

***Boesenbergia siphonantha* (Zingiberaceae), a new record for Thailand and Vietnam with notes on the molecular phylogeny**

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ABSTRACT. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. (Zingiberaceae) is newly recorded from Thailand and Vietnam. Its nomenclatural history, taxonomy, and molecular phylogeny are discussed. Figures, a comparative table and a watercolour illustration are provided.

Keywords. Andaman Islands, *Gastrochilus*, *Kaempferia*

Introduction

During field work in 2010 for the *Flora of Thailand* Project, an unusual *Boesenbergia* was discovered growing on and around limestone outcrops under deciduous forest in Kanchanaburi Province [J. Mood & P. Chalermlin M2056 (BKF)]. The plant with four, deeply plaited laminae on long petioles was highlighted by a terminal inflorescence and a single flower with a shiny lavender lip and red throat. Except for the colouration, the floral appearance was reminiscent of *Boesenbergia longiflora* (Wall.) Kuntze, due in part to the long floral tube and saccate labellum. On further examination of other plants in the Kanchanaburi population, numerous radical inflorescences were observed. Except as an abnormal occurrence, bi-positional flowering, defined here as radical and terminal inflorescences on the same element, is quite rare in Zingiberaceae. However, it is not without precedent as *Boesenbergia prainiana* (Baker) Schltr., *B. tenuispicata* K.Larsen, and *B. trangensis* K.Larsen consistently exhibit this habit. Bi-positional flowering was later observed in two other populations, a second site in Kanchanaburi [J. Mood & P. Vatcharakorn 3081 (BKF)] and in Tak Province [J. Mood & P. Vatcharakorn 3366 (BKF)].

In 2008, preceding the Thai collections, the second author discovered an unidentified *Boesenbergia* species at Cát Tiên National Park, Đồng Nai Province, Vietnam [Trần et al. 123 (SING, VNM)] during MSc research at the Vietnam National University, Hồ Chí Minh City (Trần, 2009). Later, photos of this plant were sent to JDM for possible identification, along with a leaf tissue sample for addition to the authors' ongoing molecular phylogenetic research on *Boesenbergia*. Although the taxon could not be identified immediately, the molecular results showed that it grouped in the same clade as the three aforementioned Thai collections. Later, morphological comparisons also indicated close similarity except for plant stature with the Thai collections being taller (Table 1). During further study, *Boesenbergia* specimens at AAU, BK, BKF, C, CAL, CMU, CMUB, E, K, L, P, SING, QBG, VNM and W were surveyed. The search produced three unidentified, morphologically similar collections from Thailand and one from northern Myanmar. Surprisingly, an Andaman Islands species, *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk., was also very similar.

Boesenbergia siphonantha is considered to be a rare, insular endemic from the evergreen and semi-deciduous forests of the Andaman Islands, India. It was described by Baker (1890) as *Kaempferia siphonantha* based on King's Collector 372 (K). This and three similar specimens deposited in CAL were collected by Kunstler on 21 July 1884 in the Andaman Islands during exploration for George King, then Director, Royal Botanic Garden, Calcutta. It was named to describe the flower's very long floral tube. For reasons unknown, Baker did not associate this new taxon with *Boesenbergia longiflora* which also had radical inflorescences and flowers with a long floral tube. Perhaps his statement in the *Gardeners' Chronicle* (Baker, 1894: 34) shows his sentiment: "*Gastrochilus* [*Boesenbergia* (Kuntze, 1891)] is hardly worth separating as a genus from *Kaempferia*". Consequently he classified it as a *Kaempferia* L. along with six others which had little similarity to this new species. Of these, only *Kaempferia parvula* King ex Baker and *K. elegans* Wall. have withstood the test of time and remain in *Kaempferia*.

Considering this information, the Thai and Vietnamese specimens were compared to the lectotype, protologue (Baker, 1890), and later descriptions of *Boesenbergia siphonantha* (Sabu et al., 2004; Kumar et al., 2010), all of which compared favourably. To further verify the tentative identification, a complete set of photos of the Thai collections was sent to J. Leong-Škorničkova (SING) who had firsthand knowledge of this species. Although some differences were noted and discussed, it was eventually agreed that the non-Andaman collections were representative of *Boesenbergia siphonantha sensu lato*. Unfortunately, at the time, this conclusion could not be supported phylogenetically since a tissue sample of *Boesenbergia siphonantha* from the Andaman Islands was not available. Recent molecular research by Aishwarya et al. (2015), which included *Boesenbergia siphonantha*, later allowed for inclusion of partial data here. The molecular results support the decision to identify the Thai and Vietnamese taxa as *Boesenbergia siphonantha*.

Materials and Methods

Field research. In 2008, HDT collected a voucher with silica-dried leaf tissue from a single population of *Boesenbergia siphonantha* in Vietnam. Between 2010–2015, JDM collected *B. siphonantha* vouchers with silica-dried tissue samples from three different populations in Thailand. All of the populations were thoroughly studied, documented, and photographed. Ex situ nursery cultivation was accomplished in Thailand with several living collections, providing additional information.

Molecular phylogeny. The above tissue samples were used in this analysis. Additional sequences providing the scaffold were taken from the authors' ongoing research. *Boesenbergia siphonantha Sabu 73-1* is not available in GenBank but a partial *trnK* sequence, corresponding to the *matK* genetic fingerprint region, was generously provided by M. Sabu (University of Calicut, India). ITS data were not available for that sample. A complete list of samples and GenBank accession numbers are provided in Appendix 1.

DNA extraction and analytical methods follow Mood et al. (2013) and will only be summarised here. The entire plastid *trnK* region was amplified. Amplification was done in two parts, the first using 1F and 1235R primers and the second using m1F and 2R. The nuclear ribosomal ITS (nrITS) region was amplified using the 18S-F and 26S-R primers (Prince, 2010). Data were collected on an ABI Genetic Analyzer (ThermoFisher Scientific) and trace files for each specimen were edited in Sequencher v4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Consensus sequences were exported from Sequencher for manual alignment in Se-al (Rambaut, 1996). Areas of ambiguous alignment were identified and analyses were conducted both including and excluding these data. Maximum parsimony analyses were run in PAUP* (version 4.0b10; Swofford, 2002) for each genomic data partition, firstly independently and later in combination. Branch and bound search methods were conducted in each case using “farthest” stepwise addition, saving a maximum of 100,000 trees. Branch support was also estimated using Branch and Bound parsimony bootstrap (BS) in PAUP*.

Results

The ITS analyses produced eight shortest trees based on 97 potentially phylogenetically informative characters (PPIC) with a consistency index of 0.6703 and a retention index of 0.7980 (excluding uninformative characters). The *trnK* analyses, with only 35 PPIC, generated 21 shortest trees (consistency index=0.7400, retention index=0.8750). The combined partition matrix yielded 132 PPIC and generated 18 shortest trees (consistency index=0.6598, retention index=0.5231) when the sample *Sabu 73-1* (partial *trnK* sequence only) was excluded from the analyses.

The ITS partition included four samples of *Boesenbergia siphonantha* (*M2056*, *M3081*, *M3366*, and *T123*), while the *trnK* partition included those already mentioned, plus a partial sequence from *Sabu 73-1*. Analyses of all data partitions, alone or in

combination, group the samples of *Boesenbergia siphonantha* together in a strongly supported monophyletic clade (100% bootstrap support for ITS, 98% for *trnK*, and 98% for the combined analyses). Exclusion of the partial *trnK* sequence for *Sabu 73-1* resulted in a clade bootstrap value of 100%. The sample *Sabu 73-1* is identical to the other four samples for the region sequenced. Characters supporting the monophyly include a large number of autoapomorphic substitutions distributed across the coding and non-coding regions (3 from the ITS1, 5 from the ITS2, 1 from the 5' *trnK-matK* intergenic spacer, and 4 from the *matK* coding region). A unique 3 base pair insertion was also observed in the 5' *trnK-matK* intergenic spacer.

For simplicity, only a single phylogeny is shown here; a phylogram of one of the most parsimonious trees from the combined data analyses where gaps are treated as missing data, and the sample *Sabu 73-1* was excluded (Fig. 1). Bootstrap values are provided above the branch. The ITS analyses further resolved a moderately supported (86% BS) relationship between the Vietnam sample (*T123*) and one sample from Thailand (*M3366*). The closest inferred relatives from the sampling utilised here were members of the *Boesenbergia longiflora* clade, but that relationship was only weakly supported (BS 68%). This contrasts with results of the *trnK* analyses, where *Boesenbergia albomaculata* S.Q.Tong and *B. pulcherrima* (Wall.) Kuntze were indicated as closest relatives, again with only weak support (74%).

