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Source: Annales Botanici Fennici, 56(1-3) : 19-25

Published By: Finnish Zoological and Botanical Publishing Board

URL: https://doi.org/10.5735/085.056.0104

Ann. Bot. Fennici 56: 19–25 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 16 October 2018

Taxonomic identity of *Arisaema pangii* (Araceae) inferred from molecular and morphological data with a note on its distribution

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Received 2 Feb. 2018, final version received 23 July 2018, accepted 2 August 2018

Arunkumar P.G., Manudev K.M. & Nampy S. 2019: Taxonomic identity of *Arisaema pangii* (Araceae) inferred from molecular and morphological data with a note on its distribution. — *Ann. Bot. Fennici* 56: 19–25.

The identity of *Arisaema pangii* H. Li (Araceae) has been doubtful ever since it was published. Various authors treated it under either *A. nepenthoides* or under *A. wattii*. *Arisaema pangii* is recognized as a distinct species based on morphological and molecular data, and it is placed in sect. *Nepenthoidea*. A detailed description, notes on the taxonomy and colour images are provided for easy identification.

Introduction

The genus *Arisaema* (Araceae), with about 200 taxa, is widely distributed from northeastern Africa through tropical, sub-tropical and warm temperate East Asia to North America (Govaerts *et al*. 2018). It is the largest genus of Araceae in India, with 51 species and six varieties in nine sections. During botanical surveys in Mayodia in Upper Dibang Valley district of Arunachal Pradesh between 2014 and 2016, specimens of *Arisaema* were collected that were morphologically close to *A. nepenthoides* and *A. wattii*, belonging to sect. *Nepenthoidea*. Critical examination revealed that the specimens belonged to *A. pangii*, a species described by Li (1992) from Yunnan in China. The taxonomic identity of that taxon, however, has been problematic ever since its publication. Gusman and Gusman (2006) considered it conspecific with *A. nepenthoide*s, while Li *et al.* (2010) and Govaerts *et al*. (2018) placed it under *A. wattii*. In the present study all the above species were evaluated in the field as well as in the herbarium to unravel the taxonomic status of *A. pangii.* We also used molecular data from 3′*trn*L-*trn*F spacer and *rpl*20-5′*rps*12 spacer sequences.

Material and methods

Plant material

Plant material for the present study was collected in 2014–2016 from Arunachal Pradesh, Sikkim and West Bengal in India. The specimens were identified after consulting protologues and recent literature. Detailed morphological studies

were made using all the available materials and descriptions were prepared after examining a wide range of specimens. In addition, specimens of morphologically similar taxa housed at various herbaria (ASSAM, CAL, CALI, DEV, MH) were consulted to ascertain the variation, if any. Voucher specimens were deposited in Calicut University Herbarium (CALI).

For molecular analyses, species of sect. *Nepenthoidea* (*A. nepenthoides*, *A. wattii*), sect. *Arisaema* (*A. griffithii*, *A. propinquum*, *A. speciosum*, *A. speciosum* var. *ziroense*, *A. utile*), sect. *Decipienta* (*A. rhizomatum*), sect. *Sinarisaema* (*A. consanguineum*, *A. ciliatum*), sect. *Tortuosa* (*A. murrayi*, *A. sahyadricum*, *A. tortuosum* and the species under study (*A. pangii*) were selected as ingroup and *Pinellia ternata* and *P. pedatisecta* formed the outgroup. The 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 sequences were newly generated for *A. nepenthoides*, *A. wattii* and *A. pangii* while for other species, the sequences available in NCBI were used. Sequences generated in this study are deposited in GenBank (Table 1).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from silica-dried leaves using the modified CTAB method (Doyle & Doyle 1990). 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 from chloroplast DNA (cpDNA) were amplified with primers designed by Taberlet *et al*. (1991) and Hamilton (1999) respectively to study the sequence variation. PCR amplification was performed in 25 µl reaction with TaKaRa Taq polymerase (TaKaRa Bio Inc.) using the following PCR conditions. The PCR profile for 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 included an initial denaturation for 4 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55.2 °C for 1 min and extension at 72 °C for 1 min 30 s, followed by 5 min of final extension at 72 °C. The purified PCR fragments were sequenced using the same primer for PCR amplification.

Phylogenetic analysis

The DNA sequences were firstly submitted to the Nucleotide Basic Local Alignment Search Tool (BLAST) to find the primary sequence information. Alignments were made using Muscle embedded in MEGA 7 (Kumar *et al.* 2016) and aligned sequence of 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 were assembled using MEGA 7 (Kumar *et al.* 2016). All characters were given equal weight and gaps were treated as missing data. For Maximum Likelihood (ML) and Bayesian

Table 1. List of species with voucher information and GenBank accession numbers.

