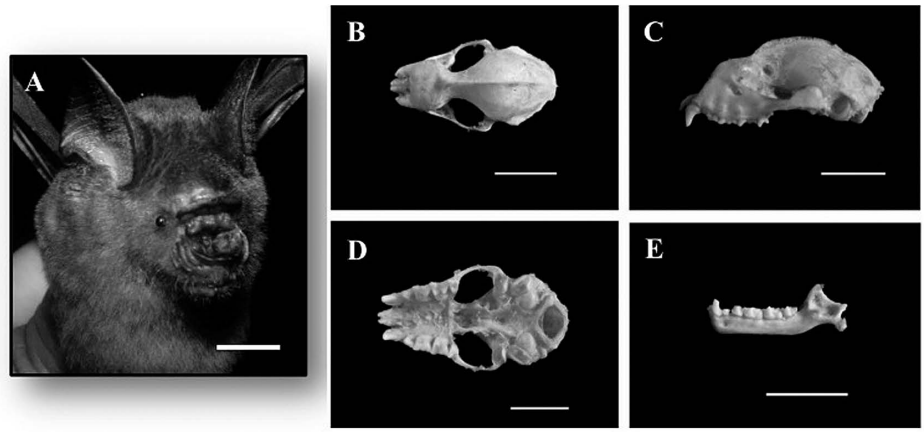

Fig. 1. Morphological characters of the intermediate roundleaf bat (*Hipposideros larvatus*) from Northeast Thailand, including external morphology (A), ventral view of skull (B), lateral view of skull (C), dorsal view of skull (D) and mandible (E) (scale bars 1.0 cm).

conditions for seven days prior to the experiments. Chromosome preparation was conducted by the squash technique from the bone marrow and testis. Conventional staining, GTG-banding and Ag-NOR banding techniques were applied to stain the chromosome (Howell and Black 1980, Rooney 2001). The length of short arms (Ls) and long arms (Ll) of the chromosome were measured and the length of total arm chromosomes (LT, LT=Ls+Ll) was recorded. The relative length (RL) and centromeric index (CI) were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiograming. External and cranial morphologies were measured for species identification (Duengkae 2006, Francis 2008, Lekagul and McNeely 1988).

Results and discussion

Chromosome number, fundamental number and karyotype of H. larvatus

The present results demonstrated that the diploid chromosome number of *H. larvatus* was

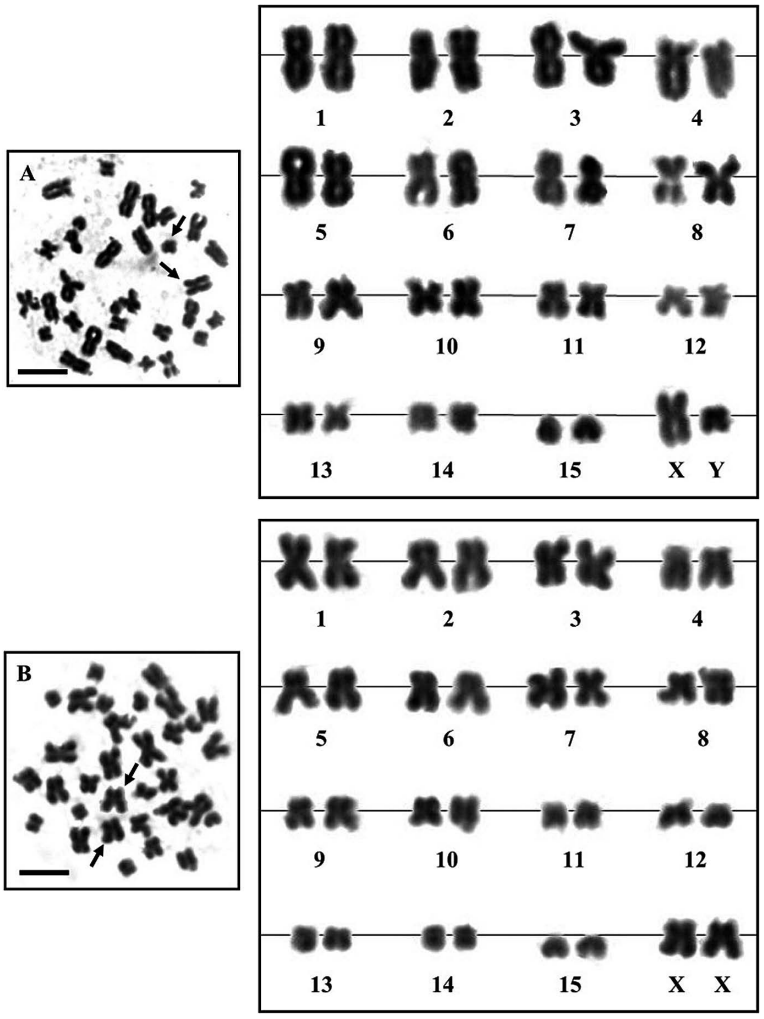

Fig. 2. Metaphase chromosome plates and karyotypes of male (A) and female (B) intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32 by conventional staining technique, arrows indicate sex chromosomes (scale bars 10 μm).

