Phylogenetic relationship and biogeography of the genus *Diestramima*, a cave cricket endemic to the Oriental realm (Rhaphidophoridae: Aemodogryllinae)

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Abstract

Background

Diestramima is only distributed in the Oriental realm, and mainly in China. Because they are apterous, these insects are good materials for studying biogeography. In this study, we reconstruct the phylogeny tree of *Diestramima* species based on maximum likelihood and Bayesian inference based on six genes, and use fossil-based molecular dating and ancestral range estimation to reconstruct the dispersal route.

Results

The results of molecular system are highly consistent with the morphological characteristics of these species. At the subgenus level, it is difficult to give a reasonable explanation on the phylogenetic tree although we found that some groups share some common characteristics. Dating and historical biogeography analyses suggest an early Miocene origin of the genus. Some ancestors of the genus *Diestramima* have been distributed in Guangxi, Yunnan province and other regions of China at this time. In the late Miocene, the genus *Diestramima* dispersed from the low mountain and hilly areas of Guizhou, Guizhou and Hunan to inland China. Quaternary glaciation determines their current geographical distribution pattern. Southeast Asia is the most likely original place of the genus *Diestramima*.

Conclusion

In phylogenetic clades of *Diestramima*, similarity in geographic distribution is as important as similarity in morphological characteristics. Combined with the results of biogeographic analysis, the two temperature rises in the Palaeocene and Miocene may have intensified the dispersal of the ancestors of the genus *Diestramima* in the Oriental realm. The temperature drop and the uplift of the Qinghai-Tibet Plateau were the main reasons for the divergence within the genus *Diestramima*. In addition, changes in humidity and changes in surface plant communities are also important factors affecting their divergence.

Introduction

Cave cricket (Rhopidophoridae Walker, 1869) as one of the oldest species of Orthoptera, are widely distributed all over the world. It is considered to be the basis of the six families and one of the oldest taxa in the Tettigoniidae clade [1, 2]. They are gregarious and typical apterous groups, which includes 10 subfamilies and 87 genera (including 1 fossil subfamily and 2 fossil genera). They mainly inhabit in dry leaves, stone crevices, caves and other dark humid environments, therefore their speciation as well as population structure have relatively strong dependence on geographical isolation [3].

Dating estimates indicated that Rhopidophoridae originated in the Cretaceous period during the Mesozoic era with the ancestral area located both in the northern and southern hemisphere. The current

distribution of Rhaphidophoridae might be explained by a combination of both dispersal and vicariance events occurred especially in the ancestral populations [4]. The radiation of Rhaphidophoridae started within the Pangaea, where the ancestor of Rhaphidophoridae occurred throughout an ancestral area including Australia, North America, and the Mediterranean region. The opening of the Atlantic Ocean promoted the divergence of North American and Mediterranean lineages while the differentiation of the southern lineages, spread from Australia, appears to be related to the fragmentation of Gondwana land [4]. The Indian subcontinent (including India and southeast Asian countries north of the Indian river) was originally part of Gondwana continent, and the initial collision between it and Eurasia probably began in the late cretaceous, and the main collision period occurred around 55–50Ma, which coincided with the isolation of the Aemodogryllinae and Rhaphidophorinae. After the collision of the Indian subcontinent and the Eurasian continent, insects from the two continents met again here, which also provided rich raw material for later Oriental insect species [5].

