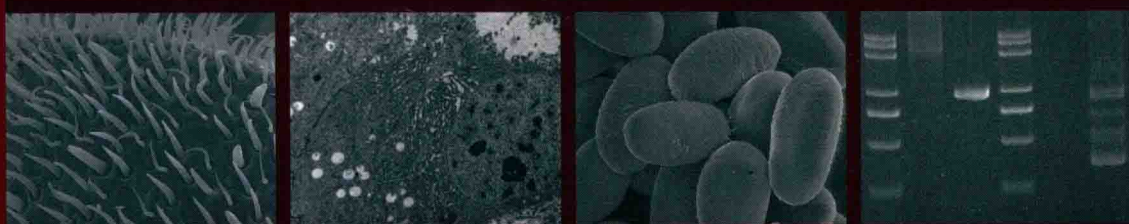吴 杰 李继莲 等著

熊蜂

生物学研究

XIONGFENG
SHENGWUXUEYANJIU

吴 杰 李继莲 等著

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本书精选了作者在熊蜂生物学研究领域具有代表性的研究论文，对熊蜂的分类、生理及环境因子3方面的研究成果进行了阐述。

　　本书可供从事蜜蜂研究和生产的相关人员参考，同时可供中小型蜂产品加工厂技术人员、蜂产品自营店店主、蜂农和养蜂爱好者参考。

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前　言

熊蜂介于高级社会性蜜蜂和独居蜂之间，属于膜翅目蜜蜂总科熊蜂属。熊蜂是自然界众多野生植物，特别是高山植物、高原植物以及一些濒危植物的重要传粉者，在保持生物多样性及维持生态系统平衡方面发挥着十分重要的作用。同时，熊蜂还具有进化程度低、趋光性差、活动起点温度低等生物学特性，比家养蜜蜂更容易适应温室环境，因而成为温室果菜传粉的理想昆虫，在设施果菜生产中发挥着十分重要的作用。

中国地域辽阔，气候与植被类型多样，具有熊蜂栖息的多种生境。据报道，全世界熊蜂种类大约有 250 种。中国现有熊蜂 120 多种，是世界上熊蜂种质资源最丰富的国家。我国对于熊蜂的研究起步较晚，1998 年，中国农业科学院蜜蜂研究所在国内首次突破了野生熊蜂人工繁育技术，并从我国本土 120 多种野生熊蜂中成功筛选出可以人工利用的熊蜂 6 种：明亮熊蜂（*Bombus lucorum*）、密林熊蜂（*Bombus hypocrita*）、兰州熊蜂（*Bombus lantschouensis*）、红光熊蜂（*Bombus ignitus*）、火红熊蜂（*Bombus pyrosoma*）和重黄熊蜂（*Bombus picipes*），并成功应用于设施果菜授粉，取得了明显的经济效益和生态效益。但是，与发达国家相比，我国本土熊蜂工厂化繁育规模小、商品化程度不高。目前，我国需要授粉的设施果菜约有 600 万公顷，熊蜂授粉技术应用的市场前景十分广阔。

近 5 年来，中国农业科学院蜜蜂研究所就我国熊蜂资源进行了调查，在调查过程中发现有一些种的分布范围很窄，如越熊蜂（*Bombus supremus*），该种群只分布在我国的青藏高原，未见有报道分布于其他国家，是高原上重要的野生植物授粉昆虫。通过近 3 年来多次的调查发现，该种数量很少，有下降的趋势。因此，开展熊蜂生物学的研究，对于进一步加强我国熊蜂资源的保护和利用也具有重要的意义。

本书是中国农业科学院蜜蜂研究所"传粉蜂生物学与授粉应用创新团队"近年来在熊蜂分类学、生理学和病虫害及农药对熊蜂的影响等方面开展系统研究的最新成果，具体包括以下内容。

第 1 部分：熊蜂分类方面的研究进展，此章节的作者有吴杰、黄家兴、安建东、刘苹等；第 2 部分：熊蜂生理方面的研究进展，此章节的作者有吴杰、李继莲、黄家兴、杨慧鹏、靳婷婷、孙冬婷、高丽娇、董捷等；第 3 部分：熊蜂与环境因子方面的研究进展，此章节的作者有李继莲、罗术东、徐龙龙、彩万志、彭文君、陈文锋、郭军等。

