Phylogeny and species differentiation of *Mollitrichosiphum* spp. (Aphididae, Greenideinae) based on mitochondrial COI and Cyt b genes

Ruiling ZHANG\(^1,2\), Xiaolei HUANG\(^1\), Liyun JIANG\(^1\), Gexia QIAO\(^1*\)

\(^1\)Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
\(^2\)Graduate University of Chinese Academy of Sciences, Beijing 100049, China

**Abstract** Phyllogenetic analyses based on mitochondrial genes were conducted to reconstruct species relationships within the aphid genus *Mollitrichosiphum* (Aphididae, Greenideinae). MP and Bayesian analysis results using COI and Cyt b datasets, and combined MP, ML and Bayesian analysis of both were consistent with a morphologically supported monophyly. Subdivision of the genus into two subgenera was strongly supported. Samples of each included species form monophyletic clade, respectively; and the result implied the valid status of related species in this genus. These results suggest some surprising hypotheses regarding the phylogeography of the genus: the uplift of the Tibetan Plateau, reorganization of major river catchments and the isolation of Hainan Island were probably important factors contributing to the diversification of species in this genus [Current Zoology 57 (6): 806–815, 2011].

**Keywords** *Mollitrichosiphum*, Geographical barrier, Phylogeny, Tibetan Plateau, Hainan Island

The genus *Mollitrichosiphum* (Aphididae, Greenideinae) was created by Suenaga in 1934, based on hind tibiae with numerous transverse ridges, 6-segmented antennae, and an elongated and pointed ultimate rostral segment. This genus contains 18 known species restricted mainly to southeast Asia, and divided into two subgenera, *Mollitrichosiphum* and *Metatrichosiphon* (Remaudière and Remaudière, 1997). Aphids of this genus typically colonize the young leaves and branches of host plants in the Betulaceae, Elaeagnaceae, Fagaceae, Juglandaceae, Lauraceae, Meliaceae, Proteaceae, Sabiaceae and Sapindaceae families, and more rarely the Simaroubaceae, Anacardiaceae and Rosaceae. This genus is polyphagous with a wide host range (Zhang and Qiao, 2010).

There are 11 known species of *Mollitrichosiphum* in China, distributed from the Himalayas to southern China and Taiwan, recognized biogeographic ‘hotspots’. Following the collision of the Indian plate with Eurasia, the Tibetan Plateau and the Himalaya region began to uplift forming high mountains and deep gorges within and around the plateau (Li et al., 1995). This significant geological event led to large-scale climate changes (Raymo and Ruddiman, 1992; An et al., 2001; Spicer et al., 2003), including a general transition towards cooler temperatures with more pronounced seasonality over large areas (Jen, 1982; Axelson et al., 1998). The profound changes in topography and climate greatly affected the evolution and distribution of fauna and flora (Ruan et al., 2005; Wang et al., 2005; Liu et al., 2006; Hou et al., 2007; Wang et al., 2007; Huang et al., 2008; Shih et al., 2009). South China was an important centre of speciation (Wu, 1965; Wang, 1992; Ying et al., 1993; Ying, 2001) because of dramatic climatic change that significantly affected the population structure and distribution patterns of many taxa (Zhu, 2008; Wang, 2009).

Although several studies have tested the effects of the uplift of the Tibetan Plateau and Pleistocene glaciation oscillations on phylogeographical patterns of vertebrate and plant species endemic to the Plateau (Zhang et al., 2005; Wang and Ge, 2006; Yang et al., 2006; Zhang and Jiang, 2006; Liu et al., 2007; Qu et al., 2009, 2010) or in southern China (Huang et al., 2007; Zhang et al., 2008; Song et al., 2009), few studies have tested the effects of geological change and climate oscillations at a larger geographical scale (e.g. considering these two regions together) and above species level. *Mollitrichosiphum* is an excellent model for the investigation of prehistoric patterns of species distribution and radiation, because
they have low dispersal capabilities compared to vertebrate groups such as birds and mammals and they are relatively abundant and easy to capture. Furthermore, species of this genus show extreme host specificity and seem to be easily affected by both geographic and climatic events.

