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Molecular phylogeny and historical biogeography of *Cyclommatus* stag beetles (Coleoptera: Lucanidae): Insights into their evolution and diversification in tropical and subtropical Asia

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Cyclommatus stag beetles (Coleoptera, Lucanidae) are very interesting insects, because of their striking allometry (mandibles can be longer than the whole body in large males of some species) and sexual dimorphism. They mainly inhabit tropical and subtropical forests in Asia. To date, there has been no molecular phylogenetic research on how these stag beetles evolved and diversified. In this study, we constructed the first phylogenetic relationship for *Cyclommatus* using multi-locus datasets. Analyses showed that *Cyclommatus* is monophyletic, being subdivided into two well-supported clades (A and B). The clade A includes the island species from Southeast Asia, and the clade B is formed by the continental species. The divergent time estimates showed these beetles split from the outgroup around 43.10 million years ago (Mya) in the late Eocene, divided during the late Oligocene (around 24.90 Mya) and diversified further during the early and middle Miocene (around 18.19 Mya, around 15.17 Mya). RASP analysis suggested that these beetles likely originated in the Philippine archipelago, then dispersed to the other Southeast Asian archipelagos, Indochina Peninsula, Southeast Himalayas, and Southern China. Moreover, relatively large genetic distance and stable morphological variations signified that the two clades reach the level of inter-generic differences, i.e., the current *Cyclommatus* should be separated in two genera: *Cyclommatus* Parry, 1863 including the clade A species, and *Cyclommatus* Didier, 1927 covering the clade B species. In addition, the evidence we generated indicated these beetles' diversification was promoted probably by both long-distance dispersal and colonization, supporting an "Upstream" colonization hypothesis. Our study provides insights into the classification, genetics and evolution of stag beetles in the Oriental region.

KEYWORDS

Lucanidae, *Cyclommatus*, molecular phylogeny, divergence time, "upstream" colonization hypothesis

1. Introduction

Cyclommatus Parry (1863) is one of the most interesting genera in the Lucanidae family, with more than 60 species, distributed across the continental area of the Indochina Peninsula, Sino-Himalaya region, Southeast Asia archipelago, and parts of New Guinea (Fujita, 2010). These stag beetles exhibit highly intraspecific variability owing to male polymorphism with impressive

mandibles, sexual dimorphism and color varieties. So far, some of them have been good models for the studies of behavior and functional morphology, or popular pets and valuably private collections (Kojima, 1996; New, 2010; Gotoh et al., 2014, 2016; Goyens et al., 2015; Liu et al., 2017). These saproxylic beetles also play an important role in forest ecosystems (Araya, 1993; Tanahashi et al., 2009; Songvorawit et al., 2017). However, the taxonomic position of the genus has been a controversial issue. Their phylogenetic relationships are lacking studies. The origin, dispersal and diversification of these beetles remain poorly known.

Historically, *Cyclommatus* taxonomy has been revised several times mostly basing on external morphology. The genus *Cyclommatus* was created by Parry in 1863. Two generic names before Parry's proposition, *Lucanus* (*Cyclophthalmus*) Hope and Westwood (1845) and *Megaloprepes* Thomson (1862), were both preoccupied names. Didier (1927) divided *Cyclommatus* Parry into two generic taxa, basing on both morphological characters and distinct geographical distributions: *Cyclommatus* (s. s.) Parry (1863), containing the species with well-developed mandibles mainly from the insular area of Southeast Asia, and *Cyclommatinus* Didier (1927) in which the species show rather uniform male mandibles and that are distributed in the continental region of Sino-Himalaya. Shortly afterwards, Nagel (1936) designated the new genus *Cyclommatellus* consisting of three species, with a peculiar sixth-antennal joint in larger male. Arrow (1938, 1950) did not agree with Didier's and Nagel's classifications that was mainly based on characters of large males, being often obsolete or absent in smaller males and females. The opinion of Arrow, i.e., *Cyclommatinus* and *Cyclommatellus* were synonyms of *Cyclommatus* Parry, was followed by most of the taxonomists (Benesh, 1960; Mizunuma and Nagai, 1994; Krajčák, 2001; Fujita, 2010; Huang and Chen, 2017). Meanwhile, Didier and Séguy (1953) did not follow Arrow's opinion in their catalogue. Maes (1992) listed the two synonymized genera as subgenera of *Cyclommatus* without any taxonomic discussion. Thus, the molecular phylogeny analysis could be very useful to resolve the morphology-based controversy. Unfortunately, only a handful of studies provided a few available molecular data about this genus (Kim and Farrell, 2015; Liu et al., 2017; Yuan et al., 2021). To provide robustly phylogenetic framework and taxonomic utility of *Cyclommatus* stag beetles, we performed a multi-locus analysis.

