

A taxonomic study of Actinodaphne (Lauraceae)  
in Southeast Asia based on multiplexed inter-  
simple sequence repeats genotyping by  
sequencing (MIG-seq) and classic DNA barcodes

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論 文 名 : A taxonomic study of *Actinodaphne* (Lauraceae) in Southeast Asia based on multiplexed inter-simple sequence repeats genotyping by sequencing (MIG-seq) and classic DNA barcodes (MIG-seq と古典的 DNA バーコードにもとづく東南アジア産クスノキ科アクチノダフネ属の分類学的研究)

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### 論 文 内 容 の 要 旨

The genera *Actinodaphne* Nees and *Neolitsea* Merr. (Lauraceae) include ~100 each spp. of evergreen trees that mainly occur in Asia, and both morphological analysis and molecular phylogenetic analysis have supported that *Actinodaphne* and *Neolitsea* are closely related to *Litsea* Lam. Both *Actinodaphne* and *Neolitsea* can be distinguished from *Litsea* by leaves that are whorled or clustered in the nodes of branches, and *Actinodaphne* and *Neolitsea* can be distinguished on the basis of flower morphology. Recent molecular phylogenetic studies have suggested that *Neolitsea* is monophyletic but *Actinodaphne* is not. However, the resolution of the phylogenetic trees generated by these studies has been relatively low, owing to limited numbers of phylogenetically informative characters. In this study, we employed multiplexed inter-simple sequence repeats genotyping by sequencing (MIG-seq) to obtain finely resolved phylogenetic trees, in addition to phylogenetic analyses using internal transcribed spacer (ITS) sequences of ribosomal DNA. Here, we describe the results from phylogenetic analyses combined with morphological studies.

In Chapter I, a new species of *Actinodaphne* (Lauraceae), *Actinodaphne lambirensis* Tagane, Yahara & Okabe is described from Lambir Hills National Park, Miri District, Sarawak, Malaysia based on a MIG-seq tree, ITS tree, and morphological observation. Because only fruiting specimens were available for *A. lambirensis*, we confirmed its position in the phylogenetic trees obtained from 22 *Actinodaphne* spp. including the type species of the genus, *A. pruinosa* Nees, and 11 *Neolitsea* spp. from Southeast Asia, MIG-seq. In addition, we reconstructed a phylogenetic tree using ITS sequences for 36 *Actinodaphne* spp. and 40 *Neolitsea* spp. that included the 22 MIG-seq samples and additional species of *Actinodaphne* for which ITS sequences were determined in previous studies. Both MIG-seq tree and ITS tree supported that *A. lambirensis* belongs to *Actinodaphne*.

In chapter II, we examined effectiveness of MIG-seq for phylogenetic reconstruction and species discovery of *Actinodaphne* and *Neolitsea* in Southeast Asia. We compared a MIG-seq tree reconstructed for 25 and 45 species of *Actinodaphne* and *Neolitsea*, respectively, with an ITS tree for 18 and 33 species of two genera. As a result, 119 of 162 (72 %) branches and 26 of 88 (30 %) branches were supported by bootstrap values of 85 % or larger in MIG-seq and ITS trees, respectively. In the 20 nodes supported by both ITS and MIG-seq trees, a bootstrap support to each node was always higher on the MIG-seq tree. In one of two

inconsistent cases between the MIG-seq tree and the ITS tree, topologies of the MIG-seq tree agreed with morphological resemblance. In the MIG-seq tree, *Actinodaphne* was separated into two clades: *Actinodaphne* 1 including *A. aff. tsaii* 1 and *A. aff. tsaii* 2, and *Actinodaphne* 2 including the other 23 spp. *Actinodaphne* 1, *Actinodaphne* 2, and *Neolitsea* were almost equally differentiated. The MIG-seq tree supported sister relationship for 18 pairs of species, and sister species of each pair are distinguished by diagnostic traits. In both genera, morphologically similar species were often not sister to each other, suggesting repeated parallel evolution of leaf traits. On the MIG-seq tree, 6 *Actinodaphne* spp. and 30 *Neolitsea* spp. did not match any described species and are likely to be undescribed species. These results showed that a highly resolved phylogenetic tree by MIG-seq is effective to discover and delimitate new species.

In chapter III, a new genus *Neoactinodaphne* Okabe, Tagane & Yahara, including two new species and a variety were described from Vietnam and Thailand. This new genus is characterized by well-developed intervening veins perpendicularly extending between secondary veins. Phylogenetic analyses based on MIG-seq showed that this new genus, having 3-merous flowers with 9 stamens, was sister to but distinct from *Neolitsea*, having 2-merous flowers with 6 stamens. Principal component analysis and a cluster analysis by Unweighted Pair Group Method using arithmetic Average were performed for a total of 67 species of *Actinodaphne* and *Neoactinodaphne* using six leaf traits: maximal number of leaves clustered on the branch top (MLC), midpoint petiole length (PL), midpoint leaf length (LL), midpoint leaf width (LW), midpoint lateral veins (LV), midpoint aspect ratio (AR). *Neoactinodaphne* is placed among species of *Actinodaphne*, showing that *Neoactinodaphne* is difficult to be distinguished from *Actinodaphne* spp by leaf shape. The MIG-seq tree showed that *A. acuminata* was placed not in *Actinodaphne* but in *Litsea*. The MIG-seq tree and morphological observations supported that eight species of *Actinodaphne* (24 %) are considered to be undescribed. Our results showed that phylogenetic analyses using MIG-seq are effective to discover and describe new species if it is combined with morphometric analyses.