Table 1. Review of cytogenetic reports of the intermediate roundleaf bat, *Hipposideros larvatus* (Chiroptera, Hipposideridae).

Species	2*n*	NF	m	sm	a	t	X	Y	NORs	References
H. larvatus	32	64	30		0	0	sm	a	—	Harada *et al.* (1982)
	32	—	—	—	—	—	sm	a	—	Hood *et al.* (1988)
	32	64 in female 63 in male	30		0	0	sm	t	—	Yi and Harada (2006)
	32	—	30		0	0	—	—	—	Volleth *et al.* (2002)
	32	—	30		0	0	sm	a	—	Mao *et al.* (2007)
	32	66	20	8	0	2	sm	sm	L(CR)8	Present study

Notes: 2*n*=diploid chromosome, NF=fundamental number (number of chromosome arm), m=metacentric, sm=submetacentric, a=acrocentric, t=telocentric chromosome, NORs=nucleolar organizer regions, L=long arm, CR=centromeric region and — =not available.

2*n*=32 (Fig. 2). It is in accordance with previous studies (Harada *et al.* 1982, Hood *et al.* 1988, Volleth *et al.* 2002, Yi and Harada 2006, Mao *et al.* 2007). The NF was 66 in both males and females, which is different from the results of Harada *et al.* (1982), who detected an NF of 64, and Yi and Harada (2006) who detected an NF of 63 in males and 64 in females. The karyotype was composed of 20 metacentric, 8 submetacentric, and 2 telocentric chromosomes. However, it is inconsistent with the results of Harada *et al.* (1982), Volleth *et al.* (2002), Yi and Harada (2006) and Mao *et al.* (2007), which revealed that *H. larvatus* has 30 metacentric/submetacentric chromosomes (bi-arm chromosomes).

The X-chromosome of the *H. larvatus* is a large submetacentric chromosome and the Y-chromosome is the small submetacentric chromosome. These features are different from that reported by Harada *et al.* (1982), Hood *et al.* (1988) and Mao *et al.* (2006) indicating that *H. larvatus* had a submetacentric X-chromosome and acrocentric Y-chromosome. Furthermore, Yi and Harada (2006) also reported that the sex chromosomes of the *H. larvatus* had a submetacentric X-chromosome and telocentric Y-chromosome (Table 1).

The karyotype of 26 species of the family Hipposideridae have been reported (Ray-Chaudhuri and Pathak 1966, Ray-Chaudhuri *et al.* 1971, Dulić and Mutere 1974, 1977, Peterson and Nagorsen 1975, Ando *et al.* 1980, Handa and Kaur 1980, Harada *et al.* 1982, 1985a, Hood *et al.* 1988, Zhang and Wan 1992, Choudhury and Patro 1993, Rautenbach *et al.* 1993, Sreepada *et al.* 1993, Rickart *et al.* 1999, Gu 2002a, 2002b, 2006, Yi and Harada 2006, Koubínová *et al.* 2010) and a limited extent of variation is evident. In most of the species studied, a karyotype with 32 bi-armed chromosomes is present and the fundamental number of autosomal arms (NFa) is 60. Within the genus *Hipposideros*, exceptions from this uniform pattern were found in *H. commersoni* from South Africa (Rautenbach *et al.* 1993) and in *H. obscurus* from the Philippines (Rickart *et al.* 1999). The karyotype of *H. commersoni* had 2*n*=52, whereas that of *H. obscurus* had 2*n*=24 (NFa=44) (Koubínová *et al.* 2010).

Within the genus *Hipposideros*, 17 out of the 22 cytogenetic species studied had 2*n*=32 (77.27%). Different karyotypes were found in *H. obscurus* (2*n*=24), *H. cyclops* (2*n*=36), *H. commersoni* (2*n*=52) and *H. gigas* (2*n*=52) (Koubínová *et al.* 2010). Robertsonian mechanisms of centric fusion/fission combined possibly with whole arm translocations have played a major role in the karyotypic evolution within the genus *Rhinolophus* (Mao *et al.* 2007) and similar mechanisms but with the opposite direction can also be proposed for the genus *Hipposideros* (Sreepada *et al.* 1993, Koubínová *et al.* 2010). The similar fundamental numbers (number of chromosome arms) found in individual karyotypes indicate that the Robertsonian rearrangements are the probable source of karyotype variation among the species of the family Hipposideridae.

