

TAXONOMIC IDENTITY OF *ARISAEMA CONDAOENSE* (ARACEAE) BASED ON NEW MORPHOLOGICAL AND MOLECULAR DATA

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SUMMARY

Arisaema condaoense V.D. Nguyen was described as a new species from Con Dao National Park, Ba Ria–Vung Tau Province, Vietnam in 2000. However, this species has been suspected of being a form of *Arisaema roxburghii* Kunth, a species widespread in the whole Indochina and Malay Peninsula. This was due to the original description based on dried specimens with male inflorescences only. Morphological characteristics of female inflorescences, which are of taxonomical importance to identify the species, have not been known. In June 2015, we re-sampled the plant in Con Dao National Park with both male and female inflorescences for detailed examination of morphological characteristics. Besides, the *matK* gene of the chloroplast genome of this species was sequenced to analyse its phylogenetic relationship with other *Arisaema* species. The gathered morphological and molecular data indicate that *A. condaoense* is certainly a distinct species, not a synonym of *A. roxburghii*. The noted morphological characteristics also provide key differences to distinguish *A. condaoense* from two other morphologically close species of sect. *Fimbriata* (i.e. *A. maxwellii* from Thailand and *A. pierreanum* from Vietnam which also share yellow spathes and several other characteristics in common), which are supported by the molecular data. Thus, the species is endemic to Con Dao National Park in Ba Ria–Vung Tau Province, Vietnam. Furthermore, this study allows us revising the description of *A. condaoense* with, for the first time, supplemental key taxonomic characteristics and illustrating colourful photographs taken from the fresh materials. A key to all eight known Vietnamese species of sect. *Fimbriata* is also given.

Keywords: Araceae, *Arisaema condaoense*, chloroplast, *Fimbriata*, *matK*

INTRODUCTION

Arisaema condaoense was first described by Nguyen (2000) as a species belonging to *Arisaema* section *Fimbriata* based on dried specimens with only male inflorescences collected from Con Dao Island, Vung Tau–Ba Ria Province (N.T. Ban and Averyanov 385, HN!, 20 June 1989). Since then, no other collections of the species have been known.

Although its specific status was *de facto* accepted in Nguyen (2005), Nguyen, Vu (2009), Luu *et al.* (2013; 2014) and Van *et al.*, (2016), Gusman, Gusman (2006) stated in their revision of the genus that *A. condaoense* was very close to the polymorphic *A. roxburghii* Kunth which was

widespread in the whole Indochina and Malay Peninsula and they questioned whether it was a form of the later or a different species. They suggested examining its fresh material for a correct identification. Until the present study, no efforts to delimit the species have been reported.

To ascertain its identity, we re-examined the questioned taxon. New fresh material of *A. condaoense* was collected in a field trip to the type location (Con Dao National Park) in June 2015 (Van Hong Thien H.T. Van 85, SGN!, approximate coordinates 8°42'47.34" N, 106°37'2.52" E, at ca. 315 m above sea level). Our subsequent analyses of its molecular and morphological data proved it to be a good species that is re-described below.

MATERIALS AND METHODS

Materials

DNA samples from nine taxa of *Arisaema* of three *Arisaema* sections (i.e. *Anomala*, *Fimbriata* and *Sinarisaema*) collected from southern regions of Vietnam (Table 1) were used in this study. All

respective vouchered specimens were deposited at SGN (Southern Institute of Ecology).

Besides, *matK* sequences of additional species from GenBank were also used in our phylogenetic analysis, including *Arisaema erubescens* (Wall.) Schott (GenBank: KT025779) - the type of the genus and *Pinellia ternata* (Thunb.) Makino (GenBank: JN090063).

Table 1. Accessions of nine taxa of *Arisaema* species sequenced in this study.

| Sequenced voucher number | Taxon | Location of sampling |
|--------------------------|---|---|
| H.T.Van 85 | <i>A. condaoense</i> | Con Dao National Park, Ba Ria-Vung Tau Province |
| H.T.Van 81 | <i>A. chauvanminhii</i> | Bu Gia Map National Park, Binh Phuoc Province |
| H.T.Van 125 | <i>A. consanguineum</i> subsp. <i>consanguineum</i> | Bidoup-Nui Ba National Park, Lam Dong Province |
| KKK 503 | <i>A. garrettii</i> | Kon Ka Kinh National Park, Gia Lai Province |
| H.T.Van 89 | <i>A. honbaense</i> | Hon Ba Nature Reserve, Khanh Hoa Province |
| H.T.Van 06 | <i>A. langbiangense</i> | Langbiang Mountain, Lam Dong Province |
| H.T. Luu 1121 | <i>A. petiolulatum</i> | Da Lat City, Lam Dong Province |
| H.T.Van 93 | <i>A. pierreanum</i> | Ba Den Mountain, Tay Ninh Province |
| H.T.Van 79 | <i>A. roxburghii</i> | Nui Cam Mountain, An Giang Province |