Species	Voucher	Collector	*trn*L-F spacer	*rpl*20-*rps*12 spacer
Arisaema ciliatum	92118a	G. Gusman	AB982306	AB982461
Arisaema consanguineum	Arisa076	J. Murata	AB982351	AB982506
Arisaema griffithii	97013	G. Gusman	AY275607	AB982471
Arisaema murrayi	Arisa029	J. Murata	AY275623	AY279160
Arisaema nepenthoides	CALI 144813	Arunkumar & Rajeevan	MK043957	MK043954
Arisaema pangii	CALI 144841	Arunkumar & Rajeevan	MK043956	MK043953
Arisaema propinquum	*s.n.*	P. Bruggeman	AB982330	AB982485
Arisaema rhizomatum	*s.n.*	J. Murata	AY248956	AY248919
Arisaema sahyadricum	Arisa010	J. Murata	AB982428	AB982583
Arisaema speciosum var. *speciosum*	H.AR.294	W. Hetterscheid	AB982333	AB982488
Arisaema speciosum var. *ziroense*	97143	G. Gusman	AB982334	AB982489
Arisaema tortuosum	Arisa077	J. Murata	AB982446	AB982601
Arisaema utile	*s.n.*	W. Hetterscheid	AB982336	AB982491
Arisaema wattii	CALI 144839	Arunkumar & Rajeevan	MK043958	MK043955
Pinellia ternata	*s.n.*	J. McClements	AY248968	AY248931
Pinellia pedatisecta	*s.n.*	J. McClements	AY275624	AY279170

Inference (BI) analysis was performed using the best-fit substitution model GTR + I + G according to Akaike's information criterion (AIC) calculated with jModelTest ver. 2.1.4 (Darriba *et al*. 2012). The GTR + I + G model selected for the combined cpDNA 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 data sets. ML analysis was performed using RAxML ver. 8.1.21 (Stamatakis 2014) implemented in raxmlGUI ver. 1.5b2 (Silvestro & Michalak 2012) with a selection of ML + rapid bootstrap and support assessment using 1000 rapid bootstraps. MrBayes ver. 3.2.1 (Ronquist *et al*. 2012) two runs with 20000 generations sampling every 1000th generation were used for BI analysis. Trees generated by the two analyses were viewed and exported in FigTree ver. 1.3.1.

Results

Morphology

A detailed morphological analysis (Table 2) revealed that *A. pangii* can be separated from *A. nepenthoides* by its trifoliolate leaves, shorter spathe limb, and non-auriculate tube mouth, with only recurved margins; and from *A. wattii* by its shiny leaves, shorter pseudostems, white, brownish-spotted spathes and revolute mouth of tube without an auricular extension.

Sequence data and phylogenetic tree

Phylogenetic analysis involved 16 nucleotide sequences of 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 including the outgroup taxa. The 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 sequences were combined and the final matrix was used in the analysis. The total length of aligned 3´*trn*L-*trn*F and *rpl*20-5´*rps*12 sequence was 1269 bp. A ML and BI phylogenetic tree was constructed based on the substitutions using the GTR + I + G model (Fig. 1). Two major clades in the ingroup (Clades I and II) were resolved with BI-PP = 1.00, ML-BP = 100%. The clades have support values BI-PP = 0.98, ML-BP = 62% in clade I and BI-PP = 1.00, ML-BP = 94% in clade II. Clade I contains the sections *Tortuosa*, *Decipienta*, *Sinarisaema* and *Nepenthoidea* and clade II contains sect. *Arisaema*. In clade I, *A. nepenthoides* and *A. wattii* are sisters with BI-PP = 0.88, ML-BP = 80% and *A. pangii* is related to *A. nepenthoides* and *A. wattii* with BI-PP = 1.00, ML-BP = 95%. The sister clade contains *A. consanguineum* and A. *ciliatum* with BI-PP = 0.97, ML-BP = 70% and a basal *A. rhizomatum* with BI-PP = 0.96, ML-BP = 75%. Sect. *Tortuosa* forms a subclade in clade I with BI-PP = 1.00, ML-BP = 80%. Among the species included in the analysis, *A. pangii* is thus closely related to *A. nepenthoides* and *A. wattii* in sect. *Nepenthoidea*.

Taxonomic conclusion

Arisaema pangii H. Li (Fig. 2)

Acta Bot. Yunnan., Suppl. 5: 8. 1992. — Type: China. Yunnan Gongshan Xian, Dulongjian Valley, Chawudan, 2600 m a.s.l., under subtropical forests, 25 March 1991 *Dulongjian Bot. Expedition 4995* (KUN1218556, digital image!)