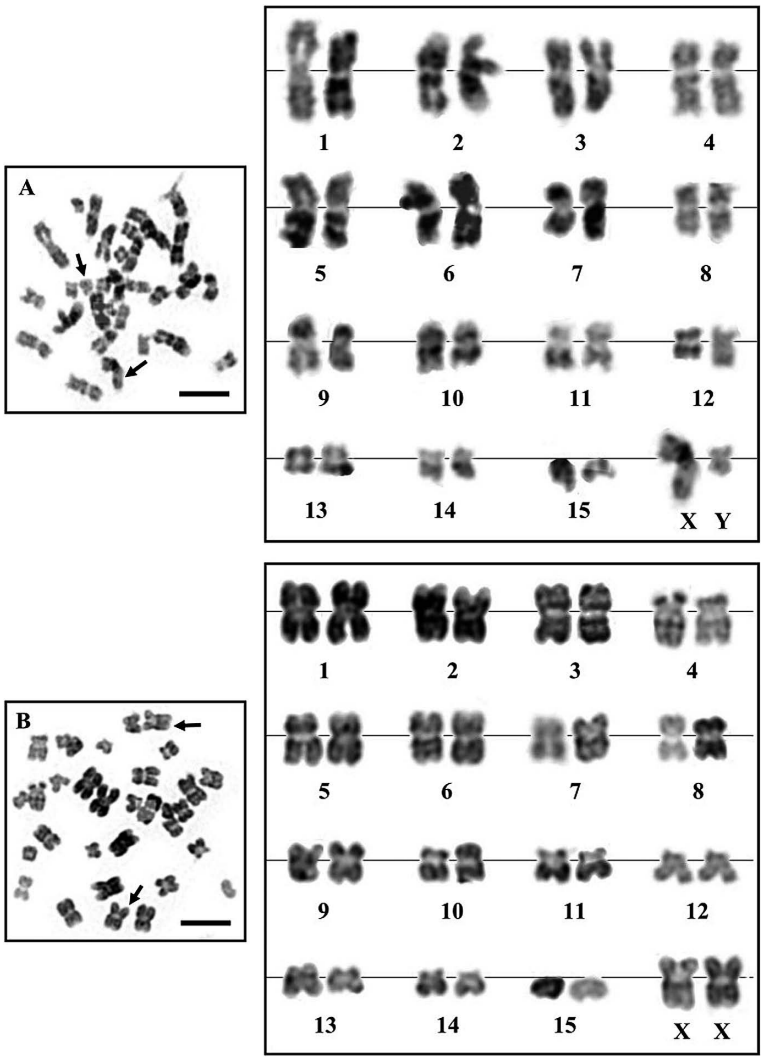

Fig. 3. Metaphase chromosome plates and karyotypes of male (A) and female (B) intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32 by GTG-banding technique, arrows indicate sex chromosomes (scale bars 10 μm).

Chromosome marker of H. larvatus

The cytogenetic study of *H. larvatus* in the present study was accomplished for the first time by using our present GTG-banding technique. GTG-banding revealed that the number of GTG-bands on one set of haploid chromosomes, which includes autosomes, X and Y chromosomes, is 159 bands. The GTG-banding technique provides defined dark band regions (heterochromatin) and light band regions (euchromatin) on a chromosome (Rooney 2001). The haploid set of *H. larvatus* consists of 15 autosomes including X and Y chromosomes. However, some chromosomes were not clearly identified because of their variation. The chromosome band scoring is represented by approximate bands that appear (Fig. 3).

This is the first cytogenetic analysis of *H. larvatus* using the Ag-NOR banding technique. The adjacent region on the long arm of the large metacentric chromosome pair 8 (centromeric NORs) showed defined NORs (Fig. 4). The objective of this technique is to detect NORs which represent the location of genes that function in ribosome synthesis. The location of NORs in several bats has

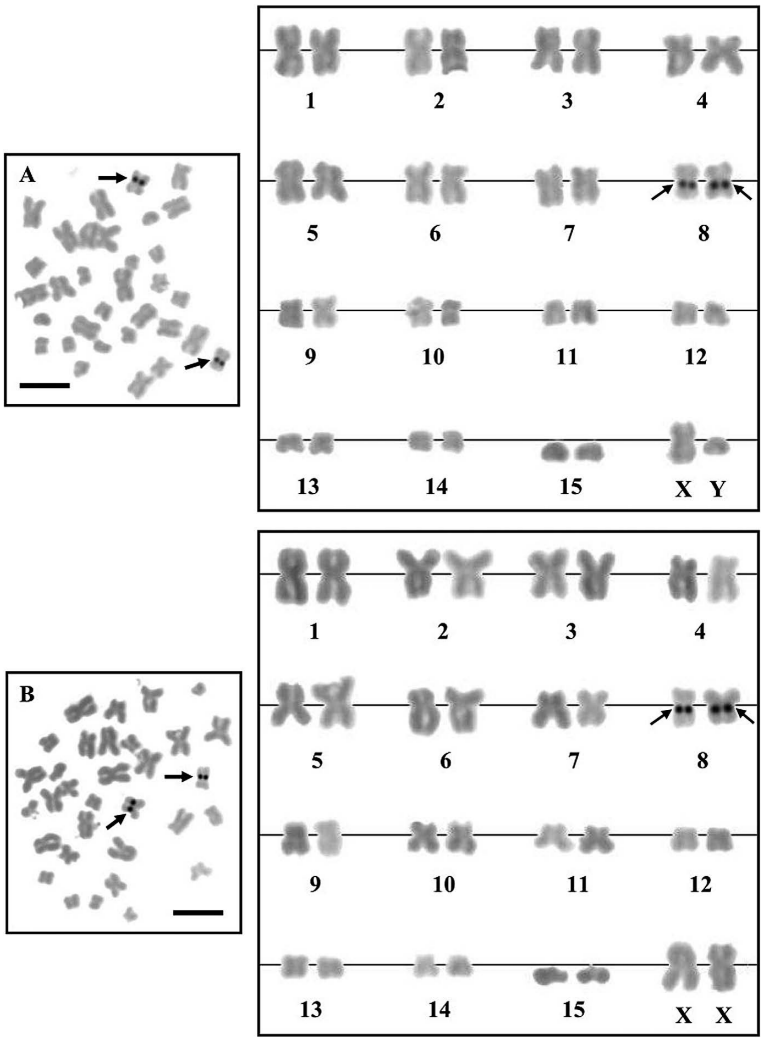

Fig. 4. Metaphase chromosome plates and karyotypes of male (A) and female (B) intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32 by Ag-NOR banding technique, arrows indicate nucleolar organizer regions/NORs (scale bars 10 μm).