限于笔者水平，书中难免有不当与疏漏之处，敬请读者批评指正！

著者
2017 年 5 月

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第1部分

熊蜂分类方面的研究进展

Newly discovered colour-pattern polymorphism of *Bombus koreanus* females (Hymenoptera: Apidae) demonstrated by DNA barcoding

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Abstract   *Bombus* (*Megabombus*) *koreanus* (Skorikov) is a long-tongued bumblebee that specialises in visiting deep flowers. To describe the distribution, colour-pattern variation and food plants of *B. koreanus,* we collected specimens and DNA cytochrome oxidase I (COI) barcodes were obtained. Bayesian phylogenetic analysis of barcodes for *Megabombus* species shows that *B. koreanus* is one of the more recently diverged species and the sister species to *Bombus consobrinus* . Our results show that specimens with previously unknown colour patterns also belong to *B. koreanus* . We describe nine different colour patterns for workers and three for queens, in which the hair of the thorax varies from black to orange-brown. However, there is a lack of covariation between COI and colour-pattern variation. *B. koreanus* has been recorded to visit 11 species of food plants showing that *B. koreanus* is polylectic. Our results demonstrate that COI barcoding is an effective method for studying closely related taxa and for recognising species with variable colour patterns.

Key Words: *Bombus koreanus* / DNA barcoding / taxonomy / colour pattern

1   INTRODUCTION

Bumblebees (genus *Bombus* Latreille) are important pollinators for many wild flowers and for economically important food crops (Velthuis and van Doorn 2006; Grixti et al. 2009; Hou et al. 2009). They are especially efficient pollinators for Leguminosae and Solanaceae, which benefit from their particular ability in buzz pollination. Since the

1980s, bumblebees have been used commercially to pollinate tomatoes, eggplants and strawberries in greenhouses because of lower production costs, increased yields and improved fruit qualities (Ravestijn and Sande 1991; Abak et al. 1995; Dimou et al. 2008). In recent years, the decline and localised extinction of wild pollinators has raised concern in different continents (Williams 1982; Goulson et al. 2006; Colla and Packer 2008). Thus, understanding the distribution of bumblebee species and their habitat requirements has become a vital activity for their conservation, which depends on accurate species identification. Here, we seek to recognise an important pollinator species in Asia.

Species identification has been difficult in bumblebees because of the high variability of colour patterns within species, with high rates of colour-pattern evolution even exceeding those in mitochondrial genes (Hines and Williams 2012). In addition, colour patterns often converge among bumblebee species depending on geography and habitat (Williams 2007; Duennes et al. 2012; Lozier et al. 2013). This is made all the more difficult because there are few reliable detailed descriptions of the diagnostic morphological characters of Asian bumblebee species, particularly for those from China, which includes about half of the world's bumblebee species (Williams et al. 2010). Therefore, it is difficult to identify species reliably, especially without access to the type specimens. Consequently, individuals with new colour patterns are easily mistaken for new species.

B. koreanus is a medium-sized and longtongued bumblebee species that belongs to the subgenus *Megabombus* Dalla Torre (Williams 1998) with morphology similar to *Bombus consobrinus* Dahlbom. Until now, females of *B. koreanus* (Skorikov) are expected to have the hair of the thorax and of metasomal terga 1—3 black and that of terga 4—5 is expected to be ochre yellow. This dark colour pattern of *B. koreanus* was first described from specimens collected in Korea by Skorikov (1933) and has been reported from both Korea and China (Skorikov 1933; Tkalcu 1961; Kim and Ito 1987; Lee and Dumouchel 1999). Sakagami (1976) later compared the morphology of *B. koreanus*, *B. hortorum* (Linnaeus), *B. argillaceus* (Scopoli), *B. consobrinus* and *B. yezoensis* Matsumura in trying to distinguish these taxa in eastern Asia. Recently, *B. koreanus* has been reported in China from Jilin province by Ruan et al. (2007), while An et al. 2008, 2011, 2014 have reported it from Shanxi, Heilongjiang, Liaoning, Beijing, Shaanxi and Gansu. However, these authors were reporting the distribution of the species based on its typical dark colour pattern. Its distribution, food plants and colourpattern polymor-phism have not been investigated in detail before.