In order to better understand the effects of prehistoric events on the origin and variation of *Mollitrichosiphum* species, clarification of the phylogenetic relationships within the genus was needed. Previous studies of this genus were alpha-taxonomic and species descriptions based on morphology alone (Zhang et al., 1983; Tao, 1990, 1999; Noordam, 1994; Zhang and Qiao, 2010). No phylogeny of this genus has been estimated. Here, we present the first molecular phylogenetic study of *Mollitrichosiphum* based on variation in the mitochondrial Cytochrome C Oxidase Subunit I (*COI*) and Cytochrome *b* (*Cyt b*) genes between seven species collected throughout their distribution ranges. Our objectives were to: (1) determine whether molecular data corroborated the existing morphological taxonomy; (2) test the apparent monophyly of the genus and elucidate phylogenetic relationships among species; and (3) determine the influence of geological changes and climate oscillations to the evolutionary history of *Mollitrichosiphum*.

1 Materials and Methods

1.1 Taxon sampling

Samples used in this study, collection localities and host plants are listed in Table 1. Samples were collected between 2000 and 2010 from throughout most of the genus’ range; except for specimens for slide-mounting that were stored in 75% ethanol for morphological examination, all other samples were stored in 95% ethanol for molecular studies. Seven species sampled in the Himalayas and southern China were used for phylogenetic analyses. Outgroups were chosen from *Cervaphis* van der Goot (Cervaphidini), *Eutrichosiphum* Essig and Kuwana (Greenideini), *Greenidea* Schouteden (Greenideini) and *Kurisakia* Takahashi (Thelaxinae). Voucher specimens from each collection were mounted on microscope slides and deposited in the National Zoological Museum of China, Chinese Academy of Sciences, Beijing.

Table 1  Aphid samples examined in this study

| Species                        | Voucher No. | Collection locality     | Host plant               | GenBank Accession Nos: COI/Cyt b |
|-------------------------------|-------------|-------------------------|--------------------------|----------------------------------|
| Ingroups                      |             |                         |                          |                                  |
| *M. tenuicorpus* (Okajima)    | 20938       | Hainan province: Jianfengling | *Castanopsis fabri* Hance (Fagaceae) | JF969334 / JF969383               |
|                               | 13361       | Yunnan province: Baoshan | *Meliosma rigida* (Sabiaceae) | JF969308 / JF969357               |
|                               | 24074       | Yunnan province: Ruili   | *Castanopsis calathiformis* (Fagaceae) | JF969339 / JF969388               |
|                               | 24067       | Yunnan province: Ruili   | *Castanopsis calathiformis* (Fagaceae) | JF969343 / JF969392               |
|                               | 14421       | Fujian province: Wuyishan | *Castanea* sp. (Fagaceae) | JF969311 / JF969360               |
|                               | 14537       | Fujian province: Wuyishan | *Castanopsis sclerophylla* (Fagaceae) | JF969313 / JF969362               |
|                               | 18506       | Hainan province: Diaoluoshan | *Cyclobalanopsis neglecta* (Fagaceae) | JF969321 / JF969370               |
|                               | 19242       | Hainan province: Changjiang | Fagaceae               | JF969327 / JF969376               |
|                               | 20866       | Hainan province: Jianfengling | Fagaceae               | JF969333 / JF969382               |
|                               | 22152       | Fujian province: Nanjing | unknown                 | JF969335 / JF969384               |
|                               | 22155       | Fujian province: Huboliao | unknown                 | JF969336 / JF969385               |
|                               | 19521       | Hainan province: Jianfengling | *Quercus* sp (Fagaceae) | JF969329 / JF969378               |
|                               | 22166       | Fujian province: Huboliao | unknown                 | JF969337 / JF969386               |
|                               | 18614       | Guangdong province: Chebaling | *Castanopsis carlesii* (Fagaceae) | JF969347 / JF969396               |
|                               | 18892       | Guangxi Auto. Reg.: Longsheng | Fagaceae               | JF969348 / JF969397               |
|                               | 20530       | Yunnan province: Simao   | *Castanopsis ferox* (Fagaceae) | JF969330 / JF969379               |
|                               | 15381       | Tibet Auto. Reg.: Motuo  | *Alnus cremastogyne* (Betulaceae) | JF969317 / JF969366               |