Moreover, all collected data show that *Cyclommatus* stag beetles are endemic or restricted to the tropical and subtropical forests, either in continental areas or islands (Krajčák, 2001; Fujita, 2010; Huang and Chen, 2017), which seems to indicate a low dispersal capacity of these beetles. Similarly, low dispersal abilities were observed in other stag beetle species, such as *Lucanus cervus* (Thomaes, 2009) and *Lucanus datunensis* (Lin et al., 2011); they also inhabit tropical and subtropical forests. Therefore, *Cyclommatus* stag beetles could be an excellent model for testing specific biogeographic hypotheses. There is an interesting topic about the origin of this genus and how it spreads from Southeast Asia islands to Southern China. To test whether the establishment of *Cyclommatus* satisfies the "Upstream" hypothesis (the colonization of continents by island fauna) or the "Downstream" hypothesis (the colonization of islands by continental fauna) (Filardi and Moyle, 2005; Jonsson et al., 2011), we also made a spatio-temporal analysis.

Considering these issues, we used a combination of six DNA regions (COI, 16S rDNA, 28S rDNA, Cytb, Wingless, ITS) of 25 representative *Cyclommatus* stag beetles in order to: (1) discuss the phylogenetic

relationship between the two clades of the *Cyclommatus* stag beetles; (2) explain the current geographical distribution pattern of *Cyclommatus* stag beetles during evolution.

2. Materials and methods

2.1. Ethics statement

The sample collection in this study was approved by the Anhui University Academic Ethics Committee, Anhui Province, China.

2.2. Taxon sampling

A total of 115 specimens of 25 taxa of *Cyclommatus* stag beetles were used in this study. Several were collected in the field by members of our research team, other specimens were donated by universities or research institutes, and some by private collectors; they were preserved in 100% ethanol at -20°C for molecular analyses. Six species closely related to *Cyclommatus* were selected: *Lucanus swinhoi* Parry, 1874, *L. maculifemoratus taiwanus* Miwa, 1936, *Noseolucanus denticulus* Boucher, 1996, *Prismognathus davidis* Deyrolle, 1878, *P. prossi* Bartolozzi and Wan, 2006 and *P. triapicalis* Houlbert, 1915; they were used for outgroup comparisons in phylogenetic analyses. According to the collecting data of the samples, we drew a distribution map of the species (Figure 1). The voucher specimens and their extracted genomic DNA are deposited at Anhui University, Hefei, China, and in addition, details of the specimen vouchers and specimen locations are listed in Supplementary Table S1.

2.3. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the thoracic muscle tissues of adult beetles preserved in absolute ethanol using the Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol. Extracted DNA was kept in a freezer at -20°C until needed for the Polymerase chain reaction (PCR). The primer sets in the present study used to amplify three mitochondrial genes 16S rDNA, COI, Cytb, and three nuclear genes 28S rDNA, ITS, and Wingless are shown in Table 1 (Simon et al., 1994; Hosoya et al., 2001; Marcilla et al., 2001; Abouheif and Wray, 2002; Balke et al., 2004; Ward and Downie, 2005; Monaghan et al., 2007; Wild and Maddison, 2008).

PCR amplification reaction was conducted in 25 μl volumes reactions, containing 12.5 μl 2 \times EasyTaq SuperMix (dye), 10 μM of each primer 1 μl , 2 μl template DNA, and 8.5 μl sterile double-distilled water (ddH₂O). The PCR amplification conditions were as follows: initial denaturation at 94°C for 2 min, followed by 35–37 cycles of denaturation at 94°C for 40 s, annealing at $52\text{--}58^{\circ}\text{C}$ for 50 s, and elongation at 70°C for 1 min, and finally 7 min extension step at 72°C . Amplifications were purified using the Template DNA Amplify Kit (Ensure Biologicals). Sequencing the successful PCR products, using the ABI PRISM Big Dye terminator v3.1 sequencing kit (Life Technologies, United States) and cycling on the ABI PRISM 3730xl automated sequencers (Life Technologies, United States) at Sangon Biotech Company, China. The sequences were deposited in GenBank with the accession number (Supplementary Table S1).