been analyzed using the Ag-NOR banding technique (Volleth 1987, Morielle-Verielle and Varella-Garcia 1988, Souza and Araújo 1990, Morielle-Verielle *et al.* 1992, Rodrigues *et al.* 2000, 2003, Santos *et al.* 2002, Noronha *et al.* 2004, Faria *et al.* 2009, Garcia and Pessôa 2010, Vilamiu *et al.* 2010, Gomes *et al.* 2012). Eukaryote ribosomal RNA genes are organized into two different gene families: a major one encoding 18S, 5.8S and 28S rRNAs, and a small one encoding 5S rRNA. The major rDNA genes are clustered at specific chromosomal sites, which are known as nucleolar organizer regions/NORs (Suzuki *et al.* 1996).

We have shown that the asymmetrical karyotype of *H. larvatus*, which has three types of chromosomes (metacentric, submetacentric and telocentric chromosomes), is an important chromosome marker. Figures 5, 6 and 7 show the standardized ideogram from conventional staining, GTG-banding, and Ag-NOR banding techniques. The idiogram shows gradually decreasing length of the chromosomes. The largest and smallest chromosomes show an approximately threefold size difference. Data of the chromosomal checks on mitotic metaphase

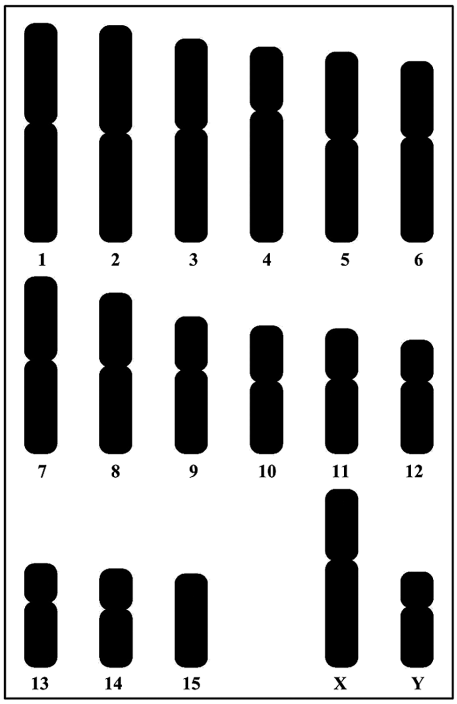

Fig. 5. Standardized idiogram of the intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32 by conventional staining technique.

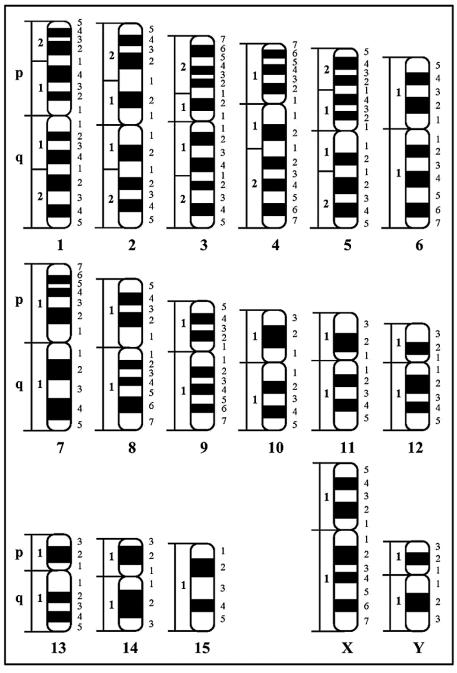

Fig. 6. Standardized idiogram of the intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32 by GTG-banding technique.

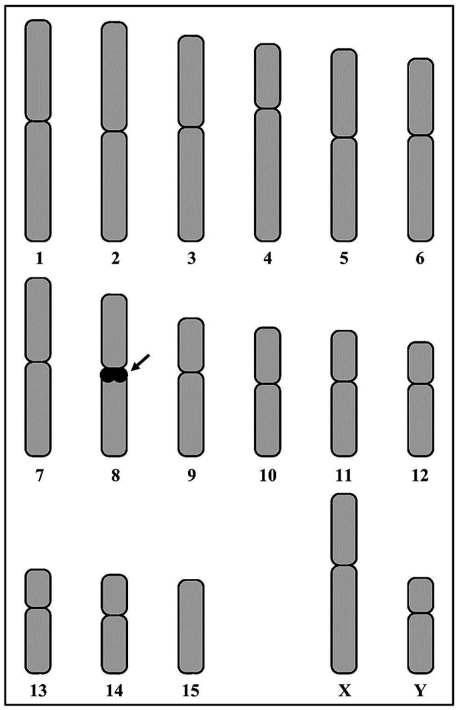

Fig. 7. Standardized idiogram of the intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32 by Ag-NOR banding technique. Arrow indicates nucleolar organizer region/NOR.