(to be continued on the next page)
| Species Voucher No. | Collection locality | Host plant | GenBank Accession Nos: COI / Cyt b |
|---------------------|---------------------|------------|---------------------------------|
| **Ingroups**        |                     |            |                                 |
| *M. nigrum* (Zhang and Qiao) | 14417 Fujian province: Wuyishan | *Elaeagnus pungens* (Elaeagnaceae) | JF969310 / JF969359 |
|                     | 19258 Guangxi Auto. Reg.: Xingan | *Ailanthus altissima* (Simaroubaceae) | JF969328 / JF969377 |
|                     | 21845 Hunan province: Mangshan | unknown | JF969341 / JF969390 |
|                     | 21856 Guangdong province: Nanling | unknown | JF969342 / JF969391 |
|                     | 18913 Guangxi Auto. Reg.: Longsheng | *Meliosma cuneifolia* (Sabiaceae) | JF969326 / JF969375 |
| *M. nandii* (Busu) | 14712 Yunnan province: Baoshan | *Ailanthus cremastogyne* (Betulaceae) | JF969315 / JF969364 |
|                     | 18382 Tibet Auto. Reg.: Tongmai | *Fagus longipetiolata* (Fagaceae) | JF969320 / JF969369 |
|                     | 15370 Tibet Auto. Reg.: Motuo | unknown | JF969316 / JF969365 |
|                     | 23101 Sichuan province: Xichang | *Psidium guajava* (Myrtaceae) | JF969345 / JF969394 |
| *M. nigrofasciatum* (Maki) | 14560 Fujian province: Wuyishan | *Lithocarpus glaber* (Fagaceae) | JF969314 / JF969363 |
|                     | 14805 Fujian province: Wuyishan | *Cyclobalanopsis glauca* (Fagaceae) | JF969346 / JF969395 |
|                     | 22101 Fujian province: Liangyeshan | *Lithocarpus glaber* (Fagaceae) | JF969331 / JF969380 |
|                     | 21966 Guangdong province: Chabaling | unknown | JF969350 / JF969399 |
|                     | 21916 Guangdong province: Nanling | unknown | JF969349 / JF969398 |
| *M. rhusae* (Ghosh) | 18511 Hainan province: Lingshui | *Helicia hainanensis* (Proteaceae) | JF969323 / JF969372 |
|                     | 18514 Hainan province: Lingshui | *Helicia hainanensis* (Proteaceae) | JF969325 / JF969374 |
|                     | 20811 Hainan province: Wuzhishan | Fagaceae | JF969331 / JF969380 |
|                     | 20858 Hainan province: Diaoluoshan | Meliaceae | JF969332 / JF969381 |
|                     | 18513 Hainan province: Diaoluoshan | Fagaceae | JF969324 / JF969373 |
|                     | 18508 Hainan province: Diaoluoshan | *Helicia hainanensis* (Proteaceae) | JF969322 / JF969371 |
| *M. luchuanum* (Takahashi) | 14414 Fujian province: Wuyishan | *Amygdalus persica* (Rosaceae) | JF969309 / JF969358 |
|                     | 14488 Fujian province: Wuyishan | *Amygdalus persica* (Rosaceae) | JF969312 / JF969361 |
|                     | 18104 Fujian province: Wuyishan | *Meliosma rigida* (Sabiaceae) | JF969319 / JF969368 |
|                     | 21910 Guangdong province: Nanling | unknown | JF969340 / JF969389 |
| *M. montanum* (van der Goot) | 16504 Tibet Auto. Reg.: Zhangmu | unknown | JF969318 / JF969367 |
|                     | 23754 Yunnan province: Qingliang | *Alnus nepalensis* (Betulaceae) | JF969338 / JF969387 |
|                     | 18324 Tibet Auto. Reg.: Linzhi | unknown | JF969344 / JF969393 |
| **Outgroups**       |                     |            |                                 |
| *Eutrichosiphum* sp. | 15506 Sichuan province: Mabian | unknown | JF969352 / JF969401 |
| *Eutrichosiphum* tattakanum (Takahashi) | 17184 Sichuan province: Miyi | unknown | JF969353 / JF969402 |
| *Greenidea* ficicola (Takahashi) | 18764 Yunnan province: Dali | *Ficus benjamina* (Moraceae) | JF969354 / JF969403 |
| *Kurisakia* querciphila (Takahashi) | 16201 Guizhou province : Leigongshan | Fagaceae | JF969355 / JF969404 |
| *Cervaphis* echinata (Hille Ris Lambers) | 18461 Hainan province: Jianfengling | *Paulownia* sp. (Sorophulariaeae) | JF969356 / JF969405 |
1.2 DNA extraction, PCR and sequencing