Arisaema nepenthoides (Wall.) Mart. *ex* Schott & Endl. *sensu* Gusman & L.Gusman, Gen. Arisaema ed. 2. 269. 2006, *pro parte*, *non* (Wall.) Mart., 1832.

Arisaema wattii Hook. f., *sensu* H. Li *et al*. in Wu *et al*., Fl. China 23: 56. 2010, *pro parte, non* Hook. f., 1893.

Table 2. Morphological comparison of *Arisaema pangii* with two related species.

Characters	*A. pangii*	*A. nepenthoides*	*A. wattii*
Plant height (cm)	< 50	< 100	< 1 m
Leaves	3-foliolate, 15–24 cm long, 7–14 cm wide	5-foliolate, 10–14.5 cm long, 2–4.5 cm wide	3-foliolate, 21–30 cm long, 5–10.5 cm wide
Lateral leaflets	Asymmetrical with oblique base	Symmetrical with cuneate base	Asymmetrical with oblique base
Length of sheathing pseudostem (cm)	4–21	51–63	28–47
Mouth of spathe tube	Not auriculate, slightly recurved	Auriculate, ca. 2 cm wide	Auriculate, ca. 2 cm wide

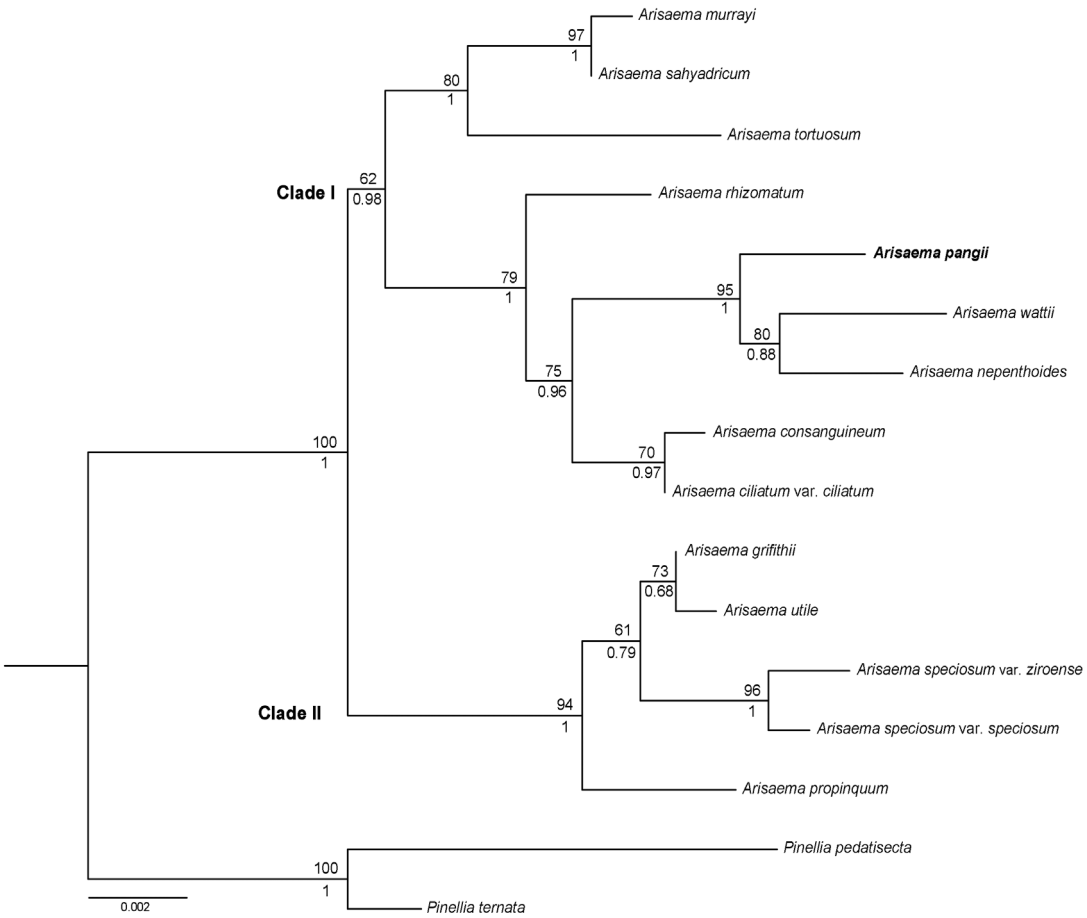

Fig. 1. Maximum Likelihood (ML) phylogram based on 3´*trn*L-*trn*F spacer region and *rpl*20-5´*rps*12 spacer region, rooted with *Pinellia* spp. ML bootstrap proportions higher than 50% and posterior probabilities from the Bayesian analysis higher than 0.68 are shown above and below branches respectively.

