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# Notes on Phylogeny of the Genus Micropera Lindl. (Orchidaceae) in Thailand

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# Notes on Phylogeny of the Genus *Micropera* Lindl. (Orchidaceae) in Thailand

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**Abstract:** The genus *Micropera* Lindl. belongs to the family Orchidaceae. It is one of the uncertainty monophyletic groups. Since the previous phylogenetic analysis employed only one species, more species are needed to reveal whether this genus forms a monophyletic group or not. Thus, this study was conducted to construct the phylogeny for the genus *Micropera* based on molecular data by adding 4 Thai *Micropera* taxa. The phylogenetic analysis was performed by using *mat*K, ITS and combined both genes, with selected taxa in *Trichoglottis* clade as outgroups. It was shown that *Micropera* is a monophyletic group and should be accepted genus. The twist rostellum is a taxonomic character for grouping among 4 Thai *Micropera* species. All taxa of the genus are required for revealing the evolution relationship of this genus.

**Keywords:** *Molecular phylogeny, Micropera, monophyletic group, Orchidaceae*

## 1. Introduction

The genus *Micropera* was established by Lindley [1] based on specimen collected from India. It had been called as *Camarotis* Lindl. for long time [2], Prain [3] and Seidenfaden and Smitinand [4]. Until Tang and Wang [5] and Garay [6] accepted the name *Micropera* again. It distributed in Tropical Asia to W. Pacific, with 22 accepted species [7]. In Thailand, Seidenfaden [8] reported this genus 4 species, *M. pallida* (Roxb.) Lindll., *M. obtusa* (Lindl.) Tang & F. T. Wang., *M. rostrata* (Roxb.) N. P. Balakr. and *M. thailandica* Garay ex. Seidenf.

The genus *Micropera* (Roxb.) Lindl. is climbing monopodial epiphytic herbs having alternate linear leaves. The flowers born in racemose or paniculate inflorescence, usually non resupinate, fleshy, usually yellow or with purple marking or pink. Sepals and petals are oblong or obovate. The labellum is spurred with a back wall callus, longitudinal septum and usuall a bilobed callus on front wall of the spur. The column is variable usually elongate into a beak-like. The pollinia has 4 unequal masses, lay on the linear stipe which has ovate viscidium at its base (Fig. 1). It is one of the uncertain generic groups [9]. Only one species was used for phylogenetic analysis, *M. pallida*. The phylogenetic position of *Micropera* embedded in the *Trichoglottis* clade [10]-[12]. To update the phylogenetic relationship of the genus *Micropera*, we aimed to add more taxa from Thailand for phylogenetic analysis, based on *mat*K and ITS to prove whether this genus is monophyletic or not.


**Fig. 1.** *Micropera pallida* Lindl. A. habit B. inflorescence C. side view flower, D. front view flower

## 2. Methods

### 2.1 Taxon selection

Four Thai species of *Micropera*, *M. pallida*, *M. obtusa*, *M. rostrata* and *M. thailandica*, were obtained from the living collection of Khlong Phai Plant Genetic Conservation in Nakhon Ratchasima Province and were used for DNA extraction Sequence data of *Trichoglottis* clade [12], *Acampe orchracea*, *A. prarmorsa*, *A. rigida*, *Adenoncos parviflora*, *Cottonia peduncularis* and *Trichoglottis triflora* were downloaded from GeneBank to be used as outgroup (Table 1).

**Table 1.** Species and DNA regions used in this study, downloaded from GenBank showing the accession numbers.

| Species | nrITS | *mat*K |
|---|---|---|
| *Acampe orchracea* | DQ091707 | DQ091314 |
| *Acampe prarmorsa* | MN517126 | JN004343 |
| *Acampe rigida* | KJ733385 | MN523477 |
| *Adenoncos parviflora* | KY966412 | AB217703 |
| *Cottonia peduncularis* | JN114477 | JN004395 |
| *Trichoglottis triflora* | KY966964 | KY966678 |

**2.2 DNA extraction**

DNA was extracted from dried leaves using the Genomic DNA Isolation Kit (Plant), (Bio-Helix, Taiwan). The amplification of the *mat*K region was performed using a primer pair, 3 9 0 F and 1 3 2 6 R [13]. The 5 0 µL amplification reaction included 25 µL OnePCR Ultra, 2.5 µL each primer (5 pmol/µL), 1 µl of template DNA and 19 µL of free water. The polymerase chain reaction (PCR) profile consisted of an initial 5 min premelting stage at 9 5 °C, followed by 3 0 cycles of 3 0 s at 9 5 °C (denaturation), 1 min at 5 5 °C (annealing), 4 0 s at 7 2 °C (extension), and a final 7 min extension at 72 °C [14]. For ITS sequences, amplification was performed using a primer pair, 17SE and 26SE [15]. The 50 µL amplification reaction included 2 5 µL OnePCR Ultra, 2 . 5 µL each primer (2 pmol/µL), 1 µL of template DNA, 1 µL of DMSO, and 1 8 µL of free water. The polymerase chain reaction (PCR) profile was performed as mentioned earlier.