China straddles Palaearctic and Oriental realm, occupies an important biogeographical position in the world. Recent years, many papers on molecular biogeography of insects have been carried out in China. Based on the phylogenetic relation and distribution of four grasshopper genera of Catantopidae from the south of China, ten areas of endemism have been recognized [6]. The order of the formation of the 10 distribution areas of these insects was as follows: southern Yunnan and Loess Plateau, Hainan Island, eastern Mountain and Taiwan Island; The Yunnan-Guizhou plateau, northern Yunnan and Qinling-Daba mountains formed at the same time, while the southern China and jiangnan hilly region formed the last. Wang *et al.* determined the phylotaxonomic relationship and differentiation time of the genus *Sinocyrtaspis* endemic to China [7]. Based on the geographic dynamics and climatological data of its ancestors, it was concluded that the centres of origin of *Sinocyrtaspis* were Hunan and north-east Guangxi. Their ancestors had two dispersal routes ending in Jiangxi and Zhejiang, after the cold period of the Miocene, some of the ancestral species remained in Guizhou while others moved out. And as a response to climate change, species altered distribution areas by moving along altitude gradients in mountain regions, whereas the anthropogenic global warming trend has promoted some species moving to high-latitude areas or caused population differentiation. However, there is still a lack of biogeographic research on the widely distributed Oriental species, especially the Rhaphidophoridae in Oriental realm.

China is the main distribution area of Rhaphidophorinae Walker, 1869 and Aemodogryllinae Jacobson, 1905. *Diestramima* Storozhenko, 1990 [8] is (Rhaphidophoroidea: Aemodogryllinae, Kevan, 1977) [9, 10] widely distributed in the Oriental realm of China (with 70% of known species) and adjacent countries. Their range is limited to the Oriental realm (tropical and subtropical mountainous hilly areas), because they usually choose wide shrub leaves as mating sites. All species of this genus has long protuberances on the seventh abdominal tergite, and the shape of the protuberances is significantly different among different species. The morphological classification system and the relationship between species are relatively clear. Therefore, *Diestramima* is a suitable candidate group for studying the speciation and diversity patterns of Oriental realm in China and the corresponding geological changes.

In this paper, we used an integrative approach to identify species, including morphology and multiple methods for molecular identification, which clarified the present taxonomic problems on *Diestramima*. We estimated the temporal and spatial divergence of this genus, and present a historical framework for the phylogeny and biogeography of these Oriental realm endemic cave crickets. And in the discussion, we try to predict the historical dispersal pattern of pure Oriental species in China from this groups.

Material And Methods

Taxon sampling

A total of 87 individuals from 19 species were collected by hand or sweep net at night. Adults were preserved in 70% ethanol in the field. The morphological identifications prior to DNA extraction were made depending on the male apex of abdomen and paraproct [10]. Samples of genus *Diestramima* were collected from twelve provinces in China Oriental realm, including Anhui, Chongqing, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangxi, Sichuan, Yunnan and Zhejiang (Fig. 1). All widespread species in China and a cross-border species distributed in China and Vietnam have been collected. Moreover, we examined a large of additional specimens and relevant literature [9–18] of Raphidophoroidea for better understanding the morphological variations of species, to aid in accurate species determination. Thus, the present sampling is the almost comprehensive one for the phylogenetic analysis in terms of both taxonomy and distribution. All diagnostic characters were photographed by OLYMPUS DP27, and arranged using Adobe Photoshop CC (Fig. 2, Fig. 3).

DNA extraction, amplification and sequencing

The total genomic DNA was extracted from the muscles of one hind leg by AxyPrep Genomic DNA Miniprep Kit (AXYGEN), according to the manufacturer's instructions. We used previously published sequences from the family Raphidophoroidea to jointly complete this sampling, including six species of the genus *Diestrammena* Brunner von Wattenwyl, 1888, four species of the genus *Rhaphidophora* Serville, 1838, twenty species of the subfamily Dolichopodainae Brunner von Wattenwyl, 1888, nineteen species of the subfamily Troglophilinae Krauss, 1879 [4]. The samples and sequence information were listed in Table S1. The following six gene regions were amplified: Mitochondrial ribosomal 16S, nuclear ribosomal 28S, mitochondrial cytochrome oxidase I (COI), ATPase catalytic subunit A-like (ACSL), cytoplasmic-like (CPL) and water dikinase-like (WDL). PCR was performed using 2×Es Taq Master Mix (Dye) manufactured by Cowin Bio (China), with a final volume of 50μL, composed of: 25μL of the 2×Es Taq Master Mix (Dye), 19μL of the ddH₂O, 2μL of forward and reverse primer each and 2μL of the sample DNA extract. The PCR annealing temperature for each gene was modified (Table S2). PCR products were directly sequenced.

