

## การระบุชนิดของพืชสกุลไก่อแดงด้วยวิธีการทางอนุชีววิทยา นำไปสู่การค้นพบพืชชนิดใหม่

### Molecular identification of cultivated *Aeschynanthus* (Gesneriaceae) leading to a discovery of a new species.

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**บทคัดย่อ :** พืชสกุลไก่อแดงเป็นพืชอิงอาศัยพืชอื่น มีประมาณ 160 ชนิด กระจายทั่วไปในเอเชียตะวันออกเฉียงใต้ มีดอกทรงหลอดสีแดงหรือเหลืองสดสวย ทำให้มีการเพาะปลูกเพื่อธุรกิจไม้ดอกไม้ประดับ การระบุชื่อชนิดและการแบ่งจำแนกระดับเชิงชั้นของพืชนี้มักจะใช้ลักษณะสัณฐานของดอกและเมล็ดเป็นหลัก ซึ่งมักเป็นปัญหาต่อนักอนุกรมวิธานและเกษตรกรผู้เพาะปลูก โดยเฉพาะเมื่อไม่อาจหาส่วนสืบพันธุ์เหล่านั้นได้ จากการใช้เทคนิคสมัยใหม่ทางอนุชีววิทยานำที่จะช่วยให้การแก้ปัญหาการระบุชื่อชนิดทำได้รวดเร็วกว่าวิธีการที่ใช้อยู่ดั้งเดิมมาก เราจึงได้ทดลองระบุชื่อชนิดของไม้เลื้อยสกุลไก่อแดงที่มีสัณฐานต่างๆ กันด้วยวิธีอนุชีววิทยา ดีเอ็นเอของบริเวณไอทีเอสของนิวเคลียร์ไรโบโซมอลดีเอ็นเอจากพืชตัวอย่างได้ถูกอ่านลำดับ และจัดเรียงกับเมทริกซ์ข้อมูลเดิมของพืชสกุลไก่อแดง 50 ชนิดเพื่อสร้างแผนภูมิต้นไม้วงศ์วานวิวัฒนาการขึ้นมาใหม่ พืชที่ใช้ศึกษาต้นหนึ่งถูกพบว่าไม่ใช่พืชสกุลไก่อแดง แต่อาจจะเป็นสกุลโคลัมเนียจากการเข้าใจผิดของคนขาย พืชอีกสี่ต้นที่เหลือได้รับการยืนยันว่าเป็นพืชสกุลไก่อแดงจริง โดยสามในสี่ต้นนี้น่าที่จะเป็น *A. radicans* เนื่องจากตำแหน่งบนแผนภูมิต้นไม้วงศ์วานวิวัฒนาการนั้นเข้ากันได้กับลักษณะสัณฐานของไก่อแดงชนิดนี้ และน่าสนใจอย่างยิ่งที่ไก่อแดงอีกต้นหนึ่งซึ่งมีดอกสีชมพูแปลกไปจากปรกติ ถูกพบว่ามีลำดับดีเอ็นเอที่เฉพาะตัวและเป็นไปได้สูงที่จะเป็นพืชชนิดใหม่ที่ยังไม่เคยถูกรายงานมาก่อนแม้ว่าจะเป็นไม้เลื้อยก็ตาม การศึกษาครั้งนี้จึงช่วยพิสูจน์ถึงประโยชน์อย่างสูงจากการใช้อนุชีววิทยาระบุชื่อชนิดของสิ่งมีชีวิตที่ยังเป็นปัญหาอยู่

**Abstract:** *Aeschynanthus* (Lipstick vines) is an epiphyte genus with c.160 species widespread in SE Asia. They have brightly red or orange tubular flowers and therefore are commercially cultivated for their splendid flowers. Species identification and sectional classification of this genus rely mostly on their flower and seed morphology. This then presents problems for both taxonomists and horticulturists, especially when the reproductive parts are usually unavailable. Using current molecular biological techniques should help clarify such identification problem of *Aeschynanthus* much quicker than a classical practice. We therefore tried to perform molecular identification on several different morphotypes of *Aeschynanthus* cultivars. Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA of the plant samples were obtained and aligned with a previously-prepared data matrix of 50 *Aeschynanthus* species to reconstruct a phylogenetic tree. One sampled cultivar was found not belonging to *Aeschynanthus*, but putatively *Columnea* sp. and possibly misunderstood by the local plant sellers. The other four cultivars were confirmed with their ITS sequences to be truly members of the genus. Three of them is plausibly *A. radicans* as their positions on the phylogenetic tree are in congruent with morphological characteristics of *A. radicans*. Very interestingly, one morphotype with unusual pinkish flowers showed unique ITS sequence characteristics and led to a high possibility of being a new undescribed species though commercially cultivated. This study therefore proves a great advantage of using molecular techniques to identify problematic taxa.