Table 2. Mean of length of short arm chromosomes (Ls), length of long arm chromosomes (Ll), total arm length of chromosomes (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI from metaphase chromosomes in 20 cells of male and female intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32.

Chromosome pair	Ls	Ll	LT	RL±SD	CI±SD	Chromosome size	Chromosome type
1	1.320	1.608	2.928	0.083±0.008	0.549±0.019	Large	Metacentric
2	1.465	1.473	2.938	0.084±0.009	0.501±0.0021	Large	Metacentric
3	1.213	1.538	2.750	0.078±0.010	0.559±0.018	Large	Metacentric
4	0.880	1.788	2.668	0.076±0.007	0.670±0.017	Large	Submetacentric
5	1.213	1.395	2.608	0.074±0.008	0.535±0.015	Large	Metacentric
6	1.013	1.398	2.410	0.069±0.005	0.580±0.018	Large	Metacentric
7	1.163	1.290	2.453	0.070±0.004	0.526±0.016	Large	Metacentric
8*	0.965	1.165	2.130	0.061±0.006	0.547±0.021	Large	Metacentric
9	0.688	1.113	1.800	0.051±0.003	0.618±0.011	Medium	Submetacentric
10	0.750	0.963	1.713	0.049±0.005	0.562±0.014	Medium	Metacentric
11	0.713	0.990	1.703	0.048±0.006	0.581±0.011	Medium	Metacentric
12	0.550	0.938	1.488	0.042±0.003	0.630±0.012	Medium	Submetacentric
13	0.510	0.885	1.395	0.040±0.002	0.634±0.013	Small	Submetacentric
14	0.520	0.765	1.285	0.037±0.001	0.595±0.019	Small	Metacentric
15	0.000	1.250	1.250	0.036±0.002	1.000±0.000	Small	Telocentric
X	0.940	1.440	2.380	0.068±0.007	0.605±0.006	Large	Submetacentric
Y	0.490	0.790	1.280	0.036±0.001	0.617±0.014	Small	Submetacentric

Remark: *=Nucleolar organizer region/NOR.

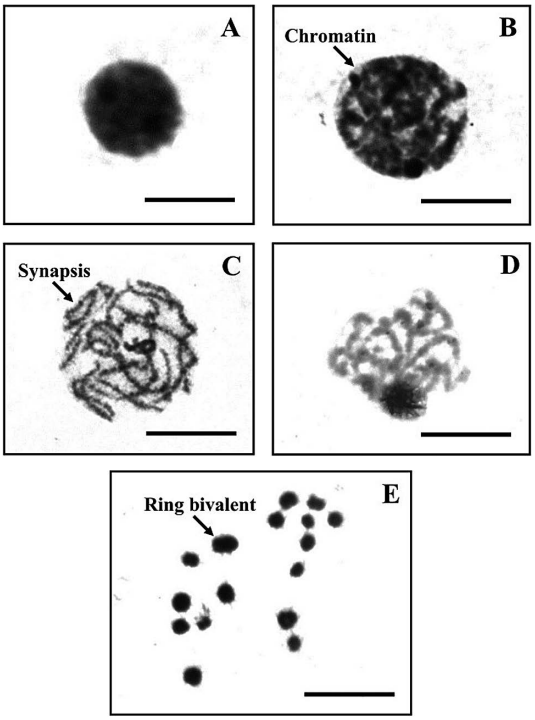

Fig. 8. Meiotic cell division of the intermediate roundleaf bat (*Hipposideros larvatus*) 2*n* (diploid)=32 on interphase (A), late leptotene (B), pachytene (C), diplotene (D), and metaphase I (E); scale bar indicates 10μm.

cells are shown in Table 2. Regarding the chromosome marker of *H. larvatus*, chromosome pair 1 is the largest metacentric and chromosome pair 15 is the smallest telocentric chromosome. The karyotype formula of *H. larvatus*is as follows:

$2n$ (32)=L_{14}^{m}+L_{2}^{sm}+M_{4}^{m}+M_{4}^{sm}+S_{2}^{m}+S_{2}^{sm}+S_{2}^{t}+sex-chromosomes

Meiotic cell division of H. larvatus

The present study on meiotic cell division of *H. larvatus* found that during metaphase I (meiosis I) the homologous chromosomes showed synapsis, which can be defined as the 16 bivalents (15 ring bivalents and 1 rod bivalent, Fig. 8E). The largest metacentric chromosome pair 1 is the largest bivalent. In interphase (Fig. 8A) and prophase I (meiosis I), we found that *H. larvatus* had the distinctness of the observable late leptotene (initiation of chromosome shrinking, Fig. 8B), pachytene (completion of chromosome synapsis, Fig. 8C), and diplotene (chiasma and crossing over, Fig. 8D).