CTAB DNA isolation technique (Vavre and Bouletreau, 1999) was used to extract genome DNA from one single individual preserved in 95% ethanol. A 700 bp fragment of COI was amplified using primers LepF (5′-ATCACAACATCTAAGATATTGG-3′) and LepR (5′-TAAAACTTGATGTCACAAATATCA-3′) from Foottit et al. (2008). PCR-amplification of about 800 bp Cyt b used primers Cp1 (5′-GATGAT-GAAATTTTGGATC-3′) and Cp2 (5′-CTAATGCAAT AACTCCTCC-3′) followed the method of Harry et al. (1998). PCRs for Cyt b were performed in 30 μl reaction volumes: 3 μl 10 × PCR buffer, 2.4 μl dNTPs, 20 μl dd H2O, 0.4 unit Taq DNA polymerase (all from TransGen Biotech, Beijing, China) and 0.6 μl 10 μM forward and reverse primers (synthesized by Invitrogen Biotech, Beijing, China). PCR-amplification for COI was the same as for Cyt b, except for 19.4 μl dd H2O and 0.8 mM primers. The thermocycling program for Cyt b consisted of 95 °C for 3 min; 35 cycles of 92 °C for 1 min, 48 °C for 1.5 min and 72 °C for 1 min. A final extension step of 10 min at 72 °C was added after cycling. PCR amplification reactions of COI used the following cycling conditions: 95 °C for 5 min; 34 cycles including denaturation at 95 °C for 0.5 min, annealing at 52 °C for 0.5 min, and extension at 72 °C for 1 min; followed by 72 °C for 10 min.

Sequencing reactions were performed using the same amplifying primers for both reactions using an ABI 3730 automated sequencer (ABI, USA).

1.3 Sequence alignment and phylogenetic analyses

Sequences were assembled using Seqman II (DNASTAR) and checked manually. The two coding genes were translated into amino acid sequences to check for the presence of stop codons that might indicate that pseudogenes had been amplified (Sanders et al., 2006). Multiple alignments were performed with Clustal X 1.81 (Thompson et al., 1997) and verified by eye. Nucleotide composition, conserved sites, variable sites, parsimony informative sites and pairwise distances were calculated using MEGA 4 (Tamura et al., 2007). DAMBE 5.2.31 (Xia et al., 2001) was used to measure sequence substitution saturation by calculating transversion / transitions versus genetic divergence (TN93 model; Tamura, 1993). A combined dataset of the COI and Cyt b genes was evaluated with the partition-homogeneity test implemented with PAUP* 4.10b (Swofford, 2002), using random taxon addition (10 replicates), tree bisection-reconnection branch-swapping, and heuristic searches with 1,000 repartitions of the data.

Phylogenetic analyses was conducted in PAUP* 4.10b (Swofford, 2002) and MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2005). Maximum parsimony (MP) analysis were conducted for the individual genes, and the combined dataset of the COI and Cyt b sequences, using a heuristic search with 1000 random sequence repetitions and tree-bisection-reconnection (TBR) branch-swapping. Consensus trees (50% majority rule) were obtained if more than one equally parsimonious tree was found. The reliability of MP trees was tested by the bootstrap approach (Felsenstein, 1985) with 1000 pseudoreplicates using the heuristic search strategy and 100 random additions of sequences in each pseudoreplicate.