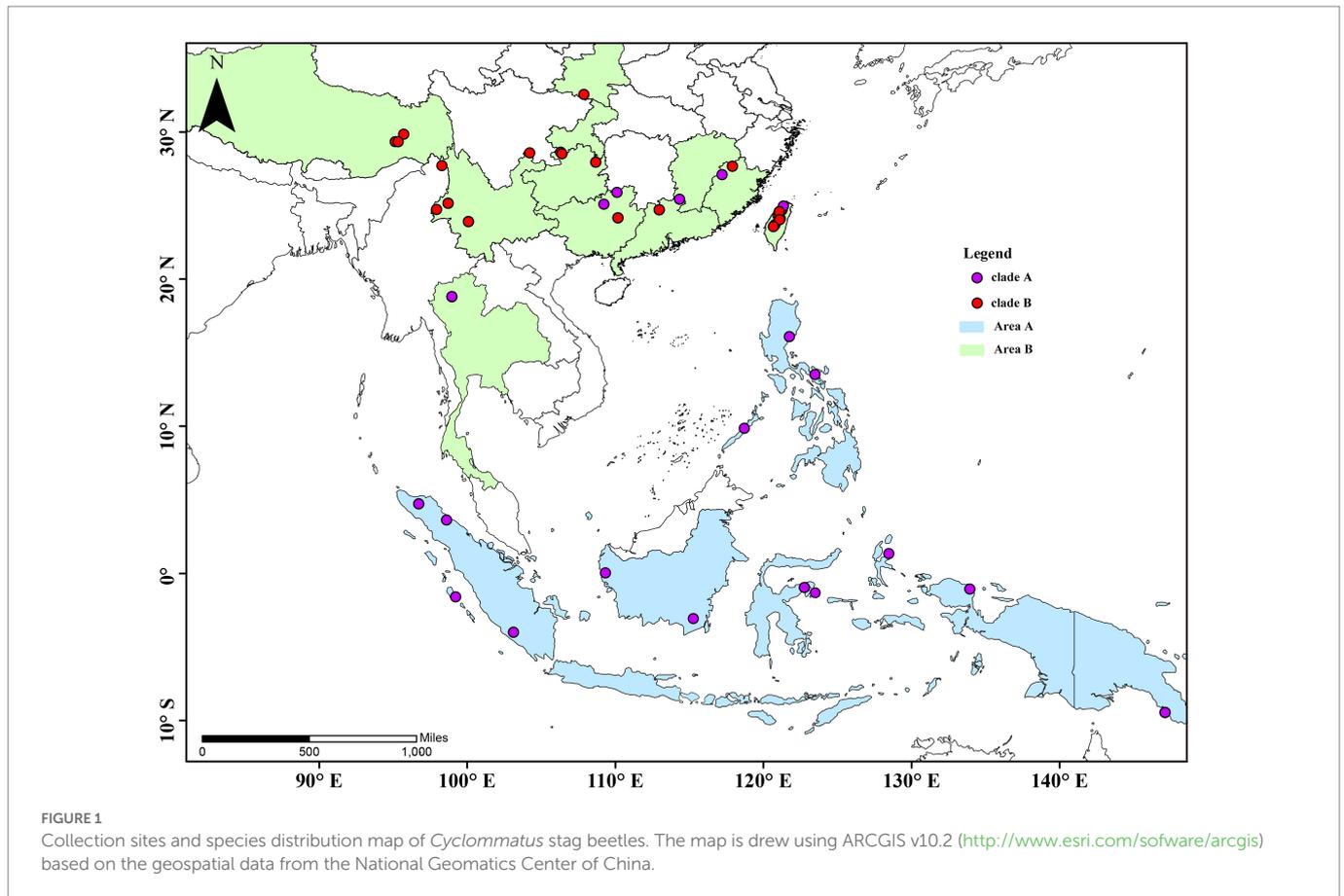


TABLE 1 Information on the primers used in this study.

Gene	Primer name	Sequence (5'-3')	Reference
COI	COI-F1	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)
	COI-R1	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994)
16S rDNA	16S-F1	CCGTTTGAAGCTCAGATCATG	Hosoya et al. (2001)
	16S-R1	TAATTATGTACCTTGTGTATCAG	Hosoya et al. (2001)
Cytb	Cytb-F2	GAGGAGCAACTGTAATTACTAA	Balke et al. (2004)
	Cytb-R2	AAAAGAAARTATCATTCAGGTTGAAT	Balke et al. (2004)
28S rDNA	28SDD	GGGACCCGTCTTGAAACAC	Monaghan et al. (2007)
	28SFF	TTACACACTCCTTAGCGGAT	Monaghan et al. (2007)
ITS	ITS-2F	CTAAGCGGTGGATCACTCGG	Marcilla et al. (2001)
	ITS-2R	GCACTATCAAGCAACACGACTC	Marcilla et al. (2001)
Wingless	Wg550F	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	Wild and Maddison (2008)
	WgAbRZ	CACTTNACYTCRCARCACCARTG	Wild and Maddison (2008)
	Wg578F	TGCACNGTGAARACYTGCTGGATG	Ward and Downie (2005)
	WgAbR	ACYTCGCAGCACCARTGGAA	Abouheif and Wray (2002)

2.4. Sequence alignment, selection of models and phylogenetic analyses

All sequences were viewed, assembled, and edited in Geneious Prime v2019.1.1.¹ Sequences for each of the six DNA regions were

¹ <https://www.geneious.com>

aligned and then inspected by eye for accuracy and trimmed to minimize missing characters in MEGA v7.0 (Kumar et al., 2016). We use PartitionFinder v2.1.1 (Lanfear et al., 2017), a small program in PhyloSuite v1.2.1 (Zhang et al., 2020), to select the best-fitting model selection and best data partition scheme. The total of six partitions were determined to be optimal using the greedy algorithm under the corrected Akaike Information Criterion (AICc): (1) 16S, (2) COI, (3) Cytb, (4) 28S, (5) ITS, (6) Wingless. Finally, best-fitting models were

selected for each partition under the AICc: the GTR + I + G model for (1), (2), (3), (4), (5), and the GTR + G model for (6).