Phylogenetic analyses

We used all *Diestramima* samples as well as the samples of adjacent genera *Diestrammena* and *Rhaphidophora* in the phylogenetic tree construction. For each fragment, DNA alignment was conducted

by MEGA7 software [19, 20]. Saturation level of sequences was verified by Xia's test [21] in dambe 7 [22]. In PhyloSuite [23], the best-fit evolutionary model GTR was derived from ModelFinder [24], and the Maximum likelihood (ML) analysis was performed in IQ-Tree [25] with 1000 ultrafast bootstrap replicates. Node support is shown as posterior probabilities and bootstrap value, as directly estimated from the majority rule consensus topology.

Divergence time estimation

The phylogenetic of *Diestramima* using BEAST v.1.8.4 [26] to reconstruct by selecting the Birth-Death tree prior and the Piecewise linear and constant root of population size model. Given the absence of reliable fossil records for *Diestramima*, we relied on secondary calibrations based on the available worldwide dated framework of Raphidophoroidea [4], we used representatives of four lineages (subfamily Dolichopodainae and Troglophilinae; genus *Diestrammena* and *Rhaphidophora*) that were sampled in this study, including 49 cave cricket species. In their conclusion, the ancestor of the Aemodogryllinae and Raphidophorinae spread from Australia to south-eastern Asia at 117Ma (95% HPD: 105–130 Ma), an estimate consistent with plate tectonics as India separated from other parts of Gondwana at 130 Ma, prior to other paleogeographic events [27]. Therefore, in our study, it was used as the first divergent calibration time to prove the purity of these Oriental realm species.

The divergence times of major nodes were estimated under uncorrelated relaxed clock by setting four fossil calibrations: (1) due to the plate tectonics as India separated from other parts of Gondwana, the ancestor of the Aemodogryllinae and Raphidophorinae spread from Australia to south-eastern Asia at 117 Ma [4]; (2) the opening of Mid-Aegean trench (9–12 Ma) separated the *Troglophilus* Krauss, 1879 species inhabiting the eastern Aegean islands and Asia Minor from the Cyclades, Crete and mainland Greece [28]; (3) the uplift of Anatolian Plateau happened 5–10 Ma due to acceleration of northward movement of the Arabian Plate [29] and separating the Anatolian species of *Troglophilus* from the Balkans ones; (4) the isolation of Crete island, inhabited by *Dolichopoda paraskevi* Boudou-Saltet, 1973 (Aegean Sea, Greece) from neighboring mainland, happened at the end of Messinian salinity crisis (5.33 Ma) [30]. Trees were sampled every 10000 generations for a total of 100 million generations. We further checked whether the effective sample size (ESS) was greater than 200 for most important parameters using Tracer v.1.7.1. The consensus tree was exported using TreeAnnotator v.1.8.4 [31], discarding the first 25% of trees as burn-in. The mean node heights and 95% highest probability density (95% HPD) were displayed in FigTree v1.4.4.

Ancestral range estimation

We defined the following six biogeographical areas of *Diestramima* according to their known distributions and the zoogeographic regionalization of China [32]: A. mountainous region of southern Yunnan (Baoshan city, Xishuangbanna Dai autonomous prefecture, south of Ailaoshan in Yuxi city, Puer city, Honghe Hani & Yi autonomous prefecture, west of Wenshan Zhuang & Miao autonomous prefecture); B. mountainous hilly region of Yunnan and Guangxi (east of Wenshan Zhuang & Miao autonomous prefecture, Baise city, Chongzuo city, Fangchenggang city, Nanning city); C. low mountainous hilly region

of Guangxi, Guizhou and Hunan (Hechi city, Liuzhou city, Guilin city, south of Qiannan Buyi & Miao autonomous prefecture, east of Qiandongnan Miao & Dong autonomous prefecture, east of Hunan province); D. Sichuan basin (most of Chongqing city and east of Sichuan province); E. Jiangnan hilly region (southeast of Hunan, Xianning city in Hubei, most of Jiangxi, north of Fujian, most of Zhejiang); F. mountainous hilly region of Fujian and Guangdong (east of Guangxi province, north of Guangdong province, south of Fujian province).