Modeltest 3.7 (Posada and Crandall, 1998) was used to determine the best-fit nucleotide substitution model for maximum likelihood (ML) analysis. The best model chosen under the Akaike Information Criterion (AIC) (Posada and Buckley, 2004) for the combined dataset was TIM+G. ML analysis were performed in PAUP* 4.10b under a heuristic search strategy with ten random additions of sequences and TBR branch swapping. Bootstrap analysis was performed under the same model with 100 pseudoreplicates, ten random addition sequences per replicate and TBR branch swapping.

Bayesian analysis was carried out separately on the COI, Cyt b and the combined COI and Cyt b datasets using the model selected by modeltest 3.7. The analysis used a random starting tree and proceeded for one million Markov chain Monte Carlo generations; trees were sampled every 100 generations. The first 2500 generations (25% of the total) were later discarded as burn-in. 50% majority-rule consensus trees were generated from the remaining trees and posterior probabilities computed.

2 Results

2.1 Sequence analysis

The alignment sequences of the COI gene include 627 sites, of which 196 were conserved, 431 were variable and 394 were parsimony-informative (for ingroups, 508 sites were conserved, 120 were variable and 114 were parsimony-informative).

On average, COI sequences were found to be A-T rich: A: 35.1%; C: 14.7%; G: 9.9%; T 40.3%. Interspecific sequence divergences were 7–12%. For the intraspecific divergences, it was 0–5% for M. tenuicollis, much lower for samples of the other species (0–0.5%) (Table 2).
A total of 670 bp nucleotides of the Cyt b gene were sequenced, containing 250 conserved, 420 variable and 344 parsimony-informative sites (for ingroups, 518, 152 and 125, respectively). Mean base compositions were: A: 36.3%; C: 11.9%; G: 9.1%; T: 42.7%. Interspecific sequence divergences were 3–12%, and the intraspecific divergences were 0–0.5% (but it was 0–5% for M. tenuicorpus samples).

The combined dataset consisted of 1297 bp (627 bp COI and 670 bp Cyt b) aligned sequences from 44 individuals of Mollitrichosiphum and five outgroup individuals. Among the dataset 312 sites were conserved, 985 sites were variable and 729 were parsimony informative sites. Results of the partition-homogeneity test (P=0.193) indicated that the COI and Cyt b gene trees reflect the same underlying phylogeny. Therefore, the combined dataset were available for phylogenetic analyses.

Substitution saturation of COI, Cyt b and the combined dataset was separately tested with DAMBE; all sequence transitions and transversions had a linear relationship indicating that the sequences are not saturated and could be used for phylogenetic analyses.

### 2.2 Phylogenetic analyses

Significant congruence was observed between the phylogenies derived from MP, ML and Bayesian analysis. The tree obtained from ML analysis of the combined dataset is shown in Figure 1. Topologies of the optimal trees for individual genes and the combined COI-Cyt b sequences were similar. Monophyly was supported (97/0.71/69), as well as the status of the subgenera Mollitrichosiphum and Metatrichosiphon.

The status of Mollitichosiphum tenuicorpus was strongly supported by MP, Bayesian and ML analysis. In this clade, the M. tenuicorpus samples show high divergence with three minor clades (A, B, C) (Fig. 1). One sample (No. 15381) from Motuo (Tibetan Autonomous Region) formed a basal branch (clade C); three samples (Nos. 13361, 24074, 24067) from Yunnan province formed the second clade (clade A) with high support (100/1.00/99). A sister group with the remaining samples from Fujian, Hainan, Guangxi, Guangdong, and one from Yunnan, grouped into clade B.

In the subgenus Metatrichosiphon clade, six species are resolved as divergent lineages with high support. M. nigrofasciatum split from the other five species first. M. luchuanum and M. rhusae were recovered as strongly supported sister groups (100/1.00/98), and then clustered together with M. nigrum (100/1.00/100). Another clade containing M. nandii and M. montanum was strongly supported (98/1.00/100).
Fig. 1  Phylogenetic relationships between *Mollitichosiphum* spp. based on maximum likelihood analysis of the combined COI and Cyt b sequence datasets

Bootstrap percentages from MP and posterior probabilities of Bayesian analysis are shown above the branch, bootstrap values from ML are shown below the branch. YN, Yunnan province; FJ, Fujian province; GD, Guangdong province; GX, Guangxi Autonomous Region; HN, Hainan province; XZ, Tibetan Autonomous Region; HuN, Hunan province; SC, Sichuan province.