Phylogenetic analyses were conducted based on maximum likelihood (ML) and Bayesian inference (BI). An ML tree was constructed using the IQ-TREE webserver² (Nguyen et al., 2015). In the ML analysis, the “automatic” option was set under the optimal evolutionary model, and the ultrafast Bootstrap approximation method with 10,000 replicates was used to construct the phylogenetic tree. We also implemented a Bayesian analysis as performed in MrBayes v3.2 (Ronquist et al., 2012; Trifinopoulos et al., 2016). Four Markov chains were run simultaneously and sampled once every 1,000 generations, for a total of 10.5×10^8 generations. The initial 25% of trees were discarded as burn-in. Analysis was settled based on the average standard deviation of frequency division being less than 0.01. Phylogenetic trees were viewed and edited in FIGTREE v1.4.4.³

2.5. Divergence time estimation

BEAST v2.6.0 (Bouckaert et al., 2014) software was used to perform the analysis for time divergence estimation, which combined two mitochondrial genes (COI and 16S rDNA). A strict molecular clock was applied in a Markov chain Monte Carlo (MCMC) simulation running for 10.5×10^8 generations with samplings every 1×10^4 generations using BEAST. For the beetles, substitution rates of 0.017 per lineage in a million years (Myr) for COI and 0.0054/lineage/Myr for 16S rDNA were found to be optimal (Papadopoulou et al., 2010). TRACER v1.7 (Rambaut et al., 2018) was used to analyze the output results of the Effective Sample Size (ESS) for the posterior distribution of estimated parameter values. Next, the first 25% of the run was discarded as burn-in, all post-burn-in trees from the four independent runs were combined using the software LOG COMBINER v2.6.0 (Bouckaert et al., 2014). Used TREE ANNOTATOR v2.5.1 (Bouckaert et al., 2014) to summarize information from the individual post-burn-in trees onto a single maximum clade credibility (MCC) tree. The final tree was described using FIGTREE v1.4.4.

2.6. Ancestral area reconstruction

To further explore the origins and dispersal routes of *Cyclommatus*, the ancestral distribution of the genus sequences was reconstructed based on the combination of COI and 16S rDNA. Ancestral range reconstruction analysis was conducted with the program RASP v4.2 (Yu et al., 2020) based on the MCC tree from the BEAST, which incorporates the following models:

Dispersal-Extinction-Cladogenesis (DEC, Ree and Smith, 2008; Massana et al., 2015), DEC + Jump parameter (DEC + J, Matzke, 2014a), Dispersal Vicariance Analysis with Likelihood implementation (DIVA LIKE, Ronquist, 1997; Matzke, 2014a), DIVALIKE + Jump parameter (DIVALIKE + J, Matzke, 2014b), Bayesian inference for discrete Areas with Likelihood implementation (BAYAREALIKE, Landis et al., 2013; Matzke, 2014a), BAYAREALIKE + Jump parameter (BAYAREALIKE + J,

Matzke, 2014b), as implemented in BioGeoBEARS (Matzke, 2013, 2014a,b; R Core Team, 2021). Choose the best model according to the corrected AICc and Akaike weights from the previously mentioned six models. The distribution range of *Cyclommatus*, was based on specimens collected information and fieldwork. Then, the areas were encoded as: (A) the main islands in Southeast Asia; (B) the mainland from the Indochina Peninsula to the southeast of the Himalayas.

3. Results

3.1. Phylogenetic relationships

Phylogenetic inferences using maximum likelihood (ML) and Bayesian inference (BI) showed a highly similar topology, which was divided into two major clades within the *Cyclommatus* stag beetles, indicated by us as clade A and clade B (Figure 2). The outgroups formed an independent clade, separated from other high support specimens. The clade A includes 36 specimens representing 19 taxa and is primarily found in the Southeast Asian Islands (Figure 2). However, *C. mniszeczki* Thomson, 1856 is also found in Southeast China, and not only on Taiwan Island. There are several species from New Guinea, such as *C. eximius* Möllenkamp, 1909, *C. imperator* Boileau, 1905, *C. pulchellus* Möllenkamp, 1900, *C. margaritae* Gestro, 1877 and *C. weinreichi* Lacroix, 1972. The species located in the Philippines include *C. alagari* De Lisle, 1968, *C. suzumurai* Mizumura and Nagai, 1991, and *C. zuberi* Parry, 1876. The remaining species, for example, *C. elaphus* Gestro, 1881, *C. truncatus* Schenk, 2000, *C. dehaani* Westwood, 1842, *C. canaliculatus* Ritsema, 1891, *C. cupreonitens* Boileau, 1901, *C. tarandus* Thunberg, 1806, and *C. metallifer* Boisduval, 1835 are mainly distributed in Indonesia. The clade B includes 79 samples representing 10 taxa distributed mainly in the continental area from Indochina to the Himalayan area. *C. bicolor* Bomans, 1991 is from Thailand and *C. scutellaris* Möllenkamp, 1912 is broadly distributed in southern China. *C. vitalisi* Pouillaude, 1913, *C. assamensis assamensis* Séguy, 1955, *C. assamensis yingjiangensis* Huang and Chen (2017), *C. nagaii* Fujita (2010) and *C. albersi* Kraatz, 1894 are all distributed in the southwestern regions of China. The remaining taxa *C. saltini* Bomans, 1991, *C. asahinai asahinai* Kurosawa, 1974 and *C. asahinai nanlingensis* Schenk, 2016 are mainly located in southeastern China (Figures 1, 2).

