Uplift-driven diversification revealed by the historical biogeography of the cockroach Cryptocercus Scudder (Blattodea: Cryptocercidae) in eastern Asia

YANLI CHE¹, QIKUN BAI¹, WENBO DENG¹, SHURAN LIAO¹, JIAJUN HE¹, SIMON Y. W. HO² and ZONGQING WANG¹

¹College of Plant Protection, Southwest University, Chongqing, China and ²School of Life and Environment Sciences, University of Sydney, Sydney, Australia

Abstract. Cryptocercus Scudder is a genus of wingless cockroaches, which spend their lives feeding within rotting wood in old-growth montane forests. Their dispersal capability is likely to be limited because they depend on the succession of temperate forests, but their distribution exhibits intercontinental disjunctions. Although the natural history and conservation biology of the North American species of the genus have received much attention, the evolution of the eastern Asian lineages remains enigmatic. To resolve the geographic patterns and evolutionary history of Cryptocercus in eastern Asia, we analysed genetic data of species from China (27 species), Korea (one species) and the Russian Far East (one species). We performed phylogenetic analyses of seven genes to infer the evolutionary relationships among species of Asian Cryptocercus. We then used Bayesian molecular dating to estimate the evolutionary timescale of the genus. Ancestral geographic distributions were reconstructed in rasp using statistical dispersal-vicariance analysis and statistical dispersal-extinction-cladogenesis. Our maximum-likelihood and Bayesian phylogenetic trees supported four major lineages of Cryptocercus, revealing a clear geographical pattern. The divergence of American and Asian lineages was inferred to have occurred 74.8 Ma (95% credibility interval: 51.1–103.8 Ma), with the diversification of Asian taxa beginning at 30.7 Ma (95% credibility interval: 22.9–40.3 Ma). The most recent common ancestor of Asian Cryptocercus taxa was inferred to have had a broad distribution in Asia. The uplift of the Qinghai-Tibet Plateau in the Palaeogene and Neogene, along with climatic oscillations in the Quaternary, probably had substantial effects on the formation of the disjunction pattern in the Cryptocercus lineages found in the Hengduan Mountains and Qin-Daba Mountains of China. We propose that the distribution of Cryptocercus has been strongly influenced by habitat fragmentation and subsequent allopatric speciation.

Introduction

Intercontinental disjunctions in the geographic distributions of taxa in eastern Asia and North America have been widely studied in plants (Wen, 1999, 2001), insects [aphids (Ren et al., 2013, 2017); butterflies (Wu et al., 2015, de Moya et al., 2017);] fish (Sun et al., 2007) and reptiles (Macey et al., 2006). A disjunct distribution is seen in the genus Cryptocercus Scudder, an uncommon, subsocial woodroach that occurs in temperate forests of North America and in China, Korea, and the Russian Far East (Cleveland et al., 1934; Nalepa, 1984; Park et al., 2002; Nalepa, 2003). All members of Cryptocercus are wingless with poor dispersal ability, and spend all stages of their life cycle in rotten wood. It is likely that their geographic distributions have been driven by palaeogeographic events that influenced their source tree hosts (Maekawa & Nalepa, 2011). Cryptocercus is an ideal model for studying deep evolutionary events, because its species share several behavioural traits with termites. Numerous
Phylogenetic studies have placed the genus as the sister group to termites (Lo et al., 2006; Inward et al., 2007; Djernæs et al., 2015; Legendre et al., 2015).

Species of Cryptocercus are distributed throughout the mountains of eastern Asia, including southwestern China, Manchuria, the Far East of Russia, and South Korea (Bey-Bienko, 1935, 1938; Grandcolas, 2000; Grandcolas et al., 2001; Grandcolas et al., 2005; Wang et al., 2015; Che et al., 2016; Bai et al., 2018). They also occur in the east and west of North America, where they are rarer than in Asia (Scudder, 1862; Nalepa et al., 1997; Burnside et al., 1999). There are nearly five times as many species of Cryptocercus in eastern Asia as in North America. This pattern is similar to those seen in vascular plants (Qian & Ricklefs, 2000), which shows greater species richness in Asia than in North America even in similar environments. Accelerated allopatric speciation in eastern Asia has been proposed to be a cause of this disparity (Qian & Ricklefs, 2000), with recent studies finding evidence of phylogeographic structure in species in North America (Clark et al., 2001; Kambhampati et al., 2002) and eastern Asia (Che et al., 2016). Although Cryptocercus is widely distributed in eastern Asia as a whole, it has a highly fragmented distribution (Che et al., 2016).