Ancestral ranges were reconstructed using RASP 4.0 [33] beta. As a guide tree for statistical dispersal-extinction-cladogenesis (S-DEC) analyses [34], we removed all outgroups and constructed a phylogenetic tree for the genus *Diestramima*. Modify and guide this tree according to the maximum credibility tree from the BEAST analysis. Ancestral areas were limited to no more than three areas. Statistical dispersal-vicariance (S-DIVA) analysis [35] were performed based on a sample of 1000 random post burn-in RASP trees and use the final tree. The maximum number of ancestral areas was limited to three.

Result

Species delimitation and phylogeny

Phylogenetic trees (Fig. 4) constructed either by BI or ML analyses had the same topology. Between species, Posterior probability (PP) of all nodes is 1 and bootstrap support values (UFB) are all above 98 except *D. (D). eurya* is 94. Based on different tree-constructing methods as well as single or combined dataset, the monophyly of the species in *Diestramima* was well-supported. The membership of each of these major clades were not strongly correlated with the distribution of their subgenera (*Baculitettix*, *Diestramima* and *Excisotettix*) [14]. The species within each clade live in roughly the same area, especially in the southwestern border region of China. *D. conica*, *D. yunnanensis*, *D. sp. 2*, *D. major*, *D. subtilis*, *D. cycla* and *D. triangulata* are in a clade, although they contain two subgenera. In the southwestern border region of China, even within the same species, samples from different regions showed great variability. We collected *D. conica* samples from Taiyanghe Reserve, Gaoligongshan and Laifengshan, and the samples from these three regions in Yunnan showed a high divergence. Similarly, *D. subtilis* samples from three regions (Xiaojie town, Gulinjing town and Ailaoshan) in Yunnan also produced this high divergence. However, widely distributed species in inland areas of China always tend together. We collected *D. beybienkoi* samples from seven regions (Jinyunshan, Emeishan, Zhangjiajie, Dujiangyan, Leigongshan, Simianshan and Yangmeiao) in five provinces (Sichuan, Chongqing, Hunan, Guizhou and Guangxi) of China, but they only divergence two clades. This divergence phenomenon is more prominent in *D. intermedia* and *D. austrosinensis*. Although the samples we collected spanned several provinces and regions in China, there were basically no divergence within their species.

Divergence time estimation

The divergence time tree is divided into two branches: Aemodogryllinae and Rhaphidophorinae from Asia, Dolichopodainae and Troglophilinae from Europe. We analyze the Asian taxa separately (Fig. 5). The

BEAST analysis indicated that the subfamily Aemodogryllinae and Rhaphidophorinae have been diverged since Cretaceous. The speciation events in genus *Diestramima* occurred from late Miocene to the early Pliocene, and most species was completed in or before early Quaternary. Following the speciation of four Yunnan and Guangxi lineages (*D. conica*, *D. major*, *D. subtilis* and *D. yunnanensis*) diverged at 8.32 Ma (95% HDP: 4.84–12.88 Ma), two clades split at 3.66 to 8.96 Ma. Both clades include species from Yunnan and Guangxi (border lineages) as well as those from inland China (inland lineages), and they further diverged at 5.84 Ma (95% HDP: 3.66–8.96 Ma) and 5.85 Ma (95% HDP: 3.67–9.10 Ma) respectively. In one clade, *D. sp. 2*, a new species we discovered on the China-Vietnam border, was diverged at 4.02 Ma (95% HDP: 2.42–6.31 Ma). At this point the border and inland lineages began to diverge. The widely distributed species *D. intermedia* was diverged at 1.02 Ma (95% HDP: 0.44–1.86 Ma). In the other clade, border and inland lineages was diverged with *D. sp. 1* and *D. lamina* at 3.52 Ma (95% HDP: 2.18–5.29 Ma). Two widely distributed species, *D. austrosinensis* and *D. beybienkoi*, was diverged at 2.04 Ma (95% HDP: 1.08–3.24 Ma) and 2.54 Ma (95% HDP: 1.49–3.84 Ma) respectively.