**Methodology:** Six *Aeschynanthus* cultivars, morphologically different more or less, were bought from Jatujak Market, Bangkok, and named *Aeschynanthus* sp.1 to sp.6. Their morphological characters were noted and digitally photographed. Fresh leaf materials of each plant were sampled. Details of DNA extraction, polymerase chain reaction (PCR) amplification, PCR cloning, and DNA sequencing strategies

are provided in the previous studies (1) (2). The genomic DNA was prepared and used as template for PCR amplification, yielding the complete ITS region (both ITS1 and ITS2 and 5.8S ribosomal DNA). The products were sequenced on both forward and reverse directions with an automated DNA sequencer. ITS sequence results were checked with GenBank nucleotide database ([www.ncbi.nih.gov](http://www.ncbi.nih.gov)) whether the sequences are really from *Aeschynanthus* or from any other genus. The *Aeschynanthus* ITS sequences were then aligned with the previous DNA data matrix (2). Phylogenetic analyses by parsimony, branch support analyses, and other sequence and tree statistics were performed as described previously (1) (2), with the program PAUP\* (3) version 4.0b. Sectional positions of the four *Aeschynanthus* cultivars suggested by the phylogenetic tree were compared with those predicted from morphological studies of the genus (4).

**Results, Discussion and Conclusion:** From the ITS sequences of six tested plants, all but one were confirmed to be truly members of the genus *Aeschynanthus* after performed BLAST searching in the GenBank nucleotide database. The only mixed-up cultivar (A. sp.6) was found possibly belonging to the genus *Columnea*. The putative name of this species could be *Columnea linearis* which gave E value=0.0, score 1037 bits, and 96% identities. *Columnea* is a New World counterpart of *Aeschynanthus*, with similar flower characteristics, but found only in tropical areas of the Americas. We reckoned that the local plant seller must have been misunderstood about the correct name of this imported plant by its misleading flowers.

For the five true *Aeschynanthus* cultivars, only the ITS sequences of A. sp.5 that gave sequencing problems from intra-individual variations in the regions as seen in the previous study (1). These awkward sequences, though unable to be put together as a complete ITS sequence, however are good enough to confirm that this plant is certainly *Aeschynanthus*. As the other four *Aeschynanthus* ITS sequences could be assembled easily and aligned nicely to the previously-prepared data matrix of 50 *Aeschynanthus* species, a new *Aeschynanthus* phylogenetic tree was then reconstructed. Semistrict consensus tree (Fig.1) was made from 336 most parsimonious trees of 54 *Aeschynanthus* and two outgroup Gesneriaceae taxa. The *Aeschynanthus* phylogeny suggested that the cultivars sp.2, sp.3, and sp.4 are sisters of *A. chrysanthus*, *A. parvifolius*, and *A. radicans* and then made the three be classified as members of the section *Aeschynanthus*. Considering their genetic similarity values from one of 336 phylogram with morphological characteristics of each plant, A. sp.3 and A. sp.4 should be recognised as the same species and probably named as *A. cf. radicans*. *Aeschynanthus radicans* is the only Thai species in this subclade and that is agreeable with the seller's comments. The plant A. sp. 2 is also similar in both DNA sequence and morphology to *A. radicans*, although some differences in their vegetative parts were observed, i.e., A. sp. 2 has reddish stems and petioles instead of green colour. While our project on collecting molecular data of all Thai *Aeschynanthus* species is still on the way, we would conclude that the sp. 2 cultivar is also *A. cf. radicans*, possibly one of its variances.

The most impressive point is about the position of *Aeschynanthus* sp. 1 on the phylogeny. This plant has already been in our deep interest because it has a very unusual, pale-pinkish flowers, the colour never been observed in the genus. In our ITS phylogenetic experiments, this pink *Aeschynanthus* was also placed in the section *Aeschynanthus* subclade which nicely agrees with its shallow-lobed calyx character. We have consulted the taxonomic expert of the genus (Mary Mendum, Royal Botanic Garden Edinburgh, United Kingdom) and there is a very high possibility that we have found a new undescribed species even though this plant is commercially cultivated. More investigations are needed on its original collection area, flower and seed morphology, esp. whether it has bubble-like cells on its seeds, an important symbol of the section member.

In conclusion, this molecular identification study of cultivated *Aeschynanthus* have shown that there is a great advantage in using simple molecular techniques, combined with phylogenetic analyses, to identify problematic taxa, especially when it is difficult to retrieve or investigate taxonomically significant organs from such organisms .