Phylogenetic studies of eastern Asian taxa have only focused on resolving the relationships within Cryptocercus or between Cryptocercus and termites, rather than on the broader patterns of speciation in Cryptocercus. Most species of Cryptocercus are restricted to the Qinghai-Tibet Plateau and adjacent areas including the Hengduan Mountains, with a few exceptions (central and northeast China). Three species occur in northeastern Asia: C. changhaiensis Bai, Wang, Wang, Lo and Che, C. relictus Bey-Bienko, and C. kyebangensis Grandcolas, of which only C. kyebangensis is endemic to South Korea. There are also several species in central China, including the Qinling Mountains and Daba Mountains: C. luanchuanensis Bai, Wang, Wang, Lo and Che, C. neixiangensis Che, Wang, Shi, Du, Zhao, Lo and Wang, C. chengkouensis Bai, Wang, Wang, Lo and Che, C. shennongjiuensis Che, Wang, Shi, Du, Zhao, Lo and Wang, C. hirtus Grandcolas and Bellés, C. waxiensis Bai, Wang, Wang, Lo and Che, and C. ningshanensis Che, Wang, Shi, Du, Zhao, Lo and Wang. The geographical origins of Cryptocercus and its various lineages are still unresolved. Grandcolas (1999) proposed that the American species evolved from Asian ancestors. However, most studies have suggested that the ancestor of Cryptocercus existed in both Asia and North America, then diverged at about the time of separation between the Eurasian and American continents (Clark et al., 2001; Maekawa et al., 2005; Che et al., 2016).

The intercontinental disjunction in Cryptocercus was possibly linked to major vicariance events in other taxa. Widely distributed Palaeogene mesophytic forests were fragmented between Eurasia and North America, and then the floras of eastern and western North America became separated (Xiang et al., 1998; Wen, 1999; Xiang et al., 2000). Cryptocercus is unlikely to have been able to disperse across large expanses of unfavourable habitat, so vicariance events and migration of host plants present the most plausible explanations for the present-day disjunctions. In Asia, the uplift of the Qinghai-Tibet Plateau affected monsoon intensity (Burbank et al., 1993; An et al., 2001; Qiang et al., 2001), driving biological diversification in central and southwestern China (Bai et al., 2018). However, the evolution of Cryptocercus in Asia remains poorly understood.

In this study, we aimed to resolve the phylogenetic relationships and evolutionary history of Cryptocercus in Asia. We analysed sequence data from four mitochondrial genes and three nuclear genes to infer the phylogeny and divergence times. Based on these data, we tested the hypothesis that the spread of their host habitat explains the divergence of Cryptocercus in eastern Asia. We also investigated the mechanisms that might have been responsible for shaping the current distribution of the genus in this region of the world.

**Materials and methods**

**Taxon sampling**

We collected samples of Cryptocercus from a total of 42 localities in China (Fig. 1; Table S1), including the mountain regions of northeastern China (Changbai Mountains), central China (Qin-Daba Mountains), and southwestern China (Hengduan Mountains: mainly Western Sichuan Plateau and Yunnan Plateau). Extensive field sampling shows that populations of Cryptocercus exist widely in forested regions from the northeast to the southwest of China. The 42 localities in northeastern China, central China, and southwestern China range from 26°N to 44°N latitude and from 99°E to 130°E longitude (Table S1). Their altitudes range from 626 m (Wunufeng, Jilin Province) to 3765 m (Shikaxueshan, Yunnan Province). We added data from Cryptocercus species that were reported from South Korea and eastern Russia, as well as from three species of Cryptocercus from North America. We compiled the unique georeferenced occurrence records of the species from sample collections. Information from the voucher specimens included the latitude, longitude and elevation of each collection location (Table S1). Using these data, we established a geographic distribution database of Chinese endemic Cryptocercus.