3 Discussion

3.1 Monophyly of genus *Mollitichosiphum*

Phylogenetic analyses (Fig. 1) based on COI, Cyt b, or combined COI-Cyt b DNA dataset all support monophyly of the genus *Mollitichosiphum* (97 bootstrap value in MP, 0.71 in Bayesian, and 69 in ML analysis). This result suggests that all species in this genus share a recent ancestor. This is also supported by evidence in morphology as hind tibia with numerous transverse ridges are a distinctive morphological characteristic of the genus that distinguishes it from others within the Greenideini (Zhang and Qiao, 2010). Transverse ridges on hind tibia are a kind of stridulatory apparatus. In the Greenideini, species of the genus *Greenideoidea*, and subgenus Paragreenidea in genus *Greenidea* also possess the same characters as *Mollitichosiphum* (Ghosh and Agarwala, 1993). However, variation in the type, number and situation of the stridulatory apparatus between genera allows *Mollitichosiphum* to be easily dis-
tungished from other genera.

3.2 Subdivision of genus Mollitrichosiphum

Based on morphological characters the genus Mollitrichosiphum can be divided into two subgenera, Mollitrichosiphum and Metatrichosiphon. Antennal setae on the flagellum of the subgenus Mollitrichosiphum have similar lengths, point in all directions and have a nearly straight radial sector. However, in Metatrichosiphon the antennal setae differ in length, the longer setae mainly point inwards and the radial sector is curved (Zhang and Qiao, 2010).

The subgenus Mollitrichosiphum contains four species (M. tenuicorpus, M. godavariense, M. nigriabdominis and M. trilokum) and Metatrichosiphon ten species (M. nigrum, M. nandii, M. nigrofasciatum, M. rhusae, M. luchuanum, M. glaucue, M. nigrum, M. niitakaensis, M. taiwanum and M. montanum) (Remaudière and Remaudière, 1997; Zhang and Qiao, 2010).

In this study we compared one species from subgenus Mollitrichosiphum and six from subgenus Metatrichosiphon. Our molecular phylogeny corroborates the existence of these two subgenera previously identified by morphological taxonomy. Three phylogenetic analyses of the combined COI-Cyt b dataset all support the status of these subgenera (Mollitrichosiphum: MP (100)/ML (89) /Bayesian (1.00); Metatrichosiphon: MP (61) / ML (80) / Bayesian (0.99).

3.3 Phylogenetic analyses of subgenus Metatrichosiphon spp.

The six Metatrichosiphon species have two main lineages, the taxonomic status of which is supported by our phylogenetic analyses. The siphunculi of M. nigrofasciatum are short, 0.26–0.36 times as long as body, while siphunculi of all five species in another clade are long, more than 0.39 times of body. Five species cluster to two clades, in the upper clade (Fig. 1) ultimate rostral segments of all species are at least twice as long as the second hind tarsal segment. M. luchuanum and M. rhusae form a sister group that then clusters with M. nigrum. All species of this major clade are found in southeastern China, with M. rhusae restricted to Hainan Island, and M. luchuanum mainly distributed in Fujian and Guangdong provinces. M. rhusae’s restricted distribution probably reflects the separation of Hainan Island from the Chinese mainland in the early Pleistocene (approximately 2 million years ago) (Zhang, 1999).

Species (M. nandii and M. montanum) in which the ultimate rostral segment is at most twice as long as the second hind tarsal segment form another clade. M. nandii and M. montanum are both confined to the Hengduan Mountain Region, which, as a possible refuge region during the Pleistocene glaciation (Li, 1998), may have influenced the development of modern aphid fauna and other biota in this region (Zhang, 1999; Huang et al., 2006). The complicated topography of this area may have promoted the divergence of these two species. Further research is needed to determine how these species evolved.