Dioecious, perennial, succulent herbs, up to 50 cm tall. Corms flattened-subglobose, 5 cm tall, 7.5–10 cm diam., proliferating, yellow-orange inside. Roots many, pinkish-purplish tinged or white. Cataphylls 3, outer 3 cm long, middle 4–9 cm, inner 13–21 cm long, outer pale to white, not mottled; inner two distally mottled as in the petiole. Leaves 2, rarely 1, trifoliolate, emerging after the inflorescence; petioles 50–72 cm long, 0.5–1.5 cm thick, pale white to pale green, often dark green-brownish, mottled with patches of longitudinal streaks; leaflets-3, sessile, elliptic to ovate or oblanceolate, margins entire, dark glossy green above, pale and glaucous beneath with purplish veins; central leaflet 15–24 cm long, 7–10.5 cm wide, cuneate at base, acute-acuminate at apex; lateral leaflets 18–23 cm long, 10–14 cm wide, obliquely cuneate at base, acuminate to caudate at apex. Pseudostem 4–21 cm long, 1.7–2.5 cm thick, wrapped by cataphylls, mottled as in petiole. Inflorescence emerging before leaves and positioned below or at same level as leaves; peduncles 17–32 cm long, 0.5–0.7 cm thick, pale white to pale green, dark greenish-brown, mottled as in petiole, exserted by 11–13 cm long from the pseudostem. Spathe 10–12 cm long; tube of spathe cylindric, funnel shaped towards mouth, 5.5–6.5 cm long, 1.5–2 cm wide, olive green with longitudinal dark purple spots or streaks, pale white inside, sometimes glaucous; mouth margins revolute, not auriculate; spathe limb ovate, 4.5–6.5 cm

