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Colorful patterns indicate common ancestry in diverged tiger beetle taxa: Molecular phylogeny, biogeography, and evolution of elytral coloration of the genus *Cicindela* subgenus *Sophiodela* and its allies ☆

Kaoru Tsuji [a], Michio Hori [b], Moe Hnin Phyu [c], Hongbin Liang [d], Teiji Sota [b,*]

[a] Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan
[b] Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan
[c] Department of Entomology and Zoology, Yezin Agricultural University, Yezin, Myanmar
[d] Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Science, Beijing 100101, China

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ABSTRACT

We investigated the phylogenetic relationships among tiger beetles of the subtribe Cicindelina (=*Cicindela* s. lat.; Coleoptera: Cicindelidae) mainly from the Oriental and Sino-Japanese zoogeographic regions using one mitochondrial and three nuclear gene sequences to examine the position of the subgenus *Sophiodela*, currently classified in the genus *Cicindela* s. str., their biogeography, and the evolution of their brilliant coloration. The subgenus *Sophiodela* was not related to the other subgenera of *Cicindela* s. str. but was closely related to the genus *Cosmodela*. In addition, the Oriental genus *Calochroa* was polyphyletic with three lineages, one of which was closely related to *Sophiodela* and *Cosmodela*. The clade comprising *Sophiodela*, *Cosmodela* and two *Calochroa* species, referred to here as the *Sophiodela* group, was strongly supported, and most species in this clade had similar brilliant coloration. The *Sophiodela* group was related to the genera *Calomera*, *Cicindela* (excluding *Sophiodela*) and *Cicindelidia*, and these were related to *Lophyra*, *Hipparidium* and *Calochroa*, except species in the *Sophiodela* group. Divergence time estimation suggested that these worldwide Cicindelina groups diverged in the early Oligocene, and the *Sophiodela* group, which is found in the Oriental and Sino-Japanese zoogeographic regions, in the mid Miocene. Some components of the elytral pattern related to maculation and coloration in the Cicindelina taxa studied contained weak, but significant, phylogenetic signals and were partly associated with habitat types. Therefore, the brilliant coloration of the *Sophiodela* was related to both phylogeny and habitat adaptation, although the function of coloration needs to be studied.

1. Introduction

Diversity in insects is partly represented by extensive variation in color patterns. Although similarities in color patterns are the first clue to recognizing relatedness among species, it has also been well documented that distantly related species exhibit similar color patterns as a result of convergence (e.g., mimicry; Wickler, 1968). In contrast, genital characters, especially of males, are often more reliable clues for discriminating closely related species and for discriminating groups of related species (e.g., genera) based on homologous genital characters. However, identifying homologous characters and correctly evaluating character states for complex genitalia are difficult tasks given that genital morphology can diversify rapidly under sexual selection (Eberhard, 1985; Hosken and Stockley, 2004). Therefore, classifications based on genital characters may not necessarily result in correct groupings of species. Thus, molecular phylogenetic analysis is ultimately needed to resolve phylogenetic relationships among diverse species of insects.

Tiger beetles (Coleoptera: Cicindelidae) are an iconic group of the hyper-diverse insect order Coleoptera, comprising approximately 2300 species (Pearson and Vogler, 2001). They provide intriguing materials for the study of character evolution, including body coloration and biogeography (Cassola and Pearson, 2000; Pearson and Vogler, 2001). Previous molecular phylogenetic studies have revealed that basal groups of Cicindelidae are confined to different continents and generally possess dark coloration, whereas the most derived group of this family, the subtribe Cicindelina (=*Cicindela* s. lat.), is widely distributed worldwide and comprises some 1000 species of various coloration (Vogler

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* Corresponding author. Fax: +81 75 753 4101.
E-mail address: sota@terra.zool.kyoto-u.ac.jp (T. Sota).