Ancestral range estimation

S-DEC and S-DIVA analyses showed a highly similar biogeographic patterns for *Diestramima* species and the results shown in Fig. 6. The reconstructed topological tree is highly consistent with the BEAST tree, the lineages located in Guangxi and Yunnan are still the first to be diverged. The ancestral range for *Diestramima* in China covers mountainous region of southern Yunnan (A) and mountainous hilly region of Yunnan and Guangxi (B). As for the two clades that followed, the ancestral distribution changed from mountainous region of southern Yunnan (A, point 152) to mountainous region of southern Yunnan and low mountainous hilly region of Guangxi, Guizhou and Hunan (AC, point 145). The ancestral distribution regions of all reconstructed species in both clades include the low mountainous hilly region of Guangxi, Guizhou and Hunan (C). The species in Sichuan basin (D) came from low mountainous hilly region of Guangxi, Guizhou and Hunan (C). Jiangnan hilly region (E) and mountainous hilly region of Fujian and Guangdong (F) greatly promoted the change of the distribution of widespread species in inland China. The main dispersal routes are indicated by arrows in Fig. 7.

Discussion

Phylogenetic relationships and systematics

Our phylogenetic results (Fig. 4), based on the combined analysis, are highly similar to the morphological analysis (Fig. 2, Fig. 3). Based on the results from our phylogenetic analyses, species in genus *Diestramima* that are morphologically similar and geographically area similar tend to be grouped together. Due to the lack of samples from Southeast Asian countries, we compared our data with the descriptions of some species of *Diestramima* by Gorochov *et al.* (Fig. 8) [10] and proposed some species that may be closely related. There is currently no description of the male *D. yunnanensis*. We compared the genital plate of female individuals (Fig. 8. F) collected in Xishuangbanna Tropical Botanical Garden Chinese Academy of Sciences with the description of this site sample by Gorochov *et al.* (Fig. 8. A46) and

found morphological consistency. Therefore, we supplemented the abdominal apex of male *D. yunnanensis* (Fig. 8. E) based on the results of collection region and phylogenetic tree. *D. yunnanensis* is highly similar to *D. propria* (Fig. 8. A31, located in Laos), except that the male has a shorter abdomen apex and paraproct. Laos borders Xishuangbanna Dai autonomous prefecture of Yunnan province in China. Similarly, Myanmar borders Baoshan city of Yunnan province in China. The abdomen apex of male *D. conica* (Fig. 8. B) is highly similar to *D. minor* (Fig. 8. A36, located in Myanmar), but has completely different shape of paraproct.

In Fig. 2, Fig. 3, Fig. 8, species in Vietnam and the area bordering China can be divided into three groups based on the shape of the male abdominal apex, which basically conclude the morphological characteristics of all species in the genus *Diestramima*. Most males have similar short and simple abdomen apex, but their paraproct are completely different in shape and length (*D. bispinosa*, *D. cyclo*, *D. hainanensis*, *D. hamata*, *D. palpata*, *D. sp. 1* and *D. subtilis*). The second group of males always has a wide and flat abdomen apex, and some of their paraproct tend to be short and blunt round, some tend to be long and pointed (*D. eurya*, *D. lamina* and *D. vietnamensis*). The male abdomen apex of the last group is significantly longer than the first two groups, and most of them are curved downwards (*D. major* and *D. sp. 2*). This male species with a more slender abdomen apex (*D. acutiapicis*, *D. austrosinensis*, *D. brevis*, *D. intermedia*) is widely distributed in the Oriental realm of inland China. Gorochov *et al.* divided three subgenera of the genus *Diestramima* according to the male paraproct and the female genital plate [14]. Paraproct of the subgenus *Baculitettix* are stick-like (rather long and thin) but with slightly or moderately higher proximal part. In the subgenus *Diestramima* and *Excisotettix*, paraproct rather short and usually high (plate-like), with distal part truncate, angular or having hooks and/or teeth (spinules); or paraproct moderately short, almost S-shaped in profile, with proximal and distal parts more or less equal to each other in height, and with angular or almost spinose apex. The difference is that the female genital plate of the subgenus *Excisotettix* with rather large posteromedian notch and angular projections around this notch. But in our current phylogenetic tree, we have not found a reasonable explanation for distinguishing these subgenera. In all our samples, we found small differences in the length and curvature of the male abdomen apex even within the same species. But the length of the paraproct, especially the shape always remains the same. Combining with the strong similarities between the male abdomen apex of many species mentioned earlier, perhaps the specialization of paraproct occupies a more dominant position in the morphological taxonomic research of the genus *Diestramima*.