3.2. Genetic distances calculation

Genetic distances (K2P-distances) using the COI gene were calculated between the clade A and clade B at 0.176 (Table 2), while the average mean genetic distance within the genus is 0.160.

3.3. Divergence-time estimation

The estimation of divergence time showed that *Cyclommatus* stag beetles (Figure 3) date back to 43.10 million years ago (Mya) in the Late Eocene [95% highest posterior density (HPD) confidence interval: 27.61–66.39 Mya]. Then, in the Late Oligocene (circa 24.90 Mya) [95% HPD, 16.24–36.55 Mya, node 1], *Cyclommatus* stag beetles were divided into clade A and clade B. Clade A showed interspecific divergence during the Early Miocene (circa (ca.) 18.19 Mya [95% HPD, 12.43–25.95

² <http://iqtree.cibiv.univie.ac.at/>

³ <http://beast.bio.ed.ac.uk/figtree>

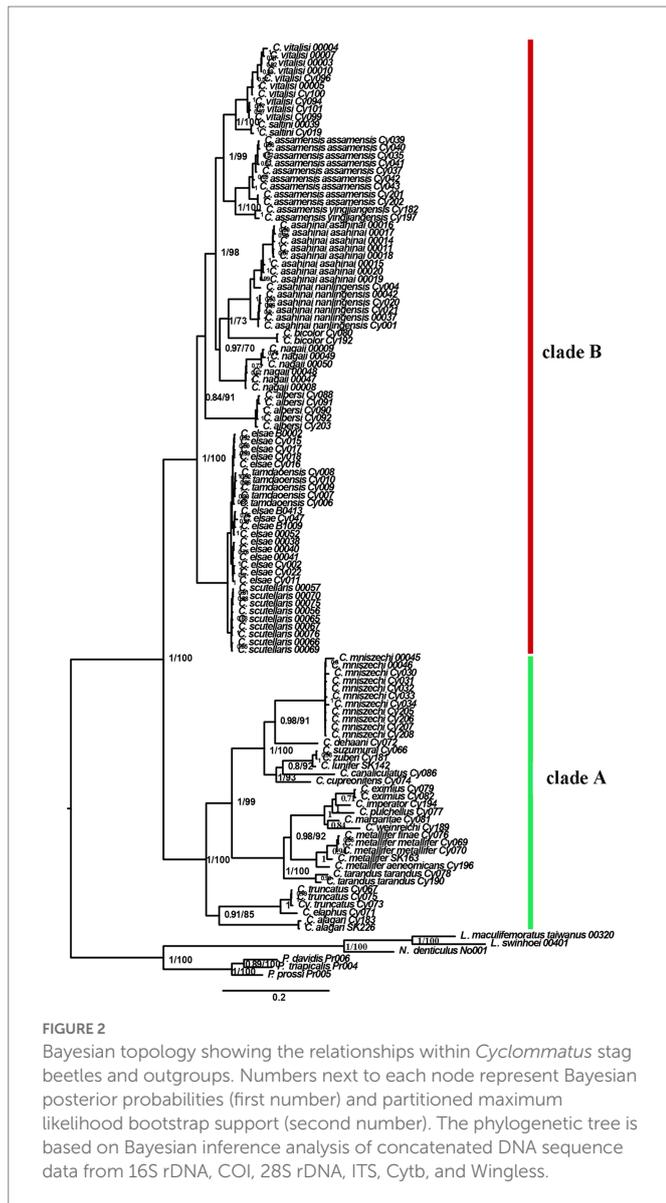


FIGURE 2 Bayesian topology showing the relationships within *Cyclommatus* stag beetles and outgroups. Numbers next to each node represent Bayesian posterior probabilities (first number) and partitioned maximum likelihood bootstrap support (second number). The phylogenetic tree is based on Bayesian inference analysis of concatenated DNA sequence data from 16S rDNA, COI, 28S rDNA, ITS, Cytb, and Wingless.

TABLE 2 The genetic distance for the *Cyclommatus* stag beetles (Kimura 2-parameter).

	Clade A	Clade B	Outgroup
Clade A			
Clade B	0.176		
Outgroup	0.217	0.213	

Mya, node 2], while clade B experienced divergence during the Middle Miocene around 15.17 Mya [95% HPD, 9.58–22.60 Mya, node 3].

3.4. Reconstruction of ancestral areas

We used the RASP to analyze the ancestral distribution area of *Cyclommatus* stag beetles. AICc scores showed that the best model for this stag beetle dataset in this analysis was the DIVALIKE model (Table 3). Based on this model analysis, at least two geographically isolated events (v) and one dispersal event could explain the genus

distribution pattern (Figure 4). Most of the other events were presumed to be speciation within the distribution range, and the distribution range of descendent species are the same as that of their ancestor (y).