Methods

Plant specimens (Table 1) were collected and processed following the Kew Botanic Garden protocol (Govaerts, Frodin, 2002). Their fresh materials were noted and photographed of morphological characteristics of vegetative and reproductive plant organs, which were later used for taxonomic comparison and species identification.

Total genomic DNA was extracted from fresh leaf tissues using a Genomic DNA Purification Mini Kit (ThermoScientific Inc, USA). The target *matK* chloroplast DNA region was amplified by polymerase chain reaction (PCR) using primers (Fazekas *et al.*, 2012). PCR were performed in an Eppendorf Mastercycler Gradient using a reaction mixture in volume of 25 µl: 12.5 µl Go Taq green master mix (Promega, USA), 1.25 µl of each forward and reverse primers (10 µM), 9.5 µl HPLC water and 0.5 µl DNA template (25 µg/ml). PCR cycles consisted of an initial denaturation for 5 min at 95°C; 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 50°C) and extension (1:30 min at 72°C); and a final extension at 72°C for 10 min. The PCR products were visualised on 1.5% agarose gel and sent for purification and direct sequencing at Nam

Khoa Biotek Company Ltd. (Vietnam) using ABI 3130 XL Sequencer.

For multiple alignments, the software ClustalW (Thompson *et al.*, 1994) was used to recognise the homology between sequences. Phylogenetic analysis was carried out with PAUP* ver. 4.0 Beta (Swofford, 2002) using Neighbor Joining methods with *Pinellia ternata* (Table 1) as an outgroup, following Jung *et al.* (2004). The statistical support for phylogenetic trees was calculated using the bootstrap method (Felsenstein, 1985) with 1,000 replicates. Bootstrap values of 50% or higher were performed to obtain cluster supports. The pairwise genetic distance (Kimura, 1980) was calculated using MEGA6 (Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

The phylogenetic results

The length of the obtained *matK* nucleotide sequences ranged from 690 to 793 bp. The entire aligned length of *matK* region was 637 bp. In sect. *Fimbriata*, the *matK* alignment contained 637 positions of which 11 were variable. Accordingly,

Arisaema roxburghii shared 5 mutations with *A. condaoense*, 4 mutations with *A. chauvanminhii* and 3 mutations with *A. honbaense* (Table 2). Nucleotides of *A. condaoense* differ absolutely from the other species at positions of 232, 351 and 540. The pairwise genetic distances between *A. roxburghii* and *A. condaoense*, *A. chauvanminhii*, *A. pierreanum* and *A. honbaense* were 0.008, 0.003, 0.005 and 0.005, respectively (Table 3).

As a result, the phylogenetic tree (Figure 1) shows that *Arisaema* species in southern Vietnam are clearly divided into three sections: *Anomala*, *Fimbriata* and *Sinarisaema* with bootstrap values of 100%, 92% and 97%, respectively. This arrangement is consistent with the results of sectioning based on

the morphological characteristics described by Gusman, Gusman (2006) as well as the molecular marker-based phylogenetic tree developed by Ohi-Toma *et al.*, (2016). In sect. *Fimbriata*, *A. condaoense* is outmost compared to the other species which have been known as distinct species in previous studies (Pham-Hoang, 2000; Luu *et al.*, 2013, 2014; Van *et al.*, 2016). The number of mutations in the *matK* region and the consequent pairwise genetic distance between *A. roxburghii* and *A. condaoense* are larger than those between *A. roxburghii* and each of the other three species in the section (Table 2 and 3). Thus, the result supported that *A. condaoense* is a good species obviously genetically different from *A. roxburghii* and the other *Arisaema* species.