**3. Analysis**

A total 10 nucleotide sequence of *mat*K, ITS and combined *mat*K and ITS of ingroup and outgroup were aligned using Bioedit ver. 7.2.5 [16] followed by manual adjustments. The evolution model was tested by using jModelTest2 on XSEDE in the CIPRES Gateway v. 3.3 (https://www.phylo.org/) [17]. The sequences of *mat*K, ITS and combined sequences fit with TVM+I, TPM1uf+I and TrN+G, respectively. Phylogenetic analyses were done using RAxML BlackBox with 1000 bootstrap replicate for maximum likelihood (ML) tree and MrBayes on XSEDE with a Markov chain Monte Carlo (MCMC) chain length of 1,000,000 was used for Bayesian analysis (BA) in the CIPES Science Gateway v. 3.3 (https://www.phylo.org/) [18]. Obtained trees were viewed and adjusted using FigTree v 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

**4. Results and discussion**

Using NCBI nucleotide BLAST (blastn) (http://blast.ncbi.nlm.nih.gov), all sequences acquired in this study were compared to GenBank database sequences, and the findings indicated that all sequences are from orchids. The aligned *mat*K, ITS and combined *mat*K and ITS matrix with gaps were 1,340, 807 and 2,149 bp long, respectively.

The phylogenetic analysis of *mat*K showed the highly statistic support of 2 clades, a group of *Adenoncos*, *Trochottis*, *Cottonia* and *Acampe* and *Micropera* clade. However, the resolution infrageneric level of the *Micropera* clade was low. *M. obtusa* and *M. thailandica* form a group with low support (Fig. 2). Based on ITS, the resolution is quite better than of *mat*K. *Micropera* spp. form a group with high bootstrap percentage (BP) and Bayesian posterior probabilities (PP), separated from the outgroup as same as of *mat*K result. While the combined genes showed the result congruent to ITS result.

The last update on *Micropera* phylogenetic position involvement was reported by Zou et al. [12], showed that the *Trichoglottis* clade consisted of *Trichoglottis* s.l., *Adenoncos*, *Acampe*, *Cottonia* and *Micropera*, congruent with Topik et al. [10],[11]. However, only one species of *Micropera*, *M. pallida*, was employed for the analysis. Adding more taxa of the genus showed a clade of *Micropera* in this study. For the generic circumscription, Pridgeon et al. [9] pointed out that the genus *Micropera* is not yet stable. Our result of the phylogenetic analysis of *mat*K, ITS and combined genes showed in Figs. 2-4, revealed that the genus *Micropera* is a monophyletic group with highly statistic support. Thus, this genus should be accepted.

The relationship in the genus based on combined ITS and *mat*K revealed that Thai *Micropera* form two groups with high support based on ITS gene, although weak bootstrap support, possibly due to low resolution from *mat*K gene. The characteristic of column twist may be a taxonomic character. Group of *M. rostrata* and *M. obtusa* has a twist rostellum as a share character while group of *M. pallida* and *M. thailandica* has rostellum not twist.

Beside of the objective of this study, focused on the genus *Micropera*, it revealed better solutions of related genera position compared to previous study [12] after adding more taxa of the *Micropera.* On the second clade, it can be seen that the genus *Acampe* is a monophyletic group with rather strong BP and PP support. However, more taxa of *Adenoncos* is needed to include for the analysis. In this study, the position of *Adenoncos* is separated out from *Acampe.*

**5. Conclusions**

This study supported the monophyly of the genus *Micropera*. ITS and and combined genes gave the similar pattern of the tree with slightly difference on *mat*K result. The tree of ITS and combine genes showed the divergent infrageneric level. The twist rostellum is a taxonomic character for this divergent, which is a synapomorphy of *M. rostrata* and *M. obtusa*. To uncover the intraspecific evolutionary connection in this genus, phylogenetic study of all *Micropera* species is required.

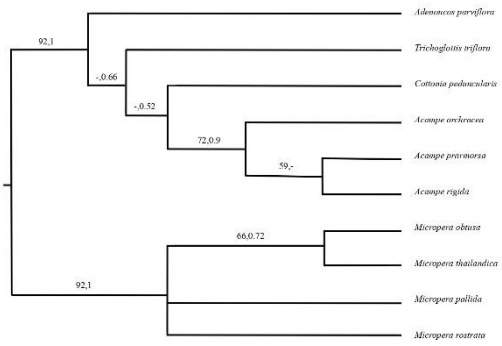


**Fig. 2**. Phylogenetic tree based on *mat*K, showing bootstrap percentage (BP) and Bayesian posterior probabilities (PP).

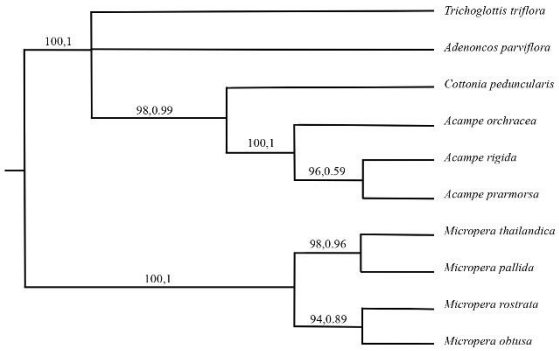


**Fig. 3**. Phylogenetic tree based on ITS, showing bootstrap percentage (BP) and Bayesian posterior probabilities (PP).

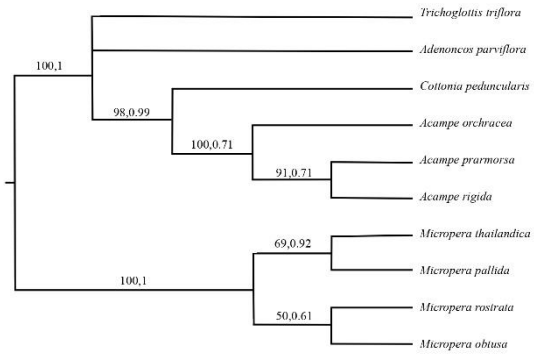


**Fig. 4**. Bootstrap consensus tree of *Micropera* based on the combined nuclear (ITS) and plastid (*mat*K) markers, showing bootstrap percentage (BP) and Bayesian posterior probabilities (PP).


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