According to this model the ancestor of *Cyclommatus* stag beetles most probably inhabited the Philippine Islands (Southeast Asian islands), then dispersed to other Southeast Asian islands, the Indochinese peninsula, the southeastern Himalayas, and finally to southern China (Figure 4). Initially, a geographic isolation event divided this population into two groups: clade A (Southeast Asia) and clade B (Southern China). Later, clade A diversified by expanding their range to the main island area of Southeast Asia, and subsequently, experienced isolation events such as the distribution of *C. mniszeczii* in southeastern China (area B). Finally, the species of clade B diversified gradually through the colonization of the mainland from the Indochina Peninsula to the Southeast Himalayas.

4. Discussion

4.1. Phylogenetic analyses

Phylogenetic inferences using ML and BI showed a highly similar topology, which confirmed that *Cyclommatus* stag beetles belong into a monophyletic group. The results are consistent with previous studies (Kim and Farrell, 2015; Tsai and Yeh, 2016; Wu, 2016), although their biological samples were not fully sufficient. The phylogenetic analysis also showed that the samples formed two genetically distinct population clusters (i.e., clade A and B). Clade A species are mainly distributed in Southeast Asian islands, while clade B covers mainly the southern part of China. Two well supported genetic separations implied that species of clade A and clade B could belong to two different taxa, respectively. Furthermore, the K2P-distance between clade A and clade B provided informative sign to assess their generic relationship. The average inter-genetic K2P distance is 0.220 (range 0.174–0.259) in Lucanidae (Wu, 2016). Within a few of closely related lucanid genera, such as between *Falcicornis* Planet, 1894 and *Dorcus* MacLeay, 1819; *Rhaetus* Parry, 1864 and *Rhaetulus* Westwood, 1871, the K2P-distances values are 0.173 and 0.174, respectively (Wu, 2016; Chen, 2018). The K2P-distance value between the clade A and clade B (=0.176) in our study suggests that the two clades could represent different genera. Additionally, morphological comparison (the terminology used in this study from Holloway (2007) of the two clades indicated some variability, and their differences were supported by Wan (2007), Fujita (2010), Huang and Chen (2017). In species of clade A, the males usually have well-developed mandibles, which can be even 2–3 times their body length (total length of head, pronotum and elytra), and in their genitalia the parameres are long and pointed; their permanently everted internal sac are relatively short, usually no more than twice the length of aedeagus; the female spermathecal gland and spermatheca are clearly separated, and the bursa copulatrix is short (e.g., in *C. mniszeczii*). In reverse, the male mandibles are less developed in the clade B species, usually not exceeding the total length of the head and pronotum; the parameres are rounded and blunt, the permanently everted internal sac relatively long, at least twice the length of aedeagus; the spermathecal gland and spermatheca of female are almost attached to the base of bursa copulatrix, and the bursa copulatrix is long (e.g., in *C. assamensis*).

In summary, the well-separated topology, relatively high genetic differences and stable morphological variations between the clade A and clade B showed that they could reach the level of inter-generic differences. Consequently, the current *Cyclommatus* stag beetles could be divided in the typical genus *Cyclommatus* Parry (1863) and the resurrected genus *Cyclommatinus* Didier (1927). Despite having no molecular data for *Cyclommatellus* Nagel (1936) in this study, we suggest to include it in *Cyclommatus* Parry (1863) basing on the morphological characters. Taxonomic notes on the genera *Cyclommatus* Parry (1863) and *Cyclommatinus* Didier (1927) are presented, which include new and revised status for *Cyclommatinus*. Type species, synonymies, generic diagnosis, updated species checklist with distribution data of the two genera are also given.

4.2. Biogeography and evolutionary trends

Our results are consistent with the “Upstream” colonization hypothesis in *Cyclommatus* stag beetles, which a general colonization from Southeast Asia islands to mainland Asia, and from tropical archipelagos to subtropical continents, with Southeast Asian being the source of mainland Asian lineages. This diversification pattern is similar to those previously found in other taxa (van der Kaars, 1991; Grismer et al., 2017; Williams et al., 2017; Chen et al., 2018; Liang et al., 2018; Poyarkov et al., 2022). Therefore, islands can be a source and a sink for continental biodiversity (Bellemain and Ricklefs, 2008; Weinell et al., 2021). The direction of species dispersal from continents to islands has also been recorded in Asia (Matuszak et al., 2016), which is the “Downstream” colonization hypothesis. However, it is necessary to synthesize the diversity patterns of other endemic genera within this large geographic range to produce a comprehensive understanding of regional source-sink dynamics between continents and islands.

Our biogeographic analysis shows that *Cyclommatus* stag beetles appear to have originated from the Late Eocene to the Early Oligocene transition (ca. 43.10 Mya). During this period, the global climate shifted from warm and humid to cold and dry, especially in Southeast Asia (Zachos et al., 2005). At the same time, the continuously humid forests shrunk and fragmented (Bain and Hurley, 2011; Buerki et al., 2013), which may have promoted the initial diversification of *Cyclommatus* stag beetles.