In recent years, with the development of molecular methods, it has become much easier and more reliable to estimate phylogenetic relationships and to identify species. Kawakita et al. (2004) estimated the phylogeny, historical biogeography and character evolution in bumblebees based on an analysis of three nuclear genes. Their study

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confirmed that *B. koreanus* belongs to the subgenus *Megabombus* and that it is the sister species to *B. consobrinus*. Cameron et al. (2007) analysed many more species using four nuclear genes and one mitochondrial gene to gain a similar but even more strongly supported result. However, these studies concentrated on the more distant relationships among taxa and used very few specimens within species to assess closer relationships near the rank of species. In contrast to the nuclear genes in these studies, cytochrome oxidase I (COI) barcodes are relatively fast evolving and conse-quently have become popular for species identification and for studying relationships near the rank of species. COI barcodes provide an easily obtained, reliable and cost-effective solution, especially for morphologically cryptic species (Hebert et al. 2003). Consequently, COI barcodes have been used to re-evaluate species, estimate phylogenetic relationships and clarify species complexes in Asian bumblebees (Williams et al. 2011, 2012a, b, 2013)and therefore should be appropriate to assess colour-pattern polymorphism within the subgenus *Megabombus* .

In this study, COI barcodes are used to explore colour-pattern polymorphism by estimating the phylogenetic relationships among samples of *B. koreanus* and its close relatives, which include the species *B. consobrinus* . The geographic distribution and food plants of *B. koreanus* are described from the new samples, published studies and museum collections. Thus, the results of this work contribute basic information that is essential to the conservation of *B. koreanus* in the wild and for its possible utilisation in crop pollination.

2 MATERIALS AND METHODS

2.1 Materials

Samples of bumblebees of the subgenus *Megabombus* were collected each year from 2005 to 2012, from July to September. The sample sites are in north, northeast and northwest China, which cover the distribution of *B. koreanus* in China expected from previous collections (Figure 1). Detailed information regarding the collection site name, date, elevation and location were recorded using a hand-held GPS (Garmin 60CS; Table I). At the same time, records and photographs of the food plants visited by *B. koreanus* were made. Specimens of bumblebees were pinned, labelled, dried and deposited in the Chinese Academy of Agricultural Sciences, Institute of Apiculture, Beijing, China. Food plants were identified from photographs by Dr. Lei Meng of China Agricultural University, and all plant names were checked against the International Plant Names Index (http://www.ipni.org, accessed 2013).

Figare 1. Distribution of *B. koreanus* based on specimen data from museum collections and literature records. *Black spots* show records of the *B. koreanus* specimens examined; *black triangles* show records of *B. koreanus* from the literature; and *grey spots* show records of all bumblebees in the IAB database. [参见：Apidologie. 2015, 46(2): 250-261.]

Table I   Material used in the phylogenetic analysis with the sample localities, collector, COI sequence length, depository and GenBank accession number. Duplicate COI sequences of *B. koreanus* were removed.

Taxon	Sample locality	Collector	Latitude	Longitude	Depository	Sequence length (bp)	Genbank acc. no.
haemorrhoidalis	TH, Doi Phu Kha NP	Charoen and Nikom	N 19.2022	E 101.079	L. Packer	658	4746 F01*
trifasciatus	CN, Xinglongcun	J. Guo	N 28.91	E 107.27	IAB	658	KJ734238
gerstaeckeri	AT, Pongau	A. Aichhorn	N 47.276	E 13.111	P. Williams	407	KJ734239
gerstaeckeri	DE, Schachen GAP	J. Voith	N 47.572	E 11.128	BSCZ	654	FBHAP779-09*
argillaceus	IR, Werk	A. Monfared	N 36.454	E 50.687	NHM	658	KJ746617
consobrinus	KR, Nodong-li	Tripotin	N 37.7013	E 128.482	P. Rasmont	658	HQ553043
consobrinus	KR, Nodong-li	Tripotin	N 37.7013	E 128.482	P. Rasmont	658	HQ553042
czerskii	CN, Majiazi	J. Huang	N 43.22	E 117.32	IAB	658	KJ734240
czerskii	CN, Majiazi	J. Huang	N 43.22	E 117.32	IAB	658	KJ734241
hortorum	CN, Nalati	Z. Zhou	N 43.19	E 84.34	IAB	658	KJ734242
hortorum	CN, Guozigou	Z. Zhou	N 44.46	E 81.11	IAB	658	KJ734243
koreanus	CN, Chengxian	J. Huang	N 33.72	E 105.61	IAB	658	KJ734244
koreanus	CN, Xiaolongmen	J. Huang	N 39.97	E 115.43	IAB	658	KJ734245
koreanus	CN, Baihuashan	J. Huang	N 40.53	E 115.75	IAB	658	KJ734246
koreanus	CN, Ciba	J. An	N 33.8	E 106.63	IAB	658	KJ734247
koreanus	CN, Ciba	J. An	N 33.8	E 106.63	IAB	658	KJ734248
koreanus	CN, Shimen	Z. Miao	N 34.41	E 106.11	IAB	658	KJ734249
koreanus	CN, Liuba	Y. Zhang	N 34.78	E 107.18	IAB	658	KJ734250
portchinsky	TY, Kars	P. Rasmont	—	—	P. Rasmont	658	KJ734251
portchinsky	TY, Artvin	M. Aytekin	N 41.076	E 42.208	IAB	658	KJ734252
religiosus	CN, Yehegu	S. Luo	N 35.51	E 106.23	IAB	658	KJ734253
religiosus	CN, Leidongping	X. Zhang	N 29.5	E 103.33	IAB	658	KJ734254
ruderatus	UK, Kent	P. Williams	N 51.35	E 1.43	IAB	658	KJ734255
ruderatus	UK, Kent	J. Huang	N 51.334	E 0.178	IAB	658	KJ746616
securus	CN, Dingri	Z. Miao	N 28.61	E 87.13	IAB	658	KJ734256
securus	CN, Xiaozhongdian	C. Liang	N 21.45	E 99.82	IAB	658	KJ734257
supremus	CN, Dachamuchang	J. Huang	N 38.69	E 99.52	IAB	658	KJ734258
supremus	CN, Xiega	Z. Miao	N 31.97	E 96.32	IAB	658	KJ734259

