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The genus *Leucophenga* (Diptera, Drosophilidae), part IV: the *ornata* species group from the East Asia, with morphological and molecular evidence (II)

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Abstract

Twenty-one species of the *Leucophenga ornata* species group are surveyed (including 12 new species): *L. atrinervis* Okada, 1968; *L. digmasoma* Lin & Wheeler, 1972; *L. multipunctata* Chen & Aotsuka, 2003; *L. nigrinervis* Duda, 1924; *L. pectinata* Okada, 1968; *L. pentapunctata* Panigrahy & Gupta, 1982; *L. quadripunctata* (de Meijere, 1908); *L. regina* Malloch, 1935; *L. trivittata* Okada, 1990; *L. acutifoliacea* **sp. nov.**; *L. albiterga* **sp. nov.**; *L. angustifoliacea* **sp. nov.**; *L. baculifoliacea* **sp. nov.**; *L. cornuta* **sp. nov.**; *L. hirticeps* **sp. nov.**; *L. latifascia* **sp. nov.**; *L. pinguifoliacea* **sp. nov.**; *L. retihirta* **sp. nov.** *L. securis* **sp. nov.**; *L. sinupenis* **sp. nov.**; *L. villosa* **sp. nov.**; these species (except for *L. baculifoliacea* **sp. nov.**) having pubescence on the distal portion of aedeagus. A key to all the examined species of the *ornata* group in this study is provided. We try to improve our species delimitation by integrating the DNA sequences data with morphological information. The intra- and interspecific pairwise K-2P (Kimura's two-parameter) distances are summarized.

Key words: DNA barcoding, *COI* gene, drosophilid, Oriental region, taxonomy

Intrduction

Huang *et al*. (2013b) surveyed 19 species of the *Leucophenga ornata* group, their aedeagus lack pubescence, mostly reticulated; these species are supported as monophyletic based on the mitochondriao *COI* sequences in phylogentic analysis. In this study, 21 species of the *ornata* group are researched, these species with pubescence on aedeagus (except for *L. baculifoliacea* **sp. nov.**), but mostly not reticulated (except for *L. retihirta* **sp. nov.** and *L. securis* **sp. nov.**). To evaluate the morphological hypotheses of these *ornata* group species, we also conduct a molecular phylogenetic analysis for them; for this, the DNA sequences of the mitochondrial cytochrome *c* oxidase I gene (*COI*) are sequenced for 85 individuals of 21 species (Table 1).

Materials and methods

Materials and morphological terminology. The samples were collected by sweeping on tussocks and tree trunks along streams in forest, then immediately immersed in 75% ethanol. All specimens are deposited in Department of Entomology, South China Agricultural University, China (SCAU). The morphological examinations of these samples were followed the process mentioned in Huang *et al.* (2013a, b).

Molecular study. The methods of DNA extraction, *COI* gene amplification and sequencing were followed in Huang *et al*. (2013a, b). The sequences were aligned with ClustalW method implemented in MEGA 5.05 (Tamura *et al*. 2011), and then the NJ (neighbor-joining) and the Bayesian analyses were used to construct the phylogenetic relationships among the species. The NJ trees were built in MEGA with K-2P distances, and the Bayesian analyses were implement in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with TIM2 substitution model and gamma-distributed rate variation among sites (+G) and a proportion of invariable sites (+I). In total, 5,000,000 generations were executed in the Bayesian analyses, 50,000 trees were obtained with default

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L. latifuscata and *L. multipunctata*, as they share similar male genitalia and show reciprocally paraphyletic relationship in the molecular phylogentic analyses. The results show that total of 15 fixed diagnostic nucleotides are found in the common sequence (641 bp) of *L. latifuscata* and *L. multipunctata* (Table 3). However, as only one *L. multipunctata* sequence is involved in the analysis, the availability of these fixed diagnostic nucleotides in the discrimination of *L. latifuscata* and *L. multipunctata* requires further examination.

In this study, the supports for the deep nodes were not robust (BP<50, PP<0.5), indicated that current topology of the phylogenetic trees were not stable. Hence, the phylogenetic relationships among examined species were still not unambiguous, even in the basal branches some ingroup taxa clustered with the outgroup taxa. Herein, we propose that to obtain the resolvable phylogentic relationship among the *ornata* group species, it is necessary to combine with more mitochondrial and nuclear loci in the phylogentic analyses.

TABLE 3. Fixed diagnostic nucleotides in the sequence of *L. multipunctata* and *L. latifascia* **sp. nov.**

Site No. in *COI* sequence of *Drosophila yakuba* strain yak_ZW162 (KF824900)	114	210	264	300	327	384	405	477	514	515	549	555	558	579	657
L. multipunctata	G	T	T	C	T	T	C	G	G	T	G	G	G	C	C
L. latifascia **sp. nov.**	A	A	A	T	A	C	T	T	A	C	T	T	A	T	T

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