Morphological descriptions of H. larvatus

The *H. larvatus* is a small insectivorous bat which has an average forearm and head-body length of 57.63±7.62 mm (45.00–64.00 mm) and 66.96±9.51 mm (51.50–79.00 mm), respectively, as examined (Table 3). The upper body is dark gray-brown to reddish-brown, whereas the underbody is slightly paler and the wing membranes are dark-brown. It has an average tail length of 29.14±5.13 mm (20.00–35.50 mm) and an average ear length of 21.08±1.79 mm (19.20–24.00 mm). The noseleaf is like a horseshoe which has three lateral leaflets and a spear-shaped internatrial septum (Fig. 1A). The average length of the tibia and hind foot are 22.46±1.92 mm (19.00–25.00 mm) and 9.81±1.06 mm (8.00–11.20 mm), respectively. The morphology of the skull was

Table 3. External, cranial and dental measurements of the intermediate roundleaf bat (*Hipposideros larvatus*) from Thailand (*n*=10).

Morphological measurement	Mean (mm)	Minimum (mm)	Maximum (mm)	SD
FA	57.63	45.00	64.80	7.26
HB	66.96	51.50	79.00	9.51
T	29.14	20.00	35.50	5.13
E	21.08	19.20	24.00	1.79
Ti	22.46	19.00	25.00	1.92
HF	9.81	8.00	11.20	1.06
GTL	24.03	21.80	25.50	1.27
CBL	21.16	19.00	22.60	1.24
CCL	20.38	18.40	22.00	1.13
ZB	13.28	12.00	14.00	0.71
BB	10.50	9.30	11.80	0.92
IC	3.20	2.90	3.60	0.22
PC	2.95	2.70	3.30	0.16
RW	8.63	7.80	9.20	0.46
ML	16.18	14.20	17.20	0.98
C–M^3	8.47	7.80	9.40	0.59
C–M_3	9.52	6.90	11.00	1.21
M^3–M^3	9.16	8.20	10.00	0.51
C^1–C^1	5.57	4.80	6.00	0.48

Remarks: Forearm length (FA), head-body length (HB), tail length (T), ear length (E), tibia length (TIB) and hind foot length (HF). The following cranial and dental measurements were taken: greatest length of skull (GTL), condylo-basal length (CBL), condylo-canine length (CCL), zygomatic breadth (ZB), braincase breadth (BB), interorbital constriction (IC), post orbital constriction (PC), mandible length (ML), rostral width (RW), maxillary toothrow (C–M^3), mandibular toothrow (C–M^3), posterior palatal width (M^3–M^3), and anterior palatal width (C^1–C^1).

measured and shown in Fig. 1B, 1C and 1D. The cranial morphometrics including greatest length of skull (GTL), condylo-basal length (CBL), condylo-canine length (CCL), zygomatic breadth (ZB), breadth of braincase (BB), interorbial constriction (IC), postorbital constriction (PC), rostral width (RW), mandible length (ML), maxillary toothrow (C–M^3), mandibulary toothrow (C–M_3), posterior palatal width (M^3–M^3), and anterior palatal width (C^1–C^1) are presented in Table 3. The skull has no orbital process. The dental formula is 1/2, 1/1, 2/2, 3/3=30. These results agree with Duengkae's (2006), Francis' (2008) and Lekagul and Mcneely's (1988) morphometric descriptions of the external morphology and skull of *H. larvatus* from Thailand.

Acknowledgments

This work was supported by Buriram Rajabhat University and the Applied Taxonomic Research Center (ATRC), Khon Kaen University grant ATRC_R5801. We wish to acknowledge the support of the Khon Kaen University Publication Clinic, Research and Technology Transfer Affairs, Khon Kaen University, for their assistance.

References

Ando, K., Tagawa, T. and Uchida, T. A. 1980. Karyotypes of Taiwanese and Japanese bats belonging to the families Rhinolophidae and Hipposideridae. Cytologia **45**: 423–432.

Bickham, J. W., McBee, K. and Schlitter, D. A. 1986. Chromosomal variation among seven species of *Myotis* (Chiroptera: Vespertilionidae). J. Mammal. **67**: 746–750.

Bogdanowicz, W. and Owen, R. D. 1998. In the Minotaur's Labyrinth: The phylogeny of the bat Family Hipposideridae. In: Kunz, T. H. and Racey, P. A. (eds.). Bats: Phylogeny, Morphology, Echolocation, and Conservation Biology.

Smithsonian Institution Press, Washington, D.C. pp. 27–42.

Bumrungsri, S., Harrison, D. L., Satasook, C., Prajukjitr, P., Thong-Aree, S. and Bates, P. J. J. 2006. A review of bat research in Thailand with eight new species records for the country. Acta Chiropt. **8**: 325–359.

Chaiyasut, K. 1989. Cytogenetics and Cytotaxonomy of the Family Zephyranthes. Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok.

Choudhury, R. C. and Patro, C. 1993. Karyotypic characteristics of an Indian Microchiroptera *Hipposideros fulvuspallidus* Anderson. Asian J. Exp. Sci. **7**: 19–23.