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and Barraclough, 1998). The genus *Cicindela* is a major group in Cicindelina, and some species exhibit brilliant coloration with a metallic luster, which is a structural coloration produced by multiple transparent reflecting layers (Shelford, 1917; Schultz, 1991). Such a brilliant metallic coloration may confuse or deceive predators in habitats with contrasting patches of illumination (Schultz, 1986, 1991). A distinct group in *Cicindela* with brilliant coloration is the subgenus *Sophiodela* confined to Asia in the Oriental and Sino-Japanese zoogeographic regions (Wiesner, 1992; zoogeographic regions follow Holt et al., 2013). A molecular phylogenetic study of Cicindelina (=*Cicindela* s. lat.) revealed that *Cicindela* (*Sophiodela*) is not related to other *Cicindela* species studied, but is sister to *Cosmodela* (Pons et al., 2004). *Cosmodela* is centered in the Oriental region and generally has brilliant coloration like that of *Sophiodela*. Although taxon sampling by Pons et al. (2004) was limited (one species from each of *Sophiodela* and *Cosmodela*; six species from *Cicindela* excluding *Sophiodela*), this result suggests that *Sophiodela* and *Cosmodela* are sister groups, distinct from *Cicindela*. Further, among diverse Oriental species of Cicindelina, the genus *Calochroa* includes species with similar coloration to that of *Sophiodela*/*Cosmodela*. *Calochroa* appears to be a heterogeneous group, considering that Rivalier (1961) recognized six groups within this genus, and it may be polyphyletic.

In this study, we aimed to reveal the phylogenetic relationships of *Sophiodela* with its related groups in the subtribe Cicindelina in terms of molecular phylogeny as suggested by clade I in Pons et al. (2004). Clade I consists of *Cicindela* (s. str.), *Cicindelidia*, *Lophyridia* (=*Calomera*), *Cosmodela*, *Calochroa*, *Hipparidium* and *Lophyra*, in addition to *Cicindela* (*Sophiodela*). Specifically, we intended to assess whether *Sophiodela* was most closely related to *Cosmodela* and a part of *Calochroa* with similar color patterns compared with the other *Cicindela* species, thus determining how similarity in color pattern in these tiger beetles reflects phylogeny rather than parallel evolution due to adaptation to habitat. In addressing the evolution of color pattern we also estimated divergence time and discussed the biogeography of clade I, which includes *Sophiodela*.

2. Materials and methods

2.1. Taxon sampling

We analyzed samples from genera/subgenera in clade I of Pons et al. (2004), namely *Cicindela*, including the subgenus *Sophiodela*, *Cicindelidia*, *Lophyridia* (=*Calomera*), *Cosmodela*, *Calochroa*, *Hipparidium* and *Lophyra*; clade I was sister to a group comprising *Jansenia*, *Chaetotaxis* and *Taenidia*, which can be regarded as outgroup taxa. We analyzed samples from all taxa except *Taenidia*; we also used *Cylindera* species (clade II in Pons et al., 2004) as potential outgroup taxa in addition to *Jansenia* and *Chaetotaxis*. Together, we used a total of 178 samples from 75 species (Table 1; see Supplementary Table S1 for details). The subgenus *Sophiodela* includes three species, *C. cyanea*, *C. chinensis* with four subspecies and *C. ferriei*. We could not include *C. cyanea* in our analysis as this species has not been collected recently. Adult beetles preserved in 99% ethanol were used for DNA extraction.