Chinese endemic species of Cryptocercus were unevenly distributed across the country, but the Hengduan Mountains region exhibited the richest diversity (18 endemic species), followed by the Qin-Daba Mountains. The Changbai Mountains region, with an area of 1964 km², has only two species of Cryptocercus. Our field investigations in the Xiaoqin’ an Mountains and south of the Hengduan Mountains found no signs of Cryptocercus. With limited geographical distributions, narrow host range and poor adaptability, Cryptocercus had very limited habitat in the temperate forests of northeastern, central and southwestern montane regions of China. All the samples of Cryptocercus used in this study were stored in absolute ethanol, then deposited in the Institute of Entomology, College of Plant Protection, Southwest University (Chongqing, China).
DNA extraction, amplification and sequencing

Sequences were obtained for seven gene fragments, consisting of mitochondrial 12S rRNA (12S), 16S rRNA (16S), and cytochrome c oxidase subunit I (COI) and subunit II (COII), and nuclear 18S rRNA (18S), 28S rRNA (28S), and histone H3. Total genomic DNA was extracted from hindleg tissues of ethanol-preserved specimens using the TIANamp Genomic DNA Kit (DP304, Tiangen, Beijing, China). These sequences were obtained using the primers given in Table S2. All reactions were carried out in volumes of 25 μL, containing 14.25 μL of ultrapure water, 2.5 μL of 10× buffer (Mg2+ Free), 2 μL of MgCl2 (25 mm), 2 μL of dNTP mixture, 1 μL of each primer, 0.25 μL of Taq polymerase, and 2 μL of DNA template.

DNA amplification was carried out on a programmable thermal cycler. The amplification protocol was 94°C for 5 min; followed by 35 cycles at 94°C for 60 s, 62°C for 90 s, and 72°C for 2 min; and a final extension at 72°C for 10 min. Polymerase chain reaction products were separated by electrophoresis on a 1% agarose gel. DNA purification and sequencing were carried out by BGI Tech (Beijing, China) using the aforementioned primers. All sequences were deposited in GenBank (accession numbers in Table S1).

Phylogenetic analyses

Sequences were aligned via the online MAFFT v.7 server (http://ma.cbrc.jp/alignment/server/) using the Q-INS-i algorithm. Manual adjustments were done in MEGA v.6.0.6 (Tamura et al., 2013). Alignments of protein-coding genes (COI, COII and H3) were manually corrected by translation into amino acids. Alignments of the ribosomal sequences (12S, 16S, 18S and 28S) were inspected visually and a small number of poorly aligned characters within the intergenic region were removed. In
combination with some samples sequenced in previous studies, we assembled a dataset comprising 31 species (Table S1). To infer the position of the root in our phylogenetic analysis and to allow informative fossil calibrations to be included for molecular dating, we included sequence data from 29 outgroup species downloaded from GenBank, including 12 other cockroaches, 15 termites and two mantids (Table S1). PARTITIONFINDER v.1.1.1 (Lanfear et al., 2012) was used to select the optimal partitioning scheme and substitution models for the data subsets. Based on the Bayesian information criterion, the dataset was partitioned into ten subsets with the following substitution models: GTR + I + G for 12S and 16S; TrNef + I + G for COI codon positions 1 and 2 and 18S; TVM + I + G for COI codon position 3 and COII codon position 3; TrN + I + G for COII codon positions 1 and 2 and 28S; K81 + I for H3 codon positions 1 and 2; and GTR + G for H3 codon position 3.

Phylogenetic analysis was performed on the combined dataset, with termite, cockroach and mantid outgroups (Table S1), using maximum likelihood (ML) and Bayesian inference. Maximum likelihood analysis was performed using RAxML v.7.7.1 (Stamatakis et al., 2008). We used a gamma distribution to model rate heterogeneity across sites. Node support was estimated using 100 bootstrap replicates. Bayesian analysis was performed using MRBAYES v.3.2 (Ronquist et al., 2012). We ran two independent sets of Markov chains, each with one cold and three heated chains, for 10^7 steps each. Samples were drawn every 1000 steps, with the first 25% of samples discarded as burn-in. Convergence was inferred when the average standard deviation of split frequencies fell below 0.01. We checked for sufficient sampling and convergence in TRACER v.1.6 (Rambaut et al., 2018) and states were combined using LOGCOMBINER in the BEAST package. A maximum-clade-credibility tree was obtained using TREANTOTATOR in the BEAST package. In our analyses, comparison of marginal likelihoods indicated decisive support for the birth-death process over the Yule process. Therefore, we only present the trees inferred using the birth-death tree prior.