Table 2. Variable nucleotide positions of the *matK* region in the five species of sect. *Fimbriata*.

| | 17 | 18 | 19 | 115 | 202 | 232 | 351 | 361 | 534 | 540 | 763 |
|-------------------------|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>A. condaoense</i> | - | C | T | T | G | A | G | G | T | T | - |
| <i>A. pierreanum</i> | C | T | C | T | G | G | A | G | A | A | - |
| <i>A. roxburghii</i> | - | C | T | C | A | G | A | G | T | A | - |
| <i>A. chauvanminhii</i> | C | C | T | T | G | G | A | G | T | A | G |
| <i>A. honbaense</i> | - | C | T | T | G | G | A | A | T | A | - |

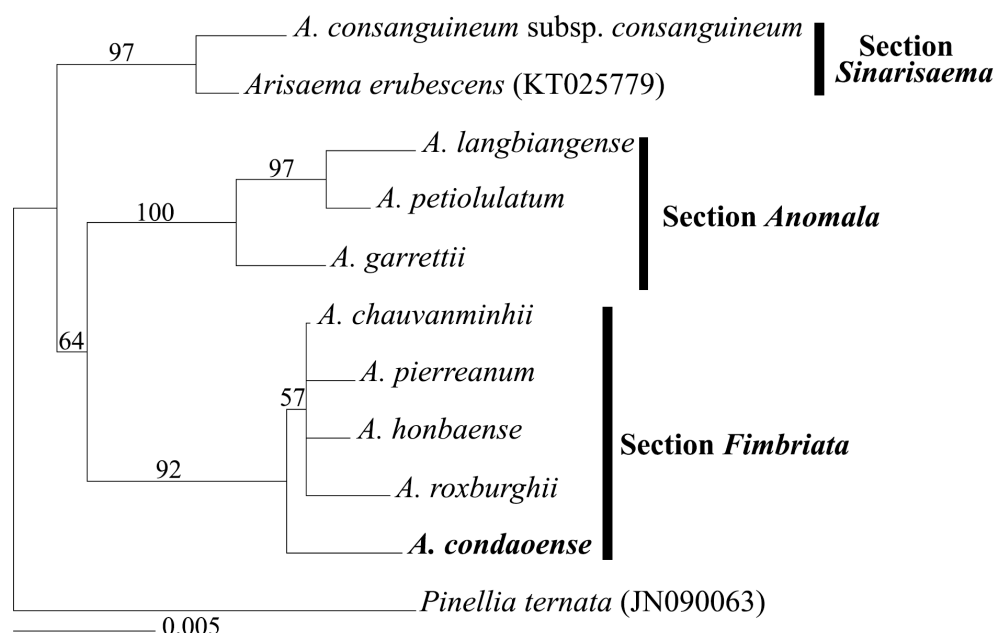


Figure 1. Phylogenetic tree of *Arisaema* species based on analysis of the *matK* nucleotide sequences. Topology was constructed using the Neighbor Joining method with the bootstrap values of 1000 replicates. Value of 50% or higher are shown above the nodes.

Table 3. Mean pairwise genetic distances between *Arisaema* taxa based on the nucleotide sequences of the *matK* region.

| | <i>A. consanguineum</i> subsp. <i>consanguineum</i> | <i>A. erubescens</i> | <i>A. langbiangense</i> | <i>A. petiolulatum</i> | <i>A. garrettii</i> | <i>A. chauvanminhii</i> | <i>A. pierreanum</i> | <i>A. honbaense</i> | <i>A. roxburghii</i> | <i>A. condaoense</i> | <i>Pinellia ternata</i> |
|--|--|----------------------|-------------------------|------------------------|---------------------|-------------------------|----------------------|---------------------|----------------------|----------------------|-------------------------|
| <i>A. consanguineum</i> subsp. <i>consanguineum</i> | | | | | | | | | | | |
| <i>A. erubescens</i> | 0.005 | | | | | | | | | | |
| <i>A. langbiangense</i> | 0.021 | 0.019 | | | | | | | | | |
| <i>A. petiolulatum</i> | 0.019 | 0.018 | 0.005 | | | | | | | | |
| <i>A. garrettii</i> | 0.018 | 0.016 | 0.010 | 0.008 | | | | | | | |
| <i>A. chauvanminhii</i> | 0.018 | 0.016 | 0.019 | 0.018 | 0.016 | | | | | | |
| <i>A. pierreanum</i> | 0.019 | 0.018 | 0.021 | 0.019 | 0.018 | 0.002 | | | | | |
| <i>A. honbaense</i> | 0.019 | 0.016 | 0.021 | 0.019 | 0.018 | 0.002 | 0.003 | | | | |
| <i>A. roxburghii</i> | 0.021 | 0.019 | 0.023 | 0.021 | 0.019 | 0.003 | 0.005 | 0.005 | | | |
| <i>A. condaoense</i> | 0.019 | 0.018 | 0.024 | 0.023 | 0.021 | 0.005 | 0.006 | 0.006 | 0.008 | | |
| <i>Pinellia ternata</i> | 0.024 | 0.023 | 0.030 | 0.028 | 0.026 | 0.026 | 0.028 | 0.028 | 0.026 | 0.028 | |