3.4 Phylogenetic analyses of Mollitrichosiphum tenuicorpus

The subgenus Mollitrichosiphum was represented in our study by one species, M. tenuicorpus. This species is widely distributed in southern China, from the Himalayas to Taiwan and has a diversity of host plants, including species from the Betulaceae, Fagaceae and Sabiaceae. In all analyses, seventeen samples of M. tenuicorpus resolved to three divergent clades, a result consistent with the genetic distance divergence of COI (Table 2). The COI distance between clades A and B, A and C, B and C is 3.2%–4.4%, 3.7%–4.6% and 5.4%–5.7%, respectively. The COI fragment we used is usually applied as standard DNA barcode to identify species and reveal cryptic diversity. According to the 2% operational threshold of sequence divergence for new species in aphids and Metazoa (Hebert et al., 2003; Fottit et al., 2008), three cryptic species can be allocated within M. tenuicorpus samples. Samples of clade A were collected in Ruili and Baoshan, Yunnan province; the single sample of No. 20530 in clade B is from Simao, Yunnan province (widespread in southern China); and clade C is comprised of samples collected in the Tibetan Autonomous Region (Fig. 3).

The earliest fossil record of Mollitrichosiphum was found in Europe and dates from the early Miocene (Wegierek and Peñalver, 2002). These fossils suggest that similar Miocene aphid fauna existed in these southern Eurasian regions, and species of this genus existed before the formation of the Tibetan Plateau. The continual development of mountains and valleys following the Plateau’s uplift strongly altered the topography of the plateau and surrounding areas (Li et al., 1981; Wu 1989). It can be hypothesized that the uplift of the Tibetan Plateau promoted the divergence of clade C from the other two clades. Previous studies also proposed that the uplift of the Himalayas and Tibetan Plateau led to speciation of many aphid species by forming geographical barriers (Huang et al., 2006, 2008).

Similarly, clades A and B are geographically separated by Big Snow Mountain, a southern extension of the Ailao Mountains, and the Lancang River. Rivers at
ZHANG RL et al.: Phylogeny and species differentiation of *Mollitrichosiphum*

**Fig. 2** Major changes in drainage basin morphology across Southeast Asia (modified from Rüber et al. (2004))

A. Drainage pattern prior to the major captures, where the Upper Yangtze, Middle Yangtze, Upper Mekong, Upper Salween, and the Tsangpo rivers drained together to the South China Sea through the paleo-Red River. B. Capture of the Upper Yangtze River by the Middle Yangtze, and of the Upper Mekong and Upper Salween rivers into their modern drainage positions.

**Fig. 3** Distribution pattern of three clades of *M. tenuicorpus*. ▲, clade A; ●, clade B; ■, clade C.

the southeast margin of the Tibetan Plateau, the Daduhe, Mekong (Lancangjiang), Salween (Nuijiang), and Tsangpo-Brahmaputra (Yalu-Tsangpo), were all once tributaries of a single southward flowing river system the Paleo-Honghe (Red River) (Fig. 2A). The uplift of the Tibetan Plateau dramatically changed major river drainages (Fig. 2B) (Clark et al., 2004). Consequently, the modern course of the Lancang River now isolates southwestern Yunnan from the rest of the province. Molecular phylogenetic studies of freshwater fish have corroborated postulated vicariant evolution caused by river capture events in southwest China (He et al., 2004; Guo et al., 2005; He and Chen 2006; Peng et al., 2006). To some extent, such events can explain the divergence of clades A and B and evidence of similar biogeographical divergence has been found in other taxa in this region (Li and Li 1992; Zhang et al., 2006; Yuan et al., 2008; Zhang et al., 2010).

Clade A species feed on the Sabiaceae and Fagaceae, whereas clade B only feed on Fagaceae and clade C on Betulaceae. Differentiation of host plants may have also been an important factor contributing to divergence.

In conclusion, molecular data confirms the monophyly of the genus *Mollitrichosiphum* and supports the subgeneric classification of *Mollitrichosiphum* and *Metatrichosiphon*. Phylogenetic patterns and genetic distances of COI indicate *Mollitrichosiphum tenuicorpus* may consist of at least three cryptic species. Geographical barriers and the differentiation of host plants may have been important factors contributing to intraspecific divergence within the genus *Mollitrichosiphum*. These results provide new data and patterns regarding the evolution of this genus; however, more research is required to further test geographical barrier and host plant differentiation hypotheses. Additional studies are also required to better understand how geological events influenced aphid species divergence and their modern distribution pattern in this region.