### Biogeographic analysis

We reconstructed the evolution of geographical ranges in *Cryptocercus* using a statistical dispersal-vicariance analysis (S-DIVA) and Bayes–Lagrange analysis (statistical dispersal-extinction-cladogenesis, S-DEC) implemented in RASP v.3.02 (Yu et al., 2015). Five areas of endemism were defined for the biogeographic analyses: (i) Western Sichuan Plateau in Hengduan Mountains; (ii) Yunnan Plateau in Hengduan Mountains; (iii) Qin-Daba Mountains; (iv) Changbai Mountains, including eastern Russia and South Korea; and (v) North America.

To account for uncertainty in the tree topology, we loaded all of the sampled trees from our BEAST analysis and discarded the first 500 trees. Outgroup taxa were excluded. For S-DIVA analyses, we used the ‘Allow Reconstruction’ option with a

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**Table 1.** Fossils used in this study for calibrating the estimates of divergence times of major clades.

| Species                          | Minimum age (Ma) | Calibration group | Soft maximum bound (97.5% probability) | Reference       |
|----------------------------------|------------------|-------------------|----------------------------------------|-----------------|
| Periplaneta houlberti            | 56               | Periplaneta + Deropeltis | 145 | Piton (1940) |
| Balatronis libanensis           | 125              | Blattidae + Tryonicida | 235 | Sendi & Azar (2017) |
| Epilampra                        | 41.3             | Epilampra + Galbliatta | 145 | Beccaloni (2014) |
| Mastotermes indet.               | 93.5             | termites excluding Mastotermes | 150 | Schlüter (1978) |
| Dolichorhinotermes dominicanus   | 16               | Dolichorhinotermes + Rhinotermes | 100 | Schlemmermeyer & Cancell (2000) |
| Coptotermes sucineus            | 16               | Coptotermes + Heterotermes | 33.9 | Emerson (1971) |
| Americus lucidus                | 13.8             | Americus + Orthogathotermes | 70 | Krishna & Grimaldi (2009) |
| All cockroaches                 | 140              | All cockroaches plus termites were constrained to be monophyletic in BEAST analyses | 311 | Labandeira (1994) |

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maximum of 100 reconstructions and three random steps, then a maximum of 1000 reconstructions for the final tree. A maximum number of five areas was allowed at each node. In S-DEC, we ran analyses with constrained ranges and with a maximum of five areas. Optimal S-DEC and S-DIVA reconstructions were summarized on the pruned maximum-clade-credibility tree from our Bayesian phylogenetic analysis.

Results

Phylogenetic analyses and haplotype network

For the concatenated dataset, our likelihood and Bayesian phylogenetic analyses yielded almost identical topologies with generally high node support (Figs 2, Fig. S1). The individuals from
eastern Asia formed a highly supported monophyletic group. The sampled taxa from North America, which include three of the five species known from this region, also formed a highly supported monophyletic group. The samples of Cryptocercus from China were grouped into four major geographic lineages (Fig. 2): clade I from Changbai Mountains, and including taxa from eastern Russia and South Korea; clade II from Qin-Daba Mountains; clade III from Western Sichuan Plateau in Hengduan Mountains; and clade IV from Yunnan Plateau in Hengduan Mountains. Clade I was placed as the sister group to the remaining Chinese members of Cryptocercus (ML bootstrap 100%, posterior probability 1.00). The topology inferred in both analyses grouped together clade II and clade III, followed by clade IV (ML bootstrap 83%, posterior probability 0.82). Samples of Cryptocercus from Hengduan Mountains formed a paraphyletic group.