Duengkae, P. 2006. Bats of Thailand the Identification Field Guide. Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok.

Dulić, B. and Mutere, F. A. 1974. The chromosomes of two bats from East Africa: *Rhinolophus clivosus* Cretzschmar, 1828 and *Hipposideros caffer* (Sundevall, 1846). Period. Biol. **76**: 31–34.

Dulić, B. and Mutere, F. A. 1977. Chromosomes of some East African bats. Säugetierkundliche Mitteilungen **25**: 231–233.

Faria, K. C., Marchesin, S. R. C., Moreira, P. R. L., Beguelini, M. R. and Morielle-Versute, E. 2009. New insights into telomeric DNA sequence $(TTAGGG)_n$ location in bat chromosomes. Genet. Mol. Res. **8**: 1079–1084.

Francis, C. M. 2008. A Field Guide to the Mammals of Thailand and South-East Asia. Asia Book, Bangkok.

Garcia, J. P. and Pessôa, L. M. 2010. Karyotypic composition of bats from the Brazilian nuclear power plant, state of Rio de Janeiro. Chiroptera Neotropical **16**: 617–628.

Gomes, A J. B., Nagamachi, C. Y., Rodrigues, L. R. R., Farias, S. G., Rissino, J. D. and Pieczrka, J. C. 2012. Karyotypic variation in *Rhinophylla pumilio* Peters, 1865 and comparative analysis with representative of two subfamilies of Phyllostomidae (Chiroptera). Comp. Cytogenet. **6**: 213–225.

Gu, X. M. 2002a. A study on the karyotype, G-bands and C-bands of *Hipposideros armiger*. Journal of Guizhou Normal University (Natural Science) **3**: 9–11.

Gu, X. M. 2002b. The karyotype analysis of *Hipposideros armiger*. Chinese Journal of Zoology **37**: 19–21.

Gu, X. M. 2006. The karyotypes of six species of bats from Guizhou. Chinese Journal of Zoology **41**: 112–116.

Handa, S. M. and Kaur, S. 1980. Chromosome studies on three species of *Hipposideros* (Hipposideridae: Chiroptera). Caryologia **33**: 537–549.

Harada, M., Minezawa, M., Takada, S., Yenbutra, S., Nunpakdee, P. and Ohtani, S. 1982. Karyological analysis of 12 species of bats from Thailand. Caryologia **35**: 269–278.

Harada, M., Yenbutra, S., Tsuchiya, K. and Takada, S. 1985a. Karyotype of seven species of bats from Thailand. Experimentia **41**: 1610–1611.

Harada, M., Yenbutra, S., Yosida, T. H. and Takada, S. 1985b. Cytogenetical study of *Rhinolophus* bats (Chiroptera, Mammalia) from Thailand. Proc. Jpn. Acad., Ser. B, Phys. Biol. Sci. **61**: 455–458.

Hood, J. S. and Baker, R. J. 1986. G- and C-banding chromosomal studies of bats of the family Emballonuridae. J. Mammal. **67**: 705–711.

Hood, J. S., Schlitter, D. A., Georgudaki, J. I., Yenbutra, S. and Baker, R. J. 1988. Chromosomal studies of bats (Mammalia: Chiroptera) from Thailand. Ann. Carnegie Mus. **57**: 99–109.

Howell, W. M. and Black, D. A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: A 1-step method. Experientia **36**: 1014–1015.

Kleiman D. G., Geist, V. and Trunipey, I. E. 2004. Grzimek's Animal Life Encyclopedia, Volume 3: Mammal II. 2nd ed. Thomson Gale, Detroit.

Koubínová, D., Sreepada, K. S., Koubek, P. and Zima, J. 2010. Karyotypic variation in rhinolophid and hipposiderid bats (Chiroptera: Rhinolophidae, Hipposideridae). Acta Chiropt. **12**: 393–400.

Lekagul, B. and McNeely, J. A. 1988. Mammals of Thailand. 2nd ed. SahaKarnBhaet, Bangkok.

Mao, X., Nie, W., Wang, J., Su, W., Ao, L., Feng, Q., Wang, Y., Volleth, M. and Yang, F. 2007. Karyotype evolution in *Rhinolophus* bats (Rhinolophidae, Chiroptera) illuminated by cross-species chromosome painting and G-banding comparison. Chromosome Res. **15**: 835–847.

Morielle-Verielle, E., Taddei, V. A. and Varella-Garcia, M. 1992. Chromosome banding studies of *Chrotopterus auritus* (Chiroptera: Phyllostomidae). Rev. Bras. Genet. **15**: 569–573.

Morielle-Verielle, E. and Varella-Garcia, M. 1988. Variability of nucleolus organizer regions inphyllostomid bats. Rev. Bras. Genet. **11**: 853–871.

Noronha, R. C. R., Nagamachi, C. Y., Pieczarka, J. C., Marques-Aguiar, S., Assis, M. F. L. and Barros, R. M. S. 2004. Meiotic of the sex chromosomes in Carollinae-Phyllostomidae (Chiroptera): NOR separates the XY_1Y_2 into two independent parts. Caryologia **57**: 1–9.