Time and space frame of *Diestramima* evolution

The results of biogeographic analysis of all genetic samples (Fig. 5) are basically consistent with the conclusions drawn by Allegrucci and Sbordoni [4]. With the gradual disintegration of Pangea, Raphidophoroidea is divided into two clades. The ancestors of Dolichopodinae and Troglophilinae are mainly distributed in Laurasia and the ancestors of Aemodogryllinae and Raphidophorinae are mainly distributed in Gondwana. With the Indian subcontinent drift and collision with Eurasia, the separation of the two clades Aemodogryllinae and Raphidophorinae were formed gradually.

In the Paleogene period, we found that the separation of genus *Diestramima* from *Diestrammena* diverged at 33.90Ma (95% HDP: 18.85–54.36 Ma) and can also be regarded as the separation of tribe Diestramimini and Aemodogryllini. During this epoch, one of the most significant climate changes on Earth since the Cenozoic era occurred: the Eocene/ Oligocene boundary cooling event. It is characterized by the rapid change of the Earth's climate from the previous Greenhouse to Icehouse, and the emergence of Ice sheets in Antarctica is accompanied by a significant drop in global temperature [36]. A drop in global temperatures may eventually lead to the divergence of Aemodogryllini, which is more resilient to cold conditions and can be dispersed to a wider area. The extreme temperature rise of the Paleocene-Eocene thermal maximum did not affect the divergence of the Aemodogryllinae and Raphidophorinae ancestors, and may have only intensified their dispersal in the Oriental realm.

In the Neogene period, the first major separation time of the genus *Diestramima* diverged at 8.32Ma (95% HDP: 4.84–12.88 Ma). At this time, temperatures began to warm during the Miocene, and various tectonic processes occurred due to the collision and compression of continental plate drift and the intensification of mountain building. Among them, the Himalayan orogeny had the most profound impact on China [37]. It transformed the planetary wind system in mainland China from early Miocene to monsoon wind system; the arid inland areas in northwest China and the monsoon areas in east China began to form. Until the late Miocene, the climate pattern of humid southeast and arid northwest was basically formed. Paleoclimate changes peaked during the Miocene and gradually shaped the modern climate patterns that prevail today [38]. The humid climate in the eastern area and the sea level caused by warming temperatures may cause some species in *Diestramima* to gradually migrate east and north, and eventually become isolated due to the frequent mountain building activities. In Fig. 5, the differentiation in *Diestramima* has a certain lag in this gradually humid and warm climate, a reasonable explanation is that their mating behavior is dependent on broadleaf shrubs or dwarf trees. Due to the monsoon climate change, the relationship between the plant communities and vegetation types in Eastern China during this period is also undergoing changes [39–42]. We found that in the males of the genus *Diestramima*, they will stay in place after the ejaculation is over and continue to lift and press the female genitalia, and the frequency and method of this behavior vary according to the species. We believe that this behavior may speed up the sperm entry into the female body while also preventing or slowing down the rate at which the female eats the spermatophore. Correspondingly, all species in *Diestramima* we have observed will choose dwarf trees, shrubs and ferns with large leaves as their venues for this mating behavior. This behavior has not been found in other species in the family Aemodogryllinae.

