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

Van Hong Thien¹, Phi Nga Nguyen², Luu Hong Truong³,⁴

¹Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City
²University of Science, Vietnam National University of Ho Chi Minh City
³Graduate University of Science and Technology, Vietnam Academy of Science and Technology
⁴Southern Institute of Ecology, Vietnam Academy of Science and Technology

To whom correspondence should be addressed. E-mail: hongtruongluu@gmail.com

Received: 21.7.2017
Accepted: 25.10.2017

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

Besides, /mk 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               | A. condaoense       | Con Dao National Park, Ba Ria-Vung Tau Province |
| H.T.Van 81               | A. chauvanminhii    | Bu Gia Map National Park, Binh Phuoc Province |
| H.T.Van 125              | A. consanguineum subsp. consanguineum | Bidoup-Nui Ba National Park, Lam Dong Province |
| KKK 503                  | A. garretii         | Kon Ka Kinh National Park, Gia Lai Province |
| H.T.Van 89               | A. honbaense        | Hon Ba Nature Reserve, Khanh Hoa Province |
| H.T.Van 06               | A. langbiangense    | Langbiang Mountain, Lam Dong Province |
| H.T.Luu 1121             | A. petiolulatum     | Da Lat City, Lam Dong Province |
| H.T.Van 93               | A. perrieanum       | Ba Den Mountain, Tay Ninh Province |
| H.T.Van 79               | A. rapxburghii      | 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 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 /mk 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 /mk nucleotide sequences ranged from 690 to 793 bp. The entire aligned length of /mk region was 637 bp. In sect. Fimbriata, the /mk 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 |
|-------------|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| A. condaoense |    | C  | T  | T   | G   | A   | G   | G   | T   | T   | -   |
| A. pierreanum   |   C | T  | C  | T   | G   | G   | A   | G   | A   | A   | -   |
| A. roxburghii   |   - | C  | T  | C   | A   | G   | A   | G   | T   | A   | -   |
| A. chauvanminhii   |   C | C  | T  | T   | G   | G   | A   | G   | T   | A   | G   |
| A. honbaense     |   - | 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.

|                  | A. consanguineum subsp. consanguineum | A. erubescens | A. langbiangense | A. petiolulatum | A. garrettii | A. chauvanminhii | A. pierreanum | A. honbaense | A. roxburghii | A. condaoense | Pinellia ternata |
|------------------|--------------------------------------|---------------|------------------|-----------------|-------------|-----------------|--------------|--------------|--------------|--------------|----------------|
| A. consanguineum subsp. consanguineum | 0.005 | | | | | | | | | | |
| A. erubescens    | 0.021 | 0.019 | | | | | | | | | |
| A. petiolulatum  | 0.019 | 0.018 | 0.005 | | | | | | | | |
| A. garrettii     | 0.018 | 0.016 | 0.010 | 0.008 | | | | | | | |
| A. chauvanminhii | 0.018 | 0.016 | 0.019 | 0.018 | 0.016 | | | | | | |
| A. pierreanum    | 0.019 | 0.018 | 0.021 | 0.019 | 0.018 | 0.018 | 0.002 | | | | |
| A. honbaense     | 0.019 | 0.016 | 0.021 | 0.019 | 0.018 | 0.02 | 0.003 | | | | |
| A. roxburghii    | 0.021 | 0.019 | 0.023 | 0.021 | 0.019 | 0.003 | 0.005 | 0.005 | | | |
| A. condaoense    | 0.019 | 0.018 | 0.024 | 0.023 | 0.021 | 0.005 | 0.006 | 0.006 | 0.008 | | |
| Pinellia ternata | 0.024 | 0.023 | 0.030 | 0.028 | 0.026 | 0.026 | 0.028 | 0.026 | 0.026 | 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 trifoliolate; 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, petiolute ca. 0.5 cm, lateral leaflets asymmetrical, with base obtuse, petiolute 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 placentaion. 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, trifoliolate 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.

Acknowledgments: The authors thank the staffs of the Southern Institute of Ecology, Vietnam Academy of Science and Technology and Industrial University of Ho Chi Minh City for valuable supports. We are grateful to Con Dao National Park Management Board, especially Mr. Le Hong Son, Head of Science and International Cooperation, Mr. Nguyen Dinh Trung and forest rangers of Con Dao National Park for their permits and help in the field.

REFERENCES

Fazekas AJ, Kuzmina L, Newmaster SG, Hollingsworth PM (2012) DNA Barcoding methods for land plants. In: Kree WJ and Erickson DL (ed) DNA Barcodes: Method and Protocols. Meth Mol Biol 858: 223–252.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Govaerts R, Frodin D (2002) *World checklist and bibliography of Araceae (and Acoraceae)*. Royal Botanic Gardens, Kew.
The genus Arisaema, A monograph for botanists and nature lovers. AR. Gantner Verlag, Ruggell, Lichtenstein.

Jung YH, Song EY, Chun SJ, Jang KC, Kim M, Kang SH, Kim SC (2004) Phylogenetic analysis of plastid trnL-trnF sequences from Arisaema species (Araceae) in Korea. *Euphytica* 138: 81–88.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 11–120.

Luu HT, Tran G, Nguyen TT, Nguyen VD (2013) *Arisaema houaense* (Araceae) - a new species from Vietnam. *Folia malayana* 14: 45–50.

Luu HT, Nguyen QD, Vu NL, Vuong DH, Kieu DT, Vo HS (2014) *Arisaema chauvaninhii* (Araceae), a new species from Vietnam. *Ann Bot Fennici* 51: 394–398.

Nguyen VD (2000) Two new species of *Arisaema* from Vietnam. *Aroideana* 23: 36–40.

Nguyen VD, in T.B Nguyen, Araceae Juss (2005) *Vietnam checklist and bibliography of Araceae*. Agriculture Publishing House, Hanoi, Vietnam: 3: 871–898.

Nguyen VD, Boyce PC (2005) Two new species of genus *Arisaema* (Araceae) from northern Vietnam. *Folia Malasiana* 6: 35–40.

Nguyen VD (2007) Two new species of the genus *Arisaema* Mart. (Araceae) described for Flora of Vietnam.

**XÁC ĐỊNH VỊ TRÍ PHÂN LOẠI LOẠI ARISAEA CONDAOENSE (ARACEAE) DƯA TRÊN DỮ LIỆU MÓI VỀ HÌNH THÁI VÀ PHÂN TỬ**

Văn Hồng Thiện¹, Nguyễn Phi Ngữ², Lưu Hồng Trương³,⁴

¹Viện Công nghệ sinh học và Thực phẩm, Trường Đại học Công nghiệp Thành phố Hồ Chí Minh
²Trường Đại học Khoa học tự nhiên, Đại học Quốc gia Thành phố Hồ Chí Minh
³Học viện Khoa học và Công nghệ, Viện Hàn lâm Khoa học và Công nghệ Việt Nam
⁴Viện Sinh thái học Miền Nam, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

TÔM 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 với phân bố rộng ở Đông Dương và bán đảo Malaya; bởi vì *A. condaoense* được mô tả dựa trên các mẫu tiêu bản khó chi có phát hoa được và thiếu một số đặc điểm quan trọng, nhất là ở phát hoa cái. Tháng 6 năm 2015, chúng tôi đã thử nghiệm cả phát hoa đực và cái của loài *Arisaema sp.* ở Vườn Quốc gia Côn Đảo. Từ đó, đoàn gen makT trên lục lạp của loài này đã được giải trình tự để phân tích, xây dựng cây phát hóa 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 cùng 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 chứng tỏ mới tả bò 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 truồi, 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.