2.2. DNA sequencing

Total genomic DNA was extracted from the thorax muscles using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Regions of the mitochondrial cytochrome oxidase subunit I (COI), nuclear 28S rDNA (28S), elongation factor 1a F2 copy (EF-1a) and wingless (wg) genes were PCR-amplified, and the PCR products were sequenced using an ABI 3031xl sequencer (Applied Biosystems, Foster City, CA, USA). Primers used for PCR and direct sequencing were as follows. COI: forward COS2183N (5′-CAR CAY YTA TTY TGR TTY TTY GG-3′) and reverse COA3107 (5′-TCT ATT ARD GGD GAD GCD CTA TCT TG-3′) (Sota and Hayashi, 2004) or forward C1J-2195 (5′-TTG ATT TTT TGG TCA TCC AGA AGT-3′) and reverse TN2N-3014 (5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′) (Simon et al., 1994); EF-1a: forward EF-1a-cicindela-F (5′-GGA CAC AGA GAT TTC ATC AAR AA-3′) and reverse EF-1a-cicindela-R (5′-CAA AGC TTC RTG RTG CAT TT-3′) (this study); 28S: forward 28S-01 (5′-GAC TAC CCC CTG AAT TTA AGC AT-3′) and reverse 28S-01R (5′-GAC TCC TTG GTC CGT GTT TCA AG-3′) (Kim et al., 2000); wg: forward LepWg1 (5′-GAR TGY AAR TGY CAY GGY ATG TCT GC-3′) and reverse ModLepWg2 (5′-ACT ICG CAR CAC CAR TGG AAT GTR CA-3′) (Brower and DeSalle, 1998). Sequences used in this study were deposited in the DNA Data Bank of Japan (DDBJ; accession numbers: AB821879–AB821950, LC020251–LC020264, LC020266–LC020396).

2.3. Phylogenetic analysis

The COI and wg gene sequences were manually aligned unambiguously, and 28S and EF-1a sequences were aligned using the program MUSCLE ver. 3.8.31 (Edgar, 2004). A maximum-likelihood (ML) analysis of the combined matrix was conducted using RAxML version 8.0.0 (Stamatakis, 2014). The sequence data were divided into 11 partitions, including three codon positions each of COI, wg and EF-1a exon, along with EF-1a intron and the entire 28S sequence. The substitution model GTR+G (general time reversible model with gamma distribution for rate heterogeneity) was applied to each partition because this model is the most general and versatile model, and no computational problems due to over-parameterization occurred in our study (see also The RAxML v.8.0.X Manual by A. Stamatakis, 2014). We used PartitionFinder v1.1.1 (Lanfear et al., 2012) to select the optimal partition scheme for 11 partitions. We used four "user schemes" with 4–11 partitions to avoid the combination of partitions from different genes. The substitution model was limited to GTR+G. All analyses using the AIC, AICc, and BIC criteria resulted in the maximally partitioned scheme. RAPID ML analysis with 1000 bootstrapping analyses was used to obtain an ML tree and bootstrap percentages of nodes. In addition, Bayesian inference (BI) of phylogeny was conducted using MrBayes version 3.2 (Ronquist et al., 2012) with the same partitioning scheme and substitution models as in the RAxML analysis. Two runs of four Metropolis-coupled Markov chain Monte Carlo (MCMC) iterations were conducted for 10 million generations with sampling of trees every 1000 generations. We ensured that the potential scale reduction factor (PSRF) approached 1 for all parameters and that the average standard deviation of split frequencies was less than 0.01. We also used Tracer version 1.6 (Rambaut et al., 2014) to ensure that convergence was reached for the posterior distributions of the parameter estimates, and the effective sample size (ESS) of these estimates was >200. A 50% majority rule consensus tree after initial 25% generation data were discarded as burn-in.

To estimate divergence time and construct a ultrametric tree for analyzing the evolution of elytron color pattern, we constructed a calibrated species tree using BEAST ver. 1.8.0 (Drummond et al., 2012). This analysis included 59 samples representing different taxa with data for all genes (except a few taxa that had no EF-1a data). Sequence partitions and substitution models were the same as in the previous ML analysis, different partitions were unlinked for the substitution models, and different genes were unlinked for the clock models, but linked for topology. For the tree prior, we used a speciation model following the Yule process. To enable calibration of divergence time, the mean clock rate of the COI gene was set to 0.0177 referring to a divergence rate of 3.54% per million years for insect mitochondrial genes, estimated based on a