

# Application of cecidomyiid galls to the systematics of the genus *Machilus* (Lauraceae) in Taiwan

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(Received October 12, 2000; Accepted September 10, 2001)

**Abstract.** The species of the genus *Machilus* (Lauraceae) in Taiwan sustain diverse cecidomyiid galls induced by the insects of the genus *Daphnephila* (Cecidomyiidae). This work examines the feasibility of applying cecidomyiid galls to the systematics of the genus *Machilus*. Amplified fragment length polymorphism (AFLP) was used to analyze the 38 gall-bearing trees of four *Machilus* taxa including *Machilus kusanoi*, *M. thunbergii*, *M. zuihoensis* var. *zuihoensis*, and *M. zuihoensis* var. *mushaensis*. The UPGMA cluster analysis of the AFLP data revealed three distinct clusters, including *M. kusanoi*, *M. thunbergii*, and *M. zuihoensis* variety complex. *Machilus zuihoensis* var. *zuihoensis* and *M. zuihoensis* var. *mushaensis* were indistinguishable from the three primer combinations. These two varieties could be considered the same taxon. PCR and DNA sequencing methods were used to analyze the nucleotide sequences of the mitochondrial 16S rDNA gene of the twenty gall midges from three types of galls from four *Machilus* taxa. The phylogenetic tree from the partial 16S rDNA sequence by UPGMA method of proportion distance revealed that the gall midges can be divided into three groups according to gall types. The phylogenetic tree cannot separate the two varieties of *M. zuihoensis* within each group. *Machilus zuihoensis* var. *zuihoensis* and *M. zuihoensis* var. *mushaensis* cannot be distinguished according to the AFLP or DNA sequencing methods, and they are more closely related to *M. thunbergii* than to *M. kusanoi*. The systematic relationships among the *Machilus* from the data of host plants are congruent with the data from the gall inducers. Results in this study imply that the gall inducers of genus *Daphnephila* provide information for resolving the plant systematic relationships based on molecular techniques.

**Keywords:** AFLP; Cecidomyiidae; DNA sequence; Insect gall; *Machilus*; Systematics.

## Introduction

The genus *Machilus* of Lauraceae is distributed in the tropical and subtropical areas of Asia (Liu et al., 1994). There are six taxa of *Machilus* in Taiwan: *Machilus kusanoi*, *M. obovatifolia*, *M. zuihoensis* var. *zuihoensis*, *M. zuihoensis* var. *mushaensis*, *M. thunbergii* and *M. japonica*. All except *M. thunbergii* and *M. japonica* are endemic species in Taiwan. Currently, plant taxonomists differ in their systematic arrangement of the *Machilus* taxa in Taiwan.

A gall is an abnormal growth on some part of a plant as a result of the activity of another organism (virus, nematode, arthropod etc.) and the gall inducer uses this structure as a shelter and nutrition source (Csóka, 1997). The genus *Machilus* plants in Taiwan have a high diversity of cecidomyiid galls induced by the insects of the genus *Daphnephila* (Cecidomyiidae). Harris (1994) demonstrated that the family Cecidomyiidae is one of the major groups of gall inducers and that most gall midges are highly host specific. The galls of phytophagous arthropods are traits associated with plants and may be

useful for separating plant taxa (Floate et al., 1996). Abrahamson et al. (1998) indicated that cynipid occurrences offer helpful information concerning some aspects of oak systematics. Our work examines the feasibility of applying cecidomyiid galls to the systematics of the genus *Machilus* (Lauraceae) in Taiwan. Amplified fragment length polymorphism (AFLP) was used to analyze the phylogeny of *Machilus* taxa. PCR and DNA sequencing methods were used to examine the phylogeny of gall inducers gathered from galled plants.

## Materials and Methods

### Gall-Bearing Plants

*Plant materials.* Thirty eight gall-bearing trees of four *Machilus* taxa including *Machilus kusanoi* (code K), *M. thunbergii* (T), *M. zuihoensis* var. *zuihoensis* (Z) and *M. zuihoensis* var. *mushaensis* (M) were sampled throughout Taiwan (Figure 1). Leaf samples from each plant were packed in a paper bag and stored in a box of silica gel desiccant. The dried leaves were used for DNA extraction. Only four *Machilus* taxa were analyzed in the preliminary study because there were not enough *M. obovatifolia* samples that are only distributed on the Hengchun Peninsula, and *M. japonica* was difficult to identify.

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