Among the samples from clade I, C. kyebangensis was more closely related to C. changbaiensis than to C. relictus based on COI sequences (Fig. 3A) and on the combined data (Figs 2, Fig. S1). The haplotype network, based on 19 COI haplotypes from 44 samples of Cryptocercus from the Changbai Mountains and eastern Russia (Table 2), revealed two separate clades: 12 haplotypes of C. relictus (Fig. 3C, A–F) and 7 haplotypes of C. changbaiensis (Fig. 3B, G–J). No haplotype was shared between these two species, but haplotypes of these two species were distributed across the Changbai Mountains. The COI

**Fig. 3.** (A) Maximum likelihood tree inferred from cytochrome c oxidase subunit I (COI) sequences of Cryptocercus from the Changbai Mountains and eastern Russia. Major nodes are labelled with bootstrap support values. (B) Haplotype network of Cryptocercus changbaiensis. (C) Haplotype network of Cryptocercus relictus. See Table 2 for further details. [Colour figure can be viewed at wileyonlinelibrary.com].

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network showed a higher level of diversity among the haplotypes of *C. relictus* than among those of *C. changbaiensis*.

**Divergence-time and biogeographic estimates**

Our BEAST analysis yielded a tree that was congruent with our likelihood-based and Bayesian phylogenetic estimates (Fig. 4). The divergence between *Cryptocercus* and termites was estimated to have occurred in the late Jurassic to early Cretaceous, 145.2 Ma (95% credibility interval 124.7–172.6 Ma). The most recent common ancestor of *Cryptocercus* lineages was estimated at 74.8 Ma (95% CI: 51.2–103.8 Ma). The clade comprising *C. changbaiensis*, *C. relictus* and *C. kyebangensis* diverged from other Asian species 30.7 Ma (95% CI: 22.9–40.3 Ma). The last phase (Fig. 4, node 4) occurred between lineages from the Western Sichuan Plateau and Qin-Daba Mountains around 24.7 Ma (95% CI: 18.5–31.9 Ma), based on the nested position of the clade from Qin-Daba Mountains.

**Discussion**

**Phylogenetic relationships of Asian Cryptocercus**

Our results confirm the broad distribution of *Cryptocercus* in Asia and suggest that the diversification of the genus has been associated with the alignment of mountain ranges. We found that eastern Asian and North American species form monophyletic groups, which is consistent with the results of previous studies (Clark et al., 2001; Grandcolas et al., 2001; Maekawa et al., 2005; Lo et al., 2006; Che et al., 2016). The broader sampling of Asian taxa in our study has allowed us to resolve the relationships among the Asian species. *Cryptocercus kyebangensis* and *C. meridianus* Grandcolas & Legendre (which was incorrectly identified as *C. primarius* Bey-Bienko) are more closely related to each other than to *C. relictus* (Maekawa et al., 2005; Lo et al., 2006), despite the geographical proximity of *C. kyebangensis* and *C. relictus*. We also found that *C. meridianus* is placed together with other lineages from the Yunnan Plateau. This contradicts one of the unexpected results previously obtained by Che et al. (2016), who grouped together *C. meridianus* from the Yunnan Plateau in the Hengduan Mountains, and *C. kyebangensis* and *C. relictus* in northeastern China, Russia, and South Korea.

### Table 2. Cytochrome c oxidase subunit I (COI) haplotypes in different populations of *Cryptocercus* from the Changbai Mountains and eastern Russia.

| Species                  | Locality abbreviation | Haplotype ID (number: sample ID, GenBank accession numbers) |
|--------------------------|-----------------------|-------------------------------------------------------------|
| *Cryptocercus relictus*  | CBSN                  | A1 (4: CBSN2, MK909543; CBS1, MK909545; CBS2, MK909546; CBS3, MK909547;), A2 (1: CBSN3, MK909544), A3 (1: CBSN1, MK909542) |
|                          | SFLC                  | B1 (6: SFLC1, KY940636; SFLC2, KY940637; SFLC3, KY940638; SFLC4, KY940639; GLZ1, KY940640; ASR, NC018132) |
|                          | GLZ                   | C1 (1: GLZ2, KY940641)                                      |
|                          | MDF                   | D1 (3: MDF1, KY940642; MDF2, KY940643; MDF3, KY940644)      |
|                          | LLK                   | E1 (1: LLK1, MK909531), E2 (1: LLK2, MK909532), E3 (1: LLK3, MK909533) |
|                          | DBG                   | F1 (1: DBG1, MK909556), F2 (1: DBG2, MK909557), F3 (1: DBG3, MK909558) |
| *Cryptocercus changbaiensis* | DJC            | G1 (9: DJC1, MK909527; DJC2, MK909528; CBSB1, MK909534; CBSB2, MK909535; CBSB3, MK909536; WTE1, MK909538; WTE2, MK909539; WTE3, MK909540; WTE 4, MK909541), G2 (1: DJC3, MK909529) |
|                          | WNF                   | H1 (3: WNF1, MK909548; WNF2, MK909549; WNF4, MK909551), H2 (1: WNF3, MK909550) |
|                          | CBS                   | I1 (2: CBS1, KY940654; CBS2, KY940655), I2 (1: CBS4, MK909537) |
|                          | YHG                   | J1 (5: YHG1, MK909552; YHG2, MK909553 YHG3, MK909554; YHG4, MK909555; DJC4, MK909530) |