Taxonomic treatment

Arisaema condaoense V.D. Nguyen, 2000. Aroideana, 23: 36, fig. 1; Govaert *et al.* 2002. World Checkl. Bibliogr. Arac. 190; Gusman and Gusman, 2002. Mon. Bot. Nat. Lov. 186; id. 2006. ed. second Rev. 209; V.D. Nguyen, 2005. in N.T. Ban Checkl. Pl. Vietn. 3: 880; V.D. Nguyen and T.C. Vu, 2009. Arac. Indoch. Agri. Pub. 3: 90. (Figure 2)

Herb deciduous, 30–40 cm high. Stem subterranean, sub-globose, ca. 4 cm in diameter and 1.5–2 cm high, with thick roots. *Cataphylls* 1–2, brown and up to 14 cm long. *Leaves* 1 or 2; petioles sheathing into pale green pseudostem at lower part, free above; pseudostem 12–26 cm long, 1–1.2 cm in diameter at base, pale green; free petiole 12–18 cm long, ca. 0.5 cm in diameter at base and ca. 0.3 cm in diameter at apex, pale green; *leaf blade* trifoliate; leaflets 11–14 cm long, 7–9 cm wide, thin, glabrous, dark green above, pale green beneath, margins undulate, apex acuminate with up to 1.5–2 cm, midrib adaxially impressed and abaxially prominent, lateral veins 16–20, collective vein at 0.2–0.5 cm from

margin, central leaflet rhombic-elliptic, with base cuneate, petiolule ca. 0.5 cm, lateral leaflets asymmetrical, with base obtuse, petiolule nearly absent. *Inflorescence* emerging from pseudostem; peduncle longer or shorter than petiole, 4.5–12 cm long, ca. 0.5 cm in diameter, pale green; *spathe tube* cylindrical, slender, 4.5–5 cm, ca. 1 cm in diameter, slightly expanded at spathe mouth, white on both surfaces, yellowish near spathe limb; mouth-margins widely recurved, *spathe limb* curved over mouth, 5–6 cm long, 2.5–3 cm wide, ovate, light yellow on both surfaces, spathe tip acute, ending in a ca. 1.5 cm thread. *Female spadix appendix* 4.5–5 cm, much exserted from spathe mouth, filiform, sinuous, 7.5–8 cm long, covered with ca. 3 mm long brown or green neuters just above the pistillate flowers, upper part naked, basally dark green, apically light yellow; female part ca. 2 cm long, cylindrical, pistils densely arranged; ovaries oval and green, 1-locular; ovules 4–5, bottle-shaped, with basal placentation. *Male spadix appendix* 4.5–5 cm much exserted from spathe mouth, naked, filiform, sinuous, 6–6.5 cm long, basally dark green, apically light yellow; male part shortly stipitate,

ca.0.4 cm long, synandrium of 3–4 stamens, loosely arranged, on 0.1 cm stipe; anthers shortly stipitate, thecae purple or cream with purple top and dehiscent by dark purple, elongated pore.

Type: *N.T. Ban and Averyanov 385* (holo- and isotypes, HN!), Con Dao National Park, Ba Ria-Vung Tau Province, 20 June 1989; *Van Hong Thien H.T. Van 85* (paratypes, SGN! & PHH!, here designated).