Taxon	Sample locality	Collector	Latitude	Longitude	Depository	Sequence length (bp)	Genbank acc. no.
sushkini	MN, Hovsgol Nuur	D. Sheppard	N 51.05	E 100.733	P. Williams	658	GU674510
sushkini	MN, Hovsgol Nuur	D. Sheppard	N 51.05	E 100.733	P. Williams	658	GU674515

* the asterisks means that the sequence was deposited in BOLD and the number is the BOLD ID

Data were also compiled from the collections of the Natural History Museum, London; Institute of Zoology, Chinese Academy of Sciences, Beijing; and China Agricultural University, Beijing, China. Locality information was obtained from specimen labels and published studies or estimated from Google Earth (V7.1.2.2041, accessed 2013).

2.2 Methods

2.2.1 DNA extraction

Specimens with different colour patterns had DNA extracted from the right foreleg or midleg using Wizard SV 96 kit (Promega Inc.), with a slight modification for the protocol. Legs were ground in a 0.5-mL Axygen tube in liquid nitrogen using a stainless steel pestle. Thereafter, total DNA was extracted following the manufacturer's protocol.

2.2.2 COI barcode sequence amplification

For PCR amplification and sequencing of the short 5′ barcode region of the mitochondrial COI gene, the universal insect primers LepF1 and LepR1 were used (Hebert et al. 2004). PCR amplification was carried out in 50μL, including 25μL PCR Mix (2×), 2μL primers (10μM) of each end and approximately 50ng of template DNA. The remaining volume was filled with ddH$_2$O. PCR amplification was done using the Eppendorf T-Gradient amplifier according to the following procedure: denaturation at 95℃ for 5min, followed by 35 cycles of 30s at 95℃, 30s at 52℃, 60s at 72℃ and a final extension at 72℃ for 10min. The amplified PCR product was then stored at 4℃ for further use. PCR products were checked under UV light after electrophoresis on a 1% agarose gel. Positive PCR products were sequenced from both ends by a commercial company (BioMed Beijing CO., LTD.).

2.2.3 Data analysis

Sequences were examined using BioEdit (version v7.2.3; http://www.mbio.ncsu.edu/BioEdit/page2.html, accessed 2013), with which the primers were also removed from both ends (Table I). Sequences were aligned with the ClustalX2 program (version 2.0; Larkin et al. 2007). Nucleotide composition and informative sites were

examined with MEGA software (version 5.1; Kimura 1980). The jModelTest (version 2.1.3) program was used to select the best nucleotide substitution model using the default parameters (Darriba, et al. 2012). The general time-reversible model with a gamma frequency distribution gave the best fit to our COI barcode dataset. We used *B. haemorrhoidalis* (Smith) and *B. trifasciatus* (Smith) as out-groups based on the results of Cameron et al. (2007). Phylogenetic relationships were estimated using the MrBayes software (version 3.2) (Ronquist et al. 2012). Two simultaneous analyses were performed using Markov Chain Monte Carlo (MCMC) simulations for 10 million generations with a sampling frequency of one in every 1000 generations. Burn-in was set to 10% of the generations, and the standard deviation of split frequencies was confirmed to be less than 0.01 (Darriba et al. 2012). Post burn-in sample trees from both replicates were combined and checked for convergence using Tracer (version 1.5.0; Rambaut 2007). The consensus tree was displayed and edited using FigTree software (version 1.4.0).