The genus *Diestramima* diverge into two clades in the late Miocene. A clade species are all from the southeast border of China and countries in Southeast Asia, including *D. conica* (similar to the species of Myanmar), *D. yunnanensis* (similar to the species of Laos), *D. subtilis* (similar to the species of Vietnam) and *D. major* (distributed in Vietnam and China), and another clade species are almost all from inland China. During this period, the uplift of the Himalayan-Tibetan Plateau led to increased mountain folds in southwestern China and further changes in the Asian seasonal pattern [43]. The ancestors of the genus *Diestramima* have dispersal to Guizhou, Hunan and deeper inland areas of China from the end of the Pliocene to the early Pleistocene. Three most widely distributed species of the genus *Diestramima* (*D.*

austrosinensis, *D. beybienkoi*, *D. intermedia*) in inland areas of China diverged almost at the same time (2.54–1.02 Ma). The temperature drop and ice sheet increase brought about by the Quaternary glaciation also affected their spread at this time. The distribution and ancestral proliferation characteristics of the genus *Diestramima* in China are very similar to the ancestral distribution characteristics of the genus *Sinocyrtaspis* [7] and the four genera of Catantopidae [6] in China. The mountainous region of southern Yunnan is one of the important origins of these grasshopper genera of Catantopidae ancestors. The centres of origin of *Sinocyrtaspis* were Hunan and north-east Guangxi, and they spread with this place as the center. This may be one of the dispersal paths of Orthoptera in China.

Conclusions

Here we according to the morphological characteristics and the phylogenetic tree constructed based on the 6 genes, phylogenetic relationship of the genus *Diestramima* is shown. At present, the interspecies morphological characteristics and molecular phylogeny relationships of all the samples have maintained extremely high consistency. However, at the subgenus level, it is difficult to give a reasonable explanation on the phylogenetic tree although we found that some groups share some common characteristics. In phylogenetic clades of *Diestramima*, similarity in geographic distribution is as important as similarity in morphological characteristics. This phenomenon may be related to their lack of wings and weak mobility. Combined with the results of biogeographic analysis, the ancestors of the genus *Diestramima* had already arrived in Guangxi and Yunnan provinces in China before the Miocene. The two temperature rises in the Palaeocene and Miocene may have intensified the dispersal of the ancestors of the genus *Diestramima* in the Oriental realm. The temperature drop and the uplift of the Qinghai-Tibet Plateau were the main reasons for the divergence within the genus *Diestramima*. In addition, changes in humidity and changes in surface plant communities are also important factors affecting their divergence. The low mountainous hilly region of Guangxi, Guizhou and Hunan is the area where the insects of this genus are most differentiated. At the early Pliocene, they may have reached most of the areas where they are now. The arrival of the Quaternary Ice Age has caused these species to undergo violent and complex evolution in inland China.

Declarations

Availability of supporting data

The data sets supporting the results of this article (Additional file 1: Tables S1 and S2) are available as online Additional file 1.

Competing interests

The authors declare no competing interests.

Authors' contributions

JSZ, YYQ and CZS collected the data, led the writing and conceived the idea, and analyzed the data; ZQH and KL led the writing. All authors read and approved the final manuscript.

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Figures

Figure 1

Distribution areas of the genus *Diestramima* in China.

Figure 2

Male abdomen apex of *Diestramima* in dorsal view. (A) *D. acutiapicis*, (B) *D. austrosinensis*, (C) *D. beybienkoi*, (D) *D. brevis*, (E) *D. conica*, (F) *D. cyclo*, (G) *D. eurya*, (H) *D. excavate*, (I) *D. guangxiensis*, (J) *D. intermedia*, (K) *D. lamina*, (L) *D. major*, (M) *D. sp. 1*, (N) *D. sp. 2*, (O) *D. subrectis*, (P) *D. subtilis*, (Q) *D. triangulate*, (R) *D. truncate*, (S) *D. yunnanensis*. Scale bar: 1.0 mm.