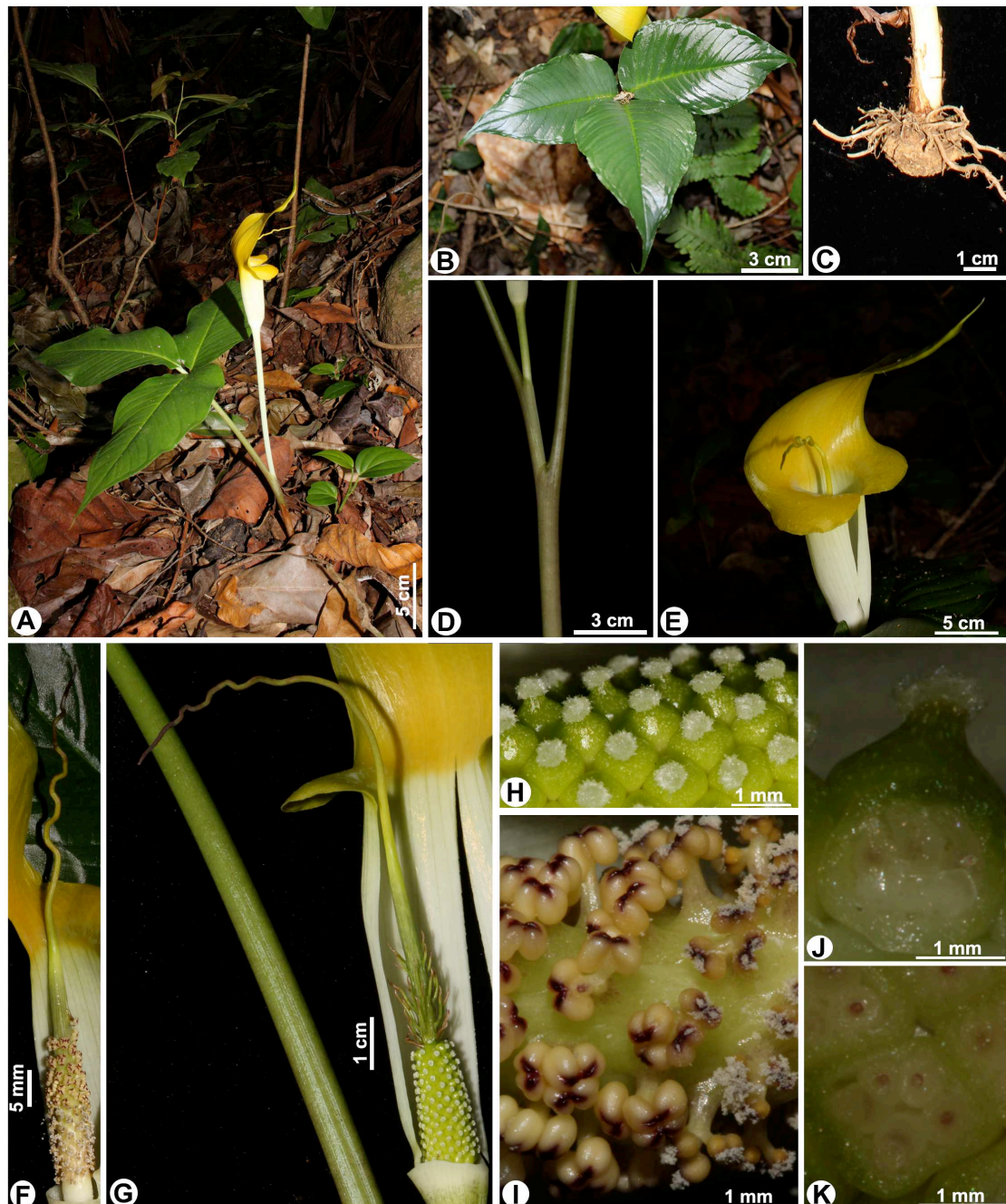


Figure 2. *Arisaema condaoense*, (A) The species in habitat, (B) Leaf blade, (C) Tuber, (D) Pseudostem, (E) Spathe, (F) Male spadix, (G) Female spadix, (H) Ovary and stigma, (I) Synandrium, (J) Longitudinal section of ovary, (K) Cross section of ovary.

Habitat: Humus soils under tropical semi-evergreen forest. Flowering was seen in June to July.

Distribution: *A. condaoense* is only known from the type location.

Notes: *A. condaoense* obviously belongs to section *Fimbriata* and it is most closely relative to *A. maxwellii*, *A. pierreanum* and *A. roxburghii* in having: deciduous habit, sub-globose, tuberous, subterranean stem, rooting around the central shoot only, 1-2 bilaterally symmetrical leaves, trifoliate leaf blade, yellow spathe, sessile, subulate and slender spadix appendage and sterile flowers (neuters) above the fertile region (at least in female spadices). However, it can be distinguished from *A. pierreanum* in having neuters on the female spadix appendix, thecae purple or cream with purple top and stamens grouped into stiped synandria (vs. naked female spadix appendix, light yellow thecae and non-grouping stamens in *A. pierreanum*). It is different from *A. maxwellii* in having a tube shorter than spathe limb, naked male spadix appendage, neuters on the base of the female spadix appendix only and always widely recurved margins of the spathe tube (vs. tube always much longer than spathe limb, bristle-like neuters on both male and female spadix appendices and hardly recurved margins of the spathe tube in *A. maxwellii*). Finally, *A. condaoense* differs from *A. roxburghii* in having yellow and not translucent spathe limb, spathe tube shorter than spathe limb and sinous spadix appendices (vs. green or sometimes yellow and translucent spathe limb, tube longer than spathe limb and not sinous spadix appendices).