3 RESULTS

3.1 COI barcode sequence analyses

We found 510 conserved sites, 81 variable sites and 67 singleton sites in the *Megabombus* COI barcode sequences. All of the sequences were 658 bp long after removing the primers from both ends. The average content of A, T, G and C was 31.9, 44.6, 13.1 and 10.4 %, respectively. We found a strong A+T bias in the COI barcode sequence, with an A+T content of 76.5 % compared to the G+C content of 23.5 %. This bias supports the interpretation that all of the COI barcoding sequences came from mtDNA.

DNA was extracted and the COI barcode sequence was amplified successfully from 30 *B. koreanus*-like specimens, representing all of the new *B. koreanus* colour patterns. Among these sequences, we found seven unique haplotypes, with six polymorphic sites (Table II). Haplotype 1 was widespread among samples from Beijing, Shanxi, Shaanxi, Hebei and Gansu provinces. Haplotype 2 is a local type found only in Gansu, haplotypes 3 and 4 were found only in Beijing and haplotypes 5, 6 and 7 were found only in Shaanxi.

Table II   Haplotypes of the COI barcode sequence among *B. koreanus* samples.

Haplotypes	Nucleotide position					
	25	88	199	235	500	565
B. koreanus 1	A	T	A	T	T	A
B. koreanus 2	G	T	A	C	T	A
B. koreanus 3	A	T	A	T	C	A
B. koreanus 4	A	A	A	T	T	A

Haplotypes	Nucleotide position					
	25	88	199	235	500	565
B. koreanus 5	G	T	A	T	T	A
B. koreanus 6	A	T	G	T	T	A
B. koreanus 7	A	T	A	T	T	G

3.2　Phylogeny of *Megabombus*

Results of phylogenetic analysis (Figure 2) showed that most of the currently recognised species form groups with high posterior probabilities, strongly supporting the monophyly of these species. However, some deep nodes with posterior probabilities <0.8 are not well supported in our results. There is a strong support (posterior probability 1)for including many *B. koreanus*-like specimens that have the new extensively orange colour patterns within a single group, which is interpreted here as the species *B. koreanus* . Our results also support *B. koreanus* as the sister species to the paler, longer-haired species, *B. consobrinus* .

3.3　Colour-pattern variation

Simple diagrammatic coding to summarise the colour variation of *B. koreanus* shows that some specimens of *B. koreanus* are quite different in colour pattern from the type specimen (Figures 3 and 4). The colour variation detected among workers is much greater than among queens. The colour pattern of the hair of the workers' thorax varies from completely black to completely orange-brown (this pale pattern is similar to all males). In addition, some workers have extensive orange-brown hair on the metasoma. The previ-ously described and common colour pattern of the hair of the thorax and of metasomal terga 1—3 is black and of terga 4—5 ochre is yellow. Figure 5 shows nine colour patterns of *B. koreanus* from Beijing, which is the highest number of colour patterns among all of the regions. There were many other slight variations in intermediate colour patterns among worker specimens from west Beijing. Most of them were collected from the tran-sition zone between the Taihang Mountain and Yanshan mountains of north China, such as from Lingshan nature reserve, Xiaowutai nature reserve and Baihuashan nature reserve.

3.4　Geographical distribution

Our revised concept of *B. koreanus,* based on COI barcodes, is of a species more broadly distributed in Korea and China (Figure 1): it extends from northeastern China up to the edge of the east Tibetan Plateau in Central China, and through Liaoning, Hebei,

