

## Development of a species-diagnostic marker and its application for population genetics studies of the stingless bee *Trigona collina* in Thailand

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**ABSTRACT.** A molecular marker for authenticating species origin of the stingless bee (*Trigona collina*) was developed. Initially, amplified fragment length polymorphism analysis was made of 11 stingless bee species using 64 primer combinations. A 316-bp band found only in *T. collina* was cloned and sequenced. A primer pair (CUTc1-F/R) was designed and tested for species-specificity in 15 stingless bee species (239 nests). The expected 259-bp fragment was consistently amplified in all *T. collina* individuals (134/134 nests, 100%). Cross-species amplification was observed in *T. pagdeni* (43/51 nests; 84.3%), but not in other species. SSCP analysis of CUTc1 unambiguously differentiated *T. collina* from *T. pagdeni*. CUTc1 generated three genotypes in Thai *T. collina* (134 nests). An AA (259/259 bp) genotype was found in all stingless bees from the north (21 nests) and northeast (32 nests), and 23/28 nests from the Central region, whereas a BB (253/253 bp) genotype was observed in most samples from peninsular Thailand (42/53 nests). Heterozygotes exhibiting the AB (253/259 bp) genotype

were observed in 5 of 28 nests from Prachuap Khiri Khan located slightly above the Kra ecotone and 11 of 53 nests originated further south of the Kra ecotone. Genotype distribution patterns of CUTc1 clearly indicated intraspecific population differentiation of Thai *T. collina*.

**Key words:** Stingless bees; *Trigona collina*; AFLP; SSCP; Species-specific marker; Population differentiation