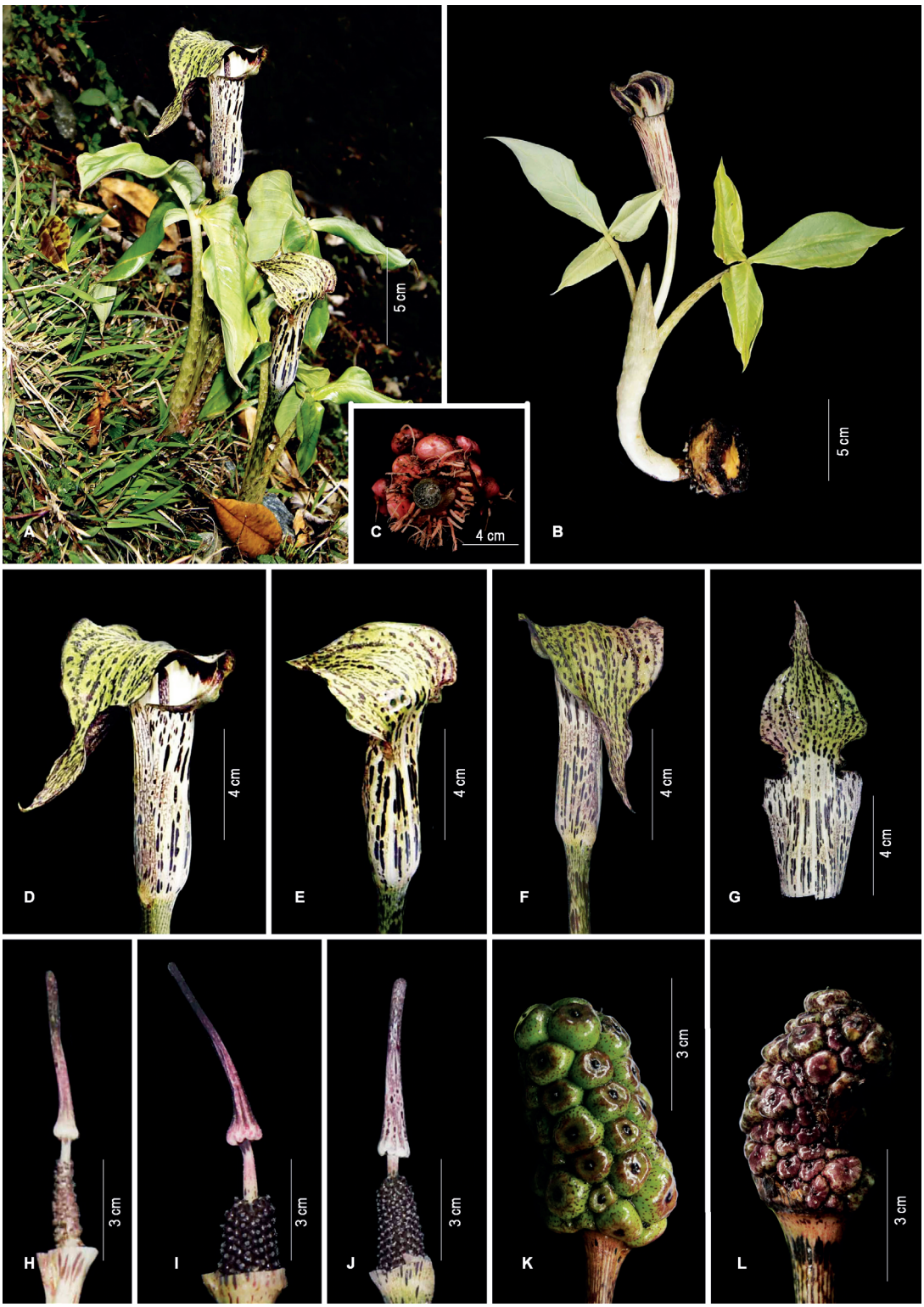

Fig. 2. *Arisaema pangii*. — **A** and **B**: Habit. — **C**: Corm. — **D**–**F**: Inflorescences. — **G**: Spathe-back view. — **H**: Male spadix. — **I** and **J**: Female spadix. — **K** and **L**: Fruiting spikes.

long, acuminate at apex, constricted at base, arched over and horizontal, olive green with dark purple streaks outside, inside dark purple with 3 prominent pale-white stripes, sometimes glaucous. Female spadix 6–7 cm long; fertile region 1.5 cm long; appendix cylindric, 4.5–5 cm long, slightly exserted from tube, stipitate, stipe to 1.2 cm long, cylindric at center, rounded at apex, pale purplish below, dark purple above, often brown mottled; pistils many, sessile, compactly arranged, bottle-shaped, green or purple and apically black; ovules 1–4; stigma white, papillate; neuters absent. Male spadix similar to female, 6–8 cm long, with a fertile region 1–1.5 cm at base; male flowers consisting of 2–3 sessile, purple anthers, scattered over or crowded at base and distant above, stipitate (stipe shorter than in female flowers); staminodes absent. Fruiting spike cylindrical, 5.5–8 cm long, 2.5–3.5 cm wide, borne on an erect or sometimes slightly curved peduncle; berries densely packed, green to brownish-green or purple when young with brownish spots all over, flat with black stigmatic region at apex, 1–4 seeded. Flowering March–May, fruiting June–July.

Habitat and distribution. In moist and shady places along roadsides, among bushes, bamboo streaks and rock crevices. China (Dulongjian Valley in Yunnan), India (Mayodia in Arunachal Pradesh).

Specimens examined: **India**. Arunachal Pradesh, *Manudev 139808* (CALI); *Manas Bhaumik 2483* (CAL); *Manudev 139831* (CALI); *Arunkumar & Hareesh 149208* (CALI); *Arunkumar & Rajeevan 144841, 144842, 144843* (CALI); *Arunkumar & Rajeevan 144837* (CALI).

Discussion

Morphological as well as molecular data support recognition of *A. pangii* within sect. *Nepenthoidea*. Li (1992) described the leaves as 3–5-foliolate in the protologue, which is in accordance with the type specimen. The illustration provided in the protologue has 3-foliolate leaves even though the plant mounted on the type sheet shows a leaf with five leaflets. Gusman and Gusman (2006) treated this taxon as a taxonomic synonym of *A. nepenthoides* as they considered the leaflet number and auriculation as a variable character. However the present collections consistently show trifoliolate leaves and that is a good character to distinguish *A. pangii* from 5-foliolate *A. nepenthoides* in the field. *Arisaema pangii* grows together with *A. wattii* and *A. nepenthoides* in Mayodia regions of Arunachal Pradesh and there are no morphological intermediates among them. In view of the morphological and molecular evidence *A. pangii* is treated here as a distinctive species.

Acknowledgements

The authors are thankful to the Head, Department of Botany, University of Calicut and the Principal, St. Joseph's College (Autonomous), Kozhikode for facilities; Mr. V.S. Hareesh, R. Rajeevan and C. Saleem, for assistance in the field and Department of Forest, Government of Arunachal Pradesh for permission. SN and APG are thankful to the Department of Science and Technology, New Delhi for financial assistance (F.No.SERB/SR/SO/PS/09/2012).

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