At the beginning of the Oligocene, the global climate began to cool sharply and sea levels dropped, leaving more islands in Southeast Asia exposed and connected, allowing species to spread southward to the islands in Southeast Asia (Tan et al., 2020). At this time, the

Qinghai-Tibet Plateau and its tectonic morphology had been initially formed, and the arid areas began to retreat inland, while most of the inland arid areas began to gradually diverge from the humid areas affected by the East Asian monsoon (Harrison et al., 1992; Kutzbach and Ruddiman, 1993; Zhisheng et al., 2001; Qiang et al., 2011). These factors may have facilitated the differentiation of *Cyclommatus* stag beetles to form two clades corresponding to clade A and clade B during the late Oligocene (ca. 24.90 Mya).

In the Late Jurassic, western Sulawesi, Java, and Borneo separated from Australia and united with the Sunda lands during the Cretaceous (Hall, 2013). During the Tertiary period, Sulawesi separated from Borneo, forming the Makassar Strait, which became a barrier for

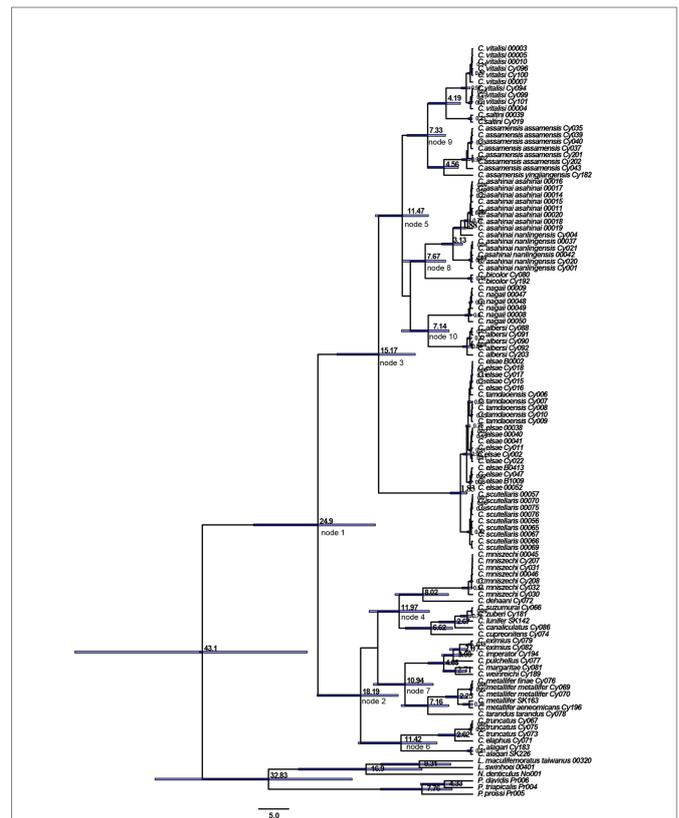


FIGURE 3 Divergence time estimation of *Cyclommatus* stag beetles based on COI and 16S rDNA gene. Divergence time estimates are represented next to the nodes (in millions of years) with horizontal bars indicating 95% highest posterior density intervals.

TABLE 3 Statistical results from RASP of *Cyclommatus* stag beetles.

Model	LnL	Number of parameters	d	e	j	AICc	AICc_wt
DEC	-8.6	2	0.0024	1.00E-12	0	21.31	0.12
DEC+J	-7.92	3	9.00E-10	4.80E-10	0.0043	22.07	0.084
DIVALIKE	-7.05	2	0.0038	1.00E-12	0	18.21	0.58
DIVALIKE+J	-7.01	3	1.50E-08	1.00E-12	0.0049	20.26	0.21
BAYAREALIKE	-14.91	2	0.0055	0.01	0	33.93	0.0002
BAYAREALIKE+J	-10.61	3	1.00E-07	1.00E-07	0.0096	27.44	0.0057

Comparison of the fit of different models of geographic-range evolution and model-specific estimates for the different parameters. LnL referred to log-likelihood; d, rate of dispersal; e, rate of extinction; j, the likelihood of founder-event speciation at cladogenesis; AICc, the corrected Akaike information criterion. The AIC model weight is used to compare all the models together to select the best one. The preferred model is indicated by the bold and red lines.