Based on the available literature (Nguyen, 2000; Pham-Hoang, 2000; Nguyen and Boyce, 2005; Gusman and Gusman, 2006; Nguyen, 2007; Luu *et al.*, 2013 and 2014; Van *et al.*, 2016) and the authors' field observations, the known Vietnamese species of *Arisaema* sect. *Fimbriata* can be distinguished by the following key:

- 1A. Spathe limb yellow
- 2A. Female spadix appendix without neuters
A. pierreanum
- 2B. Female spadix appendix with neuters
- 3A. Spathe tube shorter than spathe limb; spadix appendices sinous
A. condaoense
- 3B. Spathe tube longer than spathe limb; spadix appendices not sinous
A. roxburghii

- 1B. Spathe limb green to deep brown
- 4A. Spathe limb deep brown *A. averyanovii*
- 4B. Spathe limb plain green or basically green with dark brown staining
- 5A. Spathe mouth margins reticulated in dark brown and widely recurved *A. chauvanminhii*
- 5B. Spathe mouth margins neither reticulated nor recurved
- 6A. Spathe limb with dark cross band at base
A. ramulosum
- 6B. Spathe limb without dark cross band at base
- 7A. Spadix appendix strongly apically fluted, backwards bent
A. honbaense
- 7B. Spadix appendix naked or with short neuters, not backwards bent
A. rostratum

CONCLUSION

Based on morphological and molecular data, *A. condaoense* is obviously distinct from its close species, including *A. roxburghii*, and therefore it should be accepted as a good species endemic to Con Dao National Park of Vietnam.

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XÁC ĐỊNH VỊ TRÍ PHÂN LOẠI LOÀI *ARISAEMA CONDAOENSE* (ARACEAE) DỰA TRÊN DỮ LIỆU MỚI VỀ HÌNH THÁI VÀ PHÂN TỬ

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TÓM TẮT

Arisaema condaoense V.D. Nguyen được mô tả là một loài mới từ Vườn Quốc gia Côn Đảo thuộc tỉnh Bà Rịa-Vũng Tàu, Việt Nam, vào năm 2000. Tuy nhiên, một số nhà nghiên cứu về chi *Arisaema* đặt câu hỏi liệu đây có thực sự là một loài mới hay chỉ là một dạng của loài *A. roxburghii* Kunth vốn có phân bố rộng ở Đông Dương và bán đảo Malay; bởi vì *A. condaoense* được mô tả dựa trên các mẫu tiêu bản khô chỉ có phát hoa đực và thiếu một số đặc điểm quan trọng, nhất là ở phát hoa cái. Tháng 6 năm 2015, chúng tôi đã thu thập được cả phát hoa đực và cái của loài *Arisaema* sp. ở Vườn Quốc gia Côn Đảo. Từ đó, đoạn gen *matK* trên lục lạp của loài này đã được giải trình tự để phân tích, xây dựng cây phả hệ và so sánh với các loài *Arisaema* khác. Phân tích dữ liệu hình thái và phân tử đã chứng minh một cách chắc chắn rằng *A. condaoense* là một loài riêng biệt,

không phải là đồng danh của *A. roxburghii*, đồng thời cũng hoàn toàn khác biệt với hai loài có hình thái tương tự là *A. maxwellii* và *A. pierreanum*. Cho nên, *A. condaoense* thực sự là một loài đặc hữu của Vườn Quốc gia Côn Đảo. Ngoài ra, kết quả nghiên cứu cũng giúp chúng tôi mô tả bổ sung loài *A. condaoense* với đầy đủ các đặc điểm phân loại và hình ảnh minh họa chi tiết lần đầu tiên thu thập được từ cây tươi, đồng thời xây dựng khóa định loại cho tất cả 8 loài thuộc section *Fimbriata* đã ghi nhận được tại Việt Nam.

Từ khóa: *Araceae*, *Arisaema condaoense*, *chloroplast*, *Fimbriata*, *matK*