**Acknowledgements** We thank Lin Liu and Zhe Wang for their assistance in molecular techniques and data exploration and Qinghua Liu for providing outgroup sequences. We are grateful to those who collected samples and to Fendi Yang for producing aphid slides. Thanks to Ron Moorhouse for help in improving the English on an earlier version of this paper. This work was supported by the National Natural Sciences Foundation of China (31061160186, 30830017), National Science Funds for Distinguished Young Scientists (31025024), National Science Fund for Fostering Talents in Basic Research (J0930004), Key Laboratory of the Zoological Systematics and Evolution, Chinese Academy of Sciences (O529YX5105) and the Ministry of Science and Technology (2006FY110500).
References

An Z, Kutzbach JE, Prell WL, Porter SC, 2001. Evolution of Asian monsoons and phased uplift of the Himalayan-Tibetan plateau since Late Miocene times. Nature 411: 62–66.

Axelrod DI, Al Shehbaz I, Raven PH, 1998. History of the modern flora of China. In: Zhang AL, Wu SG, and others ed. Floristic Characteristics and Diversity of East Asian Plants: Proceedings of the First International Symposium of Floristic Characteristics and Diversity of East Asian plants. Beijing: China Higher Education Press, 43–55.

Clark MK, Schoenbohm LM, Royden LH, Whipple KK, Burchfiel BC et al., 2004. Surface uplift, tectonics, and erosion of eastern Tibet from large-scale drainage patterns. Tectonics 23: 1006–1029.

Felsenstein J, 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Footitt RG, Maw BL, von Dohlen CD, Hebert PDN, 2008. Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. Molecular Ecology Resources 8: 1189–1201.

Ghosh AK, Agarwala BK, 1993. The Fauna of India and the adjacent Countries. Homoptera, Aphiidioidea. Part 6. Subfamily: Greenideinae. Calcutta: Zoological Survey of India.

Guo X, He S, Zhang Y, 2005. Phylogeny and biogeography of Chinese sisorid catfishes re-examined using mitochondrial cytochrome b and 16S rRNA gene sequences. Molecular Phylogenetic Evolution 35: 344–362.

Harry M, Solignac M, Lachaise D, 1998. Molecular evidence for parallel evolution of adaptive syndromes in Fig-Breeding Lissocophala (Drosophilidae). Molecular Phylogenetics and Evolution 9: 3542–551.

He D, Chen Y, 2006. Biogeography and molecular phylogeny of the genus Schizotheora (Teleostei: Cyprinidae) in China inferred from cytochrome b sequences. Journal of Biogeography 33: 1448–1460.

He D, Chen Y, Chen Z, 2004. Molecular phylogeny of the specialized schizothoracine fishes (Teleostei: Cyprinidae), with their implications for the uplift of the Qinghai-Tibetan Plateau. Chinese Science Bulletin 49: 39–48.

Hebert PDN, Cywinska A, Ball SL, de Waard JR, 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B 270: 313–321.

Hou ZE, Fu JZ, Li SQ, 2007. A molecular phylogeny of the genus Gammarus (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. Molecular Phylogenetics and Evolution 45: 596–611.

Huang S, He S, Peng Z, Zhao K, Zhao E, 2008. Molecular phylogenetic analysis of endangered sharp-nosed pitviper (Deinagkistrodon acutus: Reptilia, Viperidae) in Mainland China. Molecular Phylogenetics Evolution 44(3): 942–952.

Huang XL, Qiao GX, Li FM, 2006. Diversity and distribution of apids in the Qinghai-Tibetan Plateau-Himalayas. Ecological Entomology 31: 608–615.

Huang XL, Li FM, Qiao GX, 2008. Areas of endemism and patterns of diversity for apids of the Qinghai-Tibetan Plateau and the Himalayas. Journal of Biogeography 35: 230–240.

Jen H, 1982. The uplift of the Qinghai-Xizang (Tibet) Plateau in relation to the vegetational changes in the past. Acta Phyto-