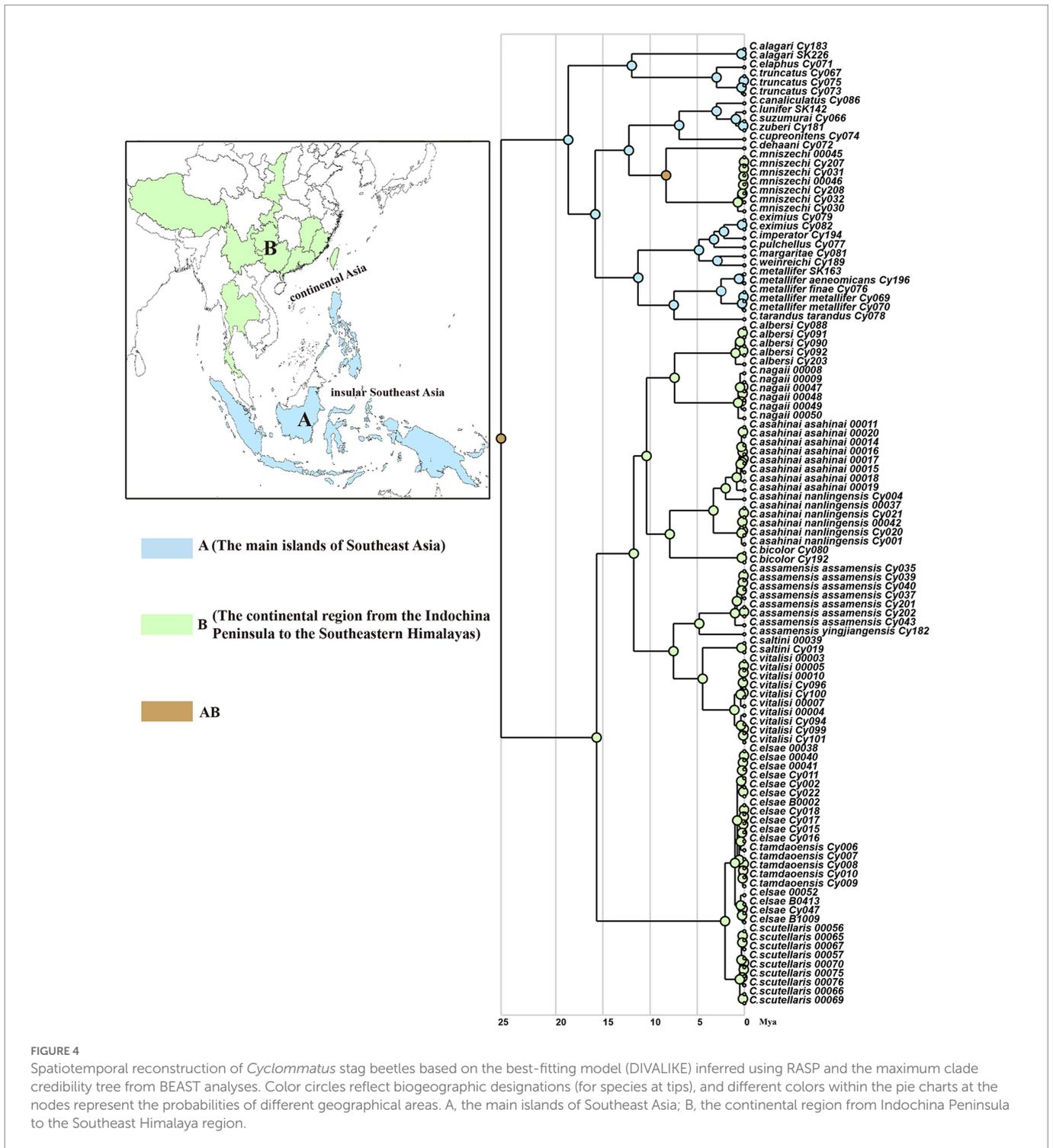


FIGURE 4 Spatiotemporal reconstruction of *Cyclommatus* stag beetles based on the best-fitting model (DIVALIKE) inferred using RASP and the maximum clade credibility tree from BEAST analyses. Color circles reflect biogeographic designations (for species at tips), and different colors within the pie charts at the nodes represent the probabilities of different geographical areas. A, the main islands of Southeast Asia; B, the continental region from Indochina Peninsula to the Southeastern Himalaya region.

species exchange between Borneo and Sulawesi with the central and northern Borneo and Palawan land surfaces in the Early Miocene (Hall, 2013). Thus, we speculated that it promoted the species diversity of the clade A through geographical isolation due to islands and straits, in the Early Miocene (ca. 18.19 Mya). After, it was the main uplift stage of the Qinghai-Tibet Plateau, the arid inland areas of Asia began to expand further (Harrison et al., 1992; Kutzbach and Ruddiman, 1993), and the monsoon gradually became stronger in the period of the Middle Miocene. Subsequently, the global temperature began to drop (Zachos et al., 2005). These events are believed to have accelerated the prosperity of most groups in the clade B of *Cyclommatus* stag beetles. In previous

studies, there are several Asian taxa with distributions which are similar to our clade B group (e.g., Che et al., 2010; Gao et al., 2013; Klaus et al., 2016; Chen et al., 2018; Yan et al., 2020).

In the late Miocene, affected by the impact of the Indian Asian plate, *Cyclommatus* stag beetles differentiated many times. *C. mniszechi*, which is an clade A taxon, gradually spread from Southeast Asian islands to the Asian continent (8.02 Mya). The southern China populations of this species resemble the clade B species in external morphology, indicating that this species adapted its phenotype to the continental habitat environment after spreading from the Taiwan island to the mainland. This species could be used

as an example of the spread to the continent from the islands. In general, our study strengthens the idea that Borneo and Indochina are evolutionary hotspots for the biodiversity in Southeast Asia (de Bruyn et al., 2014).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The animal study was reviewed and approved by Anhui University.

Author contributions

XZ and JY contributed to data curation, formal analysis, methodology, and writing of the original draft. XW supervised the manuscript writing and helped with data analysis. LZ contributed to the visualization, methodology, and investigation. LB contributed to sample collection, review and editing. All authors reviewed, edited, and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2023.974315/full#supplementary-material>

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