## References:

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**Keywords:** *Aeschynanthus*, Gesneriaceae, molecular identification, internal transcribed spacers, nuclear ribosomal DNA

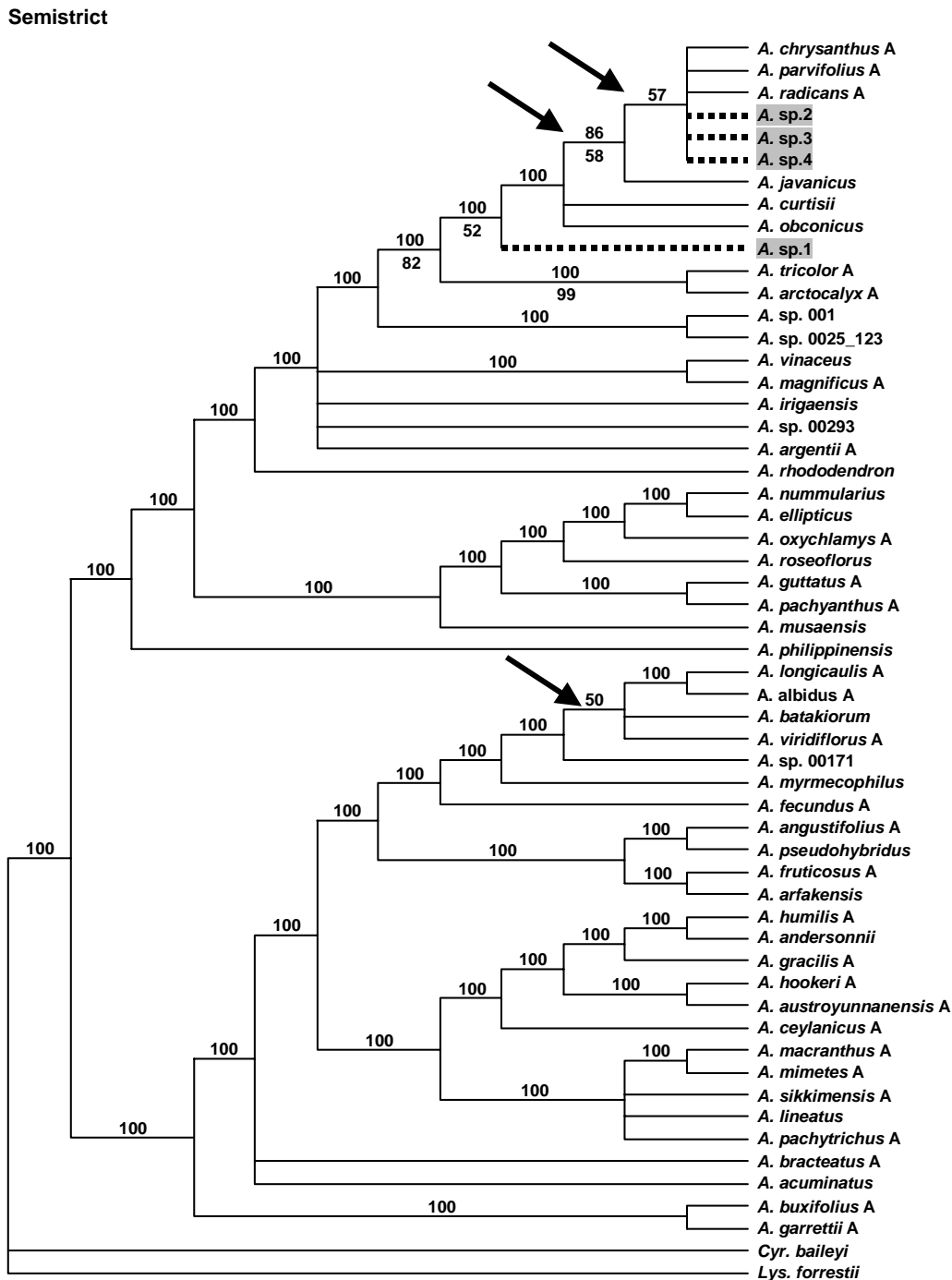


Fig. 1. Semistrict consensus of 336 most parsimonious trees (629 steps in length) for 54 *Aeschynanthus* species and two outgroup Gesneriaceae taxa (*Cyandra baileyi* and *Lysionotus forrestii*) based on parsimony analysis of the combined ITS1 and ITS2 sequence data. Four *Aeschynanthus* cultivars are highlighted in gray, also with dotted lines. The upper numbers are percentages of clade congruity between MPTs. The lower numbers are "fast" stepwise-addition Bootstrap supporting-values of 1,000 replicates for some important clades. The arrows indicate branches that collapse in the strict consensus tree. [CI = 0.6216, RI = 0.7939, RC = 0.4935]