Figure 3

Male abdomen apex of *Diestramima* in lateral view. (A) *D. acutiapicis*, (B) *D. austrosinensis*, (C) *D. beybienkoi*, (D) *D. brevis*, (E) *D. conica*, (F) *D. cycla*, (G) *D. eurya*, (H) *D. excavata*, (I) *D. guangxiensis*, (J) *D. intermedia*, (K) *D. lamina*, (L) *D. major*, (M) *D. sp. 1*, (N) *D. sp. 2*, (O) *D. subrectis*, (P) *D. subtilis*, (Q) *D. triangulate*, (R) *D. truncate*, (S) *D. yunnanensis*. Scale bar: 1.0 mm.

Figure 4

Phylogenetic relationships of the genus *Diestramima*. Maximum likelihood (ML) phylogenetic tree based on the concatenated 12S, 16S, ACSL, COI, CPL and WDL matrix sequences. Topology supports of all major nodes are indicated above branches in this order: the posterior probability and the bootstrap resampling. Different colors represent different subgenera: *Diestramima* in green; *Baculitettix* in yellow; *Excisotettix* in red. Circled in the ecological photos are the apex of these male abdomen (photograph by Zhuqing He, Yanyan Qin and Hanqiang Wang).

Figure 5

Divergence time estimated in BEAST. Blue horizontal bars show the 95% highest posterior density interval (HPD) of the estimated node age. Black circles on the nodes indicate the calibration points. Vertical bars show relevant events in that transition period of time. K, Cretaceous; N2, Pliocene; Q, Quaternary.

Figure 6

Historical biogeography of *Diestramima* as inferred by ancestral area reconstructions carried out with RASP under S-DEC and S-DIVA model. Showing estimations in color charts. The number represents the optimal distributions at each node.

Figure 7

The map represents the six major biogeographical areas and dispersal routes of *Diestramima*: mountainous region of southern Yunnan (A), mountainous hilly region of Yunnan and Guangxi (B), low mountainous hilly region of Guangxi, Guizhou and Hunan (C), Sichuan basin (D), Jiangnan hilly region (E) and mountainous hilly region of Fujian and Guangdong (F).

Figure 8

A. Descriptions of some species in *Diestramima* by Gorochov *et al.* (2015): 1, 2, *D. vietnamensis vietnamensis* (Northern Vietnam); 3–5, *D. vietnamensis saturata* (Northern Vietnam); 6–8, *D. austrosinensis* (China); 9–16, *D. palpata* (Northern Vietnam); 17, 18, *D. distincta* (China); 19–22, *D. hainanensis* (China); 23–26, *D. bispinosa* (Northern Vietnam); 27–30, *D. hamata* (Northern Vietnam); 31–33, *D. propria* (Laos); 34, 35, *D. major* (China & Vietnam); 36–38, *D. minor* (Myanmar); 39, 40, *D.*

cryptopygia (India); 41, 42, *D. intermedia* (China); 43–45, *D. tsongkhapa* (Bhutan); 46, *D. yunnanensis* (China); 47, *D. champasak* (Laos). Male abdominal apex from side (1, 6, 17, 34, 36, 39) and from above (18, 32, 35, 37, 40); same without lower part from above / behind (2, 7) and from side (9, 19, 23, 27, 31); female abdominal apex from above (4); female genital plate from below (5, 8, 12, 22, 26, 30, 38, 42, 45–47); seventh abdominal tergite and hind part of sixth one in male from above (10, 20, 24, 28); apical part of posteromedian process of male seventh abdominal tergite from behind and slightly below (11, 21, 25, 29, 33); hind part of female seventh abdominal tergite (13) and its posteromedian part (14, 15) from above; female paraproct from side (16); posteromedian process of seventh abdominal tergite (43) and paraproct (44) in male from side. B–E. apex of male abdomen in lateral view: B. *Diestramima conica*; C. *Diestramima subtilis*; D. *Diestramima* sp. 1; E. *Diestramima yunnanensis*. F. female genital plate of *D. yunnanensis*, ventral view. Scale bar: 1.0 mm.

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