*CBSN, Changbaishanbei; SFLC, Shuangfenglinchang; GLZ, Gaolingzi; MDF, Mudanfeng; LLK, Laokulihu; DBG, Dabinggou; DJC, Dongjingcheng; WNF, Wuniufeng; CBS, Changbaishan; YHG, Yanghugou. Further details of localities are given in Table S1.*
Our analyses yielded strong support for the four major lineages of Chinese Cryptocercus, which show a distinctive geographical pattern. However, Cryptocercus species from the Yunnan Plateau in the Hengduan Mountains do not exhibit a closer relationship to the clade from the Western Sichuan Plateau in the Hengduan Mountains. This is inconsistent with previous results showing that all of the Cryptocercus species from the Hengduan Mountains grouped together, with the exception of C. meridianus (Che et al., 2016). Here we found that samples of Cryptocercus from the Hengduan Mountains...
(including Western Sichuan Plateau and Yunnan Plateau) are paraphyletic because of the inclusion of Cryptocercus species from the Qin-Daba Mountains. By contrast, Che et al. (2016) found that members of Cryptocercus from the Hengduan Mountains were rendered paraphyletic by the inclusion of all other Asian Cryptocercus species.

**Global biogeography of Cryptocercus**

Our divergence dating analyses indicate that Cryptocercus split from its sister lineage in the late Jurassic to early Cretaceous at 145.2 Ma (95% CI: 124.7–172.6 Ma). This is broadly consistent with recent estimates of 145 Ma (Misof et al., 2014), 140 Ma (Tong et al., 2015), 145.8 Ma (Che et al., 2016) and 146.4 Ma (Wang et al., 2017) for this evolutionary event, but slightly younger than the range of divergence times (166–185 Ma) estimated by Djernæs et al. (2015). Clark et al. (2001) inferred that the ancestor of extant Cryptocercus inhabited temperate deciduous forests at high northern latitudes during the Jurassic (145–201 Ma), before a general cooling during the Cretaceous (66–145 Ma) forced the community to move southwards in Asia and North America. The 74.8 Ma (95% CI: 51.2–103.8 Ma) divergence time of the Nearctic and Palaeartic Cryptocercus lineages overlaps with the range of divergence times (58.7–77.8 Ma) estimated by Maekawa et al. (2005) and is largely consistent with the estimate of 115–70 Ma by Clark et al. (2001). However, it is slightly older than the 55.1 Ma (95% CI: 41.6–72.3 Ma) estimated by Che et al. (2016).

In our reconstruction of ancestral locations, we found an initial vicariance event between American and Asian lineages at 74.8 Ma. This is inconsistent with the hypothesis that the American lineage evolved from Asian ancestors (Grandcolas, 1999). Therefore, we speculate that Cryptocercus spread in both Eurasia and the Americas in the early Cretaceous to early Palaeocene. There was a temperate deciduous flora from the Cretaceous, but there were substantial changes in the composition of plant assemblages in the Eocene (Budantsev, 1994; Hang, 2002). Asia and North America began to drift apart during the Cretaceous (66–145 Ma), but remained intermittently connected by land bridges (King, 1958). Because individuals of Cryptocercus are wingless and live in rotten wood at all stages of their life cycle (Nalepa, 1984; Park & Choe, 2003), their migration is likely to have been dependent on the succession of temperate forests rather than on long-distance dispersal. In response to continuous changes in climate since the Palaeogene, large assemblages of temperate flora moved southwards into low latitudes (Wen, 1999; Milne, 2006; Guo et al., 2008). The cooling might have driven American and Asian lineages from the Bering land bridge, leading to population divergence and speciation (Milne & Abbott, 2002).