Beijing, Shanxi, Henan, Shaanxi and Gansu Provinces. From our collections, *B. koreanus* has an elevation range of 500—2000m (mean 1104m), with its greatest abun-dance in habitats from 1000 to 1500m. This pattern of elevational distribution is not affected substantially by latitude or longitude.
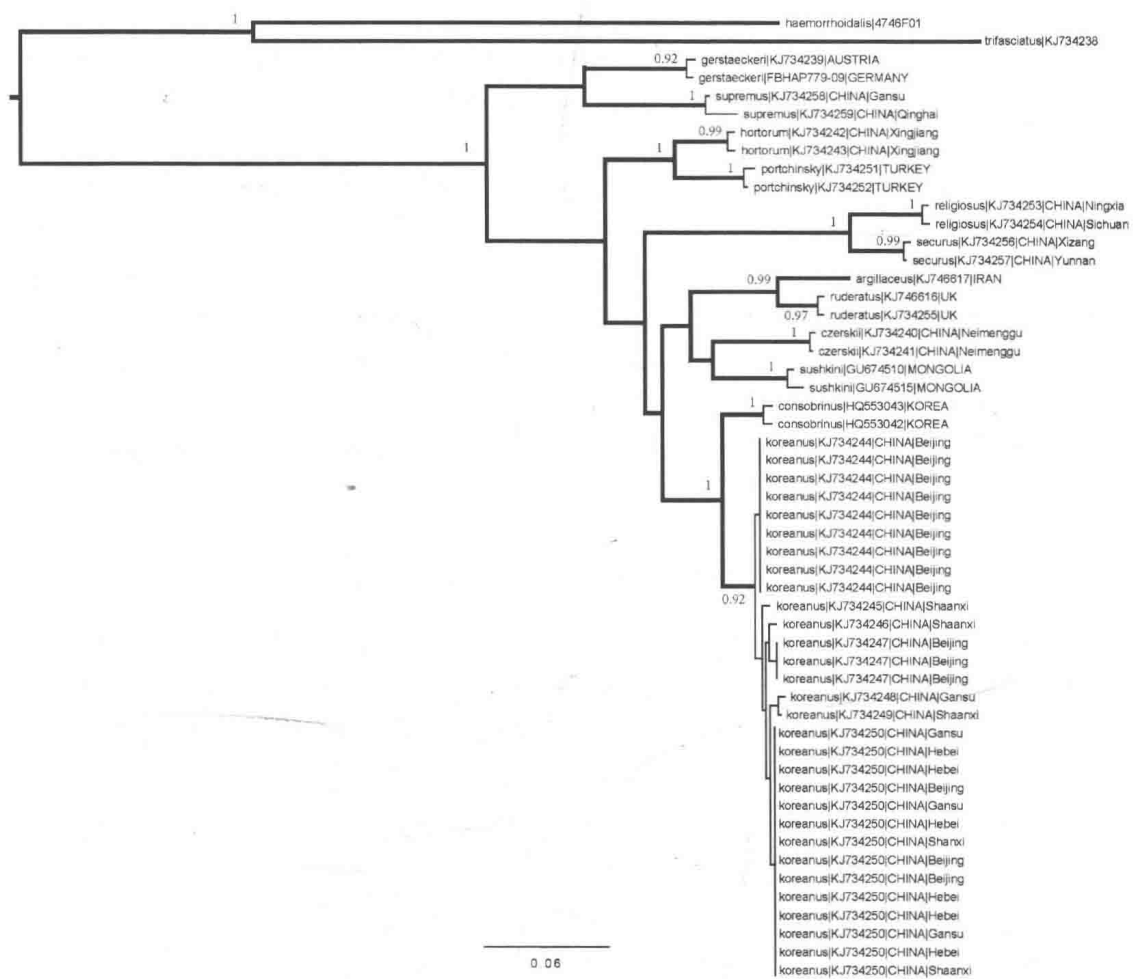

Figure 2. Estimate of the phylogenetic relationships within the subgenus *Megabombus* from COI barcode sequences by MrBayes. The *numbers* at *nodes* are the posterior probability support values. The *grey box* covers *B. koreanus* as recognised here. The *scale bar* represents 0.06 expected substitutions per nucleotide site.

3.5 Forage plants

B. koreanus is known to be a long-tongued bumblebee species that can collect nectar and pollen from many species of plants, making it polylectic. According to data for forage plants collected between 2005 and 2012 in China, this species is confirmed to visit 11 plant species (*Cosmos bipinnatus Cav., Cirsium leo Nakai et Kitag.; Cucurbita moschata Duch., Cucumis sativus Linn.; Vicia cracca Linn.; Impatiens balsamina Linn.; Elsholtzia ciliate Hyland.; Leonurus sibiricus Linn.; Malva cathayensis M. G. Gilbert, Y. Tang et*

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