Peterson, R. L. and Nagorsen, D. W. 1975. Chromosomes of fifteen species of bats (Chiroptera) from Kenya and Rhodesia. Life Science Occasional Papers, Royal Ontario Museum **27**: 1–14.

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.

Ray-Chaudhuri, S. P. and Pathak, S. 1966. Studies on the chromosomes of bats: List of worked out Indian species of

Chiroptera. Mammalian Chromosome Newsletter **22**: 206.
Ray-Chaudhuri, S. P., Pathak, S. and Sharma, T. 1971. Karyotypes of five Indian species of Microchiroptera. Caryologia **24**: 239–245.
Rickart, E. A., Mercier, J. A. and Heahey, L. R. 1999. Cytogeography of Philippine bats (Mammalia: Chiroptera). Proc. Biol. Soc. Wash. **112**: 453–469.
Rodrigues, L. R. R., Barros, R. M. S., Assis, M. F. L., Marques-Aguiar, S.A., Pieczarka, J. C. and Nagamachi, C. Y. 2000. Chromosome comparison between two species of *Phyllostomus* (Chiroptera-Phyllostomidae) from Eastern Amazonia, with some phylogenetic insights. Genet. Mol. Biol. **23**: 595–599.
Rodrigues, L. R. R., Barros, R. M. S., Assis, M. F. L., Marques-Aguiar, S.A., Pieczarka, J. C. and Nagamachi, C. Y. 2003. Comparative cytogenetics of two phyllostomids bats: A new hypothesis to the origin of the rearranged X chromosome from *Artibeus lituratus* (Chiroptera, Phyllostomidae). Caryologia **56**: 413–419.
Rooney, D. E. 2001. Human Cytogenetics: Constutitional Analysis: A Practical Approach. Oxford University Press, London.
Santos, N., Fagundes, V., Yonenaga-Yassuda, Y. and Souza, M. J. 2002. Localization of rRNA genes in Phyllostomidae bats reveals silent NORs in *Artibeus cinereus*. Heredity **136**: 137–143.
Schober, W. and Grimmberger, E. 1993. Bats of Britain and Europe. Hamlyn Publishing Group Limited, London.
Simmons, N. B. 2005. Order Chiroptera. In: Wilson, D. E. and Reeder, D. M. (eds.). Mammal Species of the World: A Taxonomic and Geographic Reference. 3rd edition. The Johns Hopkins University Press, Baltimore. pp. 312–529.
Souza, M. J. and Araújo, M. R. C. 1990. Conservation pattern of the G-bands and diversity of C-banding patterns and NORs in Stenodermatinae (Chiroptera-Phyllostomatidae). Rev. Bras. Genet. **13**: 255–268.
Sreepada, K. S., Naidu, K. N. and Gururaj, M. E. 1993. Trends of karyotypic evolution in the genus *Hipposideros* (Chiroptera: Mammalia). Cytobios **75**: 49–57.
Supanuam, P., Tanomtong, A., Khunsook, S., Pinthong, K. and Sanoamuang, L. 2012. Karyological analysis and morphometric of the lesser Asiatic house bat, *Scotophilus kuhlii* (Chiroptera, Vespertilionidae). Cytologia **77**: 401–411.
Suzuki, H., Sakurai, S. and Matsuda, Y. 1996. Rat 5S rRNA spacer sequence and chromosomal assignment of the gene to the extreme terminal region of chromosome 19. Cytogenet. Cell Genet. **72**: 1–4.
Varella-Garcia, M., Morielle-Versute, E. and Taddei, V. A. 1989. A survey of cytogenetic data on Brazilian bats. Rev. Bras. Genet. **12**: 761–793.
Vilamiu, R. P., Corrêa, M. M. O., Pessôa, L. M., Oliveira, J. A. and Oliveira, L. F. B. 2010. The karyotype of *Noctilio albiventris* (Chiroptera, Noctilionidae) from the northern Pantanal of Braziland its taxonomic implications. Mastozool. Neotrop. **17**: 219–224.
Volleth, M. 1987. Difference in the location of nucleolus organizer regions in European vespertilionid bats. Cytogenet. Cell Genet. **44**: 186–197.
Volleth, M., Heller, K. G., Pfeiffer, R. A. and Hameister, H. 2002. A comparative ZOO-FISH analysis in bats elucidates the phylogenetic relationship between Megachiroptera and five Microchiroptera families. Chromosome Res. **10**: 477–497.
Wilson, D. E. and Cole, F. R. 2000. Common Names of Mammals of the World. Smithsonian Institution Press, Washington, D.C.
Wu, Y., Harada, M. and Motokawa, M. 2009. Taxonomy of *Rhinolophus yunnanensis* Dobson, 1872 (Chiroptera: Rhinolophidae) with a description of a new species from Thailand. Acta Chiropt. **11**: 237–246.
Yi, W. and Harada, M. 2006. Karyology of seven species of bats (Mammalia: Chiroptera) from Guangdong, China. Acta Theriologica Sinica **26**: 403–406.
Zhang, W. and Wan, M. 1992. Chromosomal analysis of *Taphozous melanopogon* and *Hipposideros armiger*. Acta Theriologica Sinica **12**: 306–307.