**Biogeography of Asian Cryptocercus**

We inferred three vicariance events for Asian Cryptocercus without the occurrence of any dispersal events. This supports a scenario in which Cryptocercus was widely distributed in eastern Asia in the late Palaeocene to the early Oligocene, which does not contradict the view that the common ancestor of extant C. relictus and C. kyebangensis was distributed in east China or northeast China before migrating into the Korean Peninsula (Park et al., 2004). Our date estimates suggest that the diversification of the genus in Asia took place within the past 30 Ma.

Owing to the restricted dispersal capability of Cryptocercus, the past migrations of its lineages might have depended on the succession of temperate forests. There was a temperate-subtropical humid vegetation zone in northern China in the Palaeocene to Eocene (Sun & Wang, 2005), dominated by the modern Betulaceae, and some Cupressaceae, Cedrus and Podocarpus taxa with modern subtropical montane gymnosperm trees (Song & Tsao, 1976; Sun et al., 1980). Our sampling information confirmed these taxa to be hosts of Cryptocercus. There was a dry subtropical flora in central China and evergreen forests in south China in the late Cretaceous to Palaeocene (Tao, 1992), dominated by subtropical evergreen or deciduous broadleaved forests. These included many known hosts of Cryptocercus, such as Betula, Salix, and Zizyphus. Although there was an arid belt stretching across China in the Palaeocene, most of this region was humid in the Neogene as a result of Asian monsoon circulation (Sun & Wang, 2005), which would have been beneficial for the survival of Cryptocercus.

Our phylogenetic results show that the basal split in the eastern Asian lineage led to a clade from the Changbai Mountains, including northeastern China, Korea, and eastern Russia, and a clade from central and southeastern China, with extensions to the Hengduan Mountains. There is a distance of <1500 km between the Changbai Mountains and central and southeastern China, but a climatic barrier existed between these locations during the Eocene. It was warm and humid in northern China, but the Eocene arid belt in central China remained (Sun & Wang, 2005). Since the Oligocene, the collision of the Indian and Eurasian plates, with the associated uplift of the Himalayas, has led to the strengthening of monsoonal circulation (Allegre et al., 1984; An et al., 2001). The Qinghai-Tibetan Plateau experienced a rapid uplift around 17–25 Ma (Harrison et al., 1992; Guo et al., 2002; Spicer et al., 2003) and raised the mean altitude of the plateau to >2000 m (Shi et al., 1999). This intensified the change in climate and led to a fragmentation of forest communities (Harrison et al., 1992; An et al., 2001; Guo et al., 2002; Spicer et al., 2003), effecting a vicariance event between the lineages from the Changbai Mountains and other regions. At the same time, there was uplift-driven diversification in central and southwestern China.

At present, more than half of the species of Cryptocercus (18 species) occur in the Hengduan Mountains, which are regarded as a temperate biodiversity hotspot (Xing & Ree, 2017). Various studies have found that the uplift of the Hengduan Mountains region took place primarily during the Himalayan orogeny, between the late Miocene and the late Pliocene (Sun et al., 2011; Wang et al., 2012; Wang et al., 2014). The combination of orogenic movement and subsequent glaciation led to a terrain characterized by alpine ridges and deep river canyons (Yang
et al., 1983; Li & Zhang, 2010). This partitioned habitat is likely to have hindered gene flow and favoured rapid speciation in Cryptocercus in the Hengduan Mountains (Yunnan Plateau and Western Sichuan Plateau).

In the Qin-Daba Mountains in central China, Himalayan orogeny and climatic oscillations from the Palaeogene fragmented habitats and increased the population subdivisions in Cryptocercus. As the highest mountains in central China, the Qinling Mountains presented an important climatic and hydrological barrier between northern and southern China and restricted the northward migration of Cryptocercus. Thus, diversification is likely to have been driven by habitat fragmentation, as well as climatic oscillations and extensive uplifts of the Qinghai-Tibet Plateau.

We estimate that the lineages from the Changbai Mountains and other regions in China began to diverge in the Oligocene. Compared with other Chinese lineages, there is a lower degree of genetic differentiation among Cryptocercus from northeastern Asia, where only three species are found (C. relictus, C. changbaiensis and C. kyebangensis). We dated their most recent common ancestor to the early Oligocene (23.2 Ma), which is earlier than the time of 7.5–17.4 Ma estimated by Park et al. (2004). We dated the split between C. changbaiensis and C. kyebangensis at 11.8 Ma. Park et al. (2004) inferred that the common ancestral population of C. relictus and C. kyebangensis would have been distributed in northeastern China, and then migrated into South Korea through the connected mountains from Manchuria to South Korea or the Yellow Sea basin. Our phylogenetic results suggest that C. changbaiensis is the sister group of C. kyebangensis, not C. relictus. Based on our field investigations, we found that C. changbaiensis and C. relictus are widely distributed around the Changbai Mountains. The common ancestral populations in Manchuria might have been widely distributed during the early Miocene, but were not the result of long-distance dispersal events.

Although the present-day distributions of C. relictus and C. changbaiensis partly overlap, the two species are not found at the same sites. The divergence time (c. 2 Ma) between populations of these two species in the Changbai Mountains falls within the early Pleistocene glaciations (Cui et al., 2011). The relatively unusual demographic scenario can be explained partly by continental uplifting, glacial extinction events (Adams & Faure, 1996), and the volcanic orogeny of the Changbai Mountains between 0.6 and 15 Ma (Wan, 2012), which eventually led to the formation of the current distribution pattern of Cryptocercus in Manchuria. There is only a river barrier (Songhua River) between the Changbai Mountains and the Xiaoxinganling Mountains (Fig. 1), and we cannot differentiate the ecological conditions between these two regions. We did not find any evidence of Cryptocercus in the Xiaoxinganling Mountains.

The present-day distribution of Cryptocercus, resulting from a series of vicariance events dating back to the glacial period, has been strongly influenced by the Westerlies in winter and the Indian monsoon in summer (An et al., 2001; Zhang et al., 2009). Because the monsoons became blocked by the Gaoligong-Nushan Mountains in the Yunnan Plateau, the climate of the west side of the Gaoligong Mountains and the Yunnan Plateau became hot and humid. The climate of the eastern Gaoligong-Nushan Mountains region was cold and dry, whereas the climate of Western Sichuan Plateau was warm and humid. These conditions created ecological barriers that restricted the survival of Cryptocercus and shaped the current distribution of the genus.

Conclusions

Our study has shown that the present-day distribution of Cryptocercus represents a case of intercontinental variance, rather than dispersal from eastern Asia to North America or vice versa. Historical biogeographic analysis has highlighted the wide distribution of the genus in eastern Asia from the late Palaeocene to the early Oligocene, and that their migration might have been dependent on the succession of temperate forests. Our results are consistent with the hypothesis that the glacial periods of the Palaeogene and Neogene promoted and intensified allopatric species migration and formation in eastern Asia. We propose that the species richness and endemism in southwestern China resulted primarily from allopatric speciation driven by complex topography during the late Palaeogene and Neogene. These findings contribute to our understanding of the biogeographic processes that led to the distribution and diversification of wingless insects. Although our sampling included many of the mountainous regions of eastern Asia, additional sampling will help to improve our understanding of how temperate species of Cryptocercus in this part of the world responded to glacial-interglacial climate oscillations and complex geological conditions.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Maximum-likelihood tree inferred from combined sequences of 12S, 16S, COI, COII, 18S, 28S, and H3 genes. Bootstrap support values are displayed at nodes. Outgroups are not shown. See Table S1 for location abbreviations.

Fig S2. Reconstruction of the ancestral distributions of Cryptocercus using statistical dispersal-vicariance analysis. The map shows the biogeographic areas, namely: (A) Western Sichuan Plateau in Hengduan Mountains; (B) Yunnan Plateau in Hengduan Mountains; (C) Qin-Daba Mountains; (D) Changbai Mountains (note that the red clade includes samples from eastern Russia and South Korea; and (E) North America. The inferred evolutionary timescale is shown below the tree.

Table S1. Species included in this study, with details of families, references, collecting localities, and GenBank accession numbers.

Table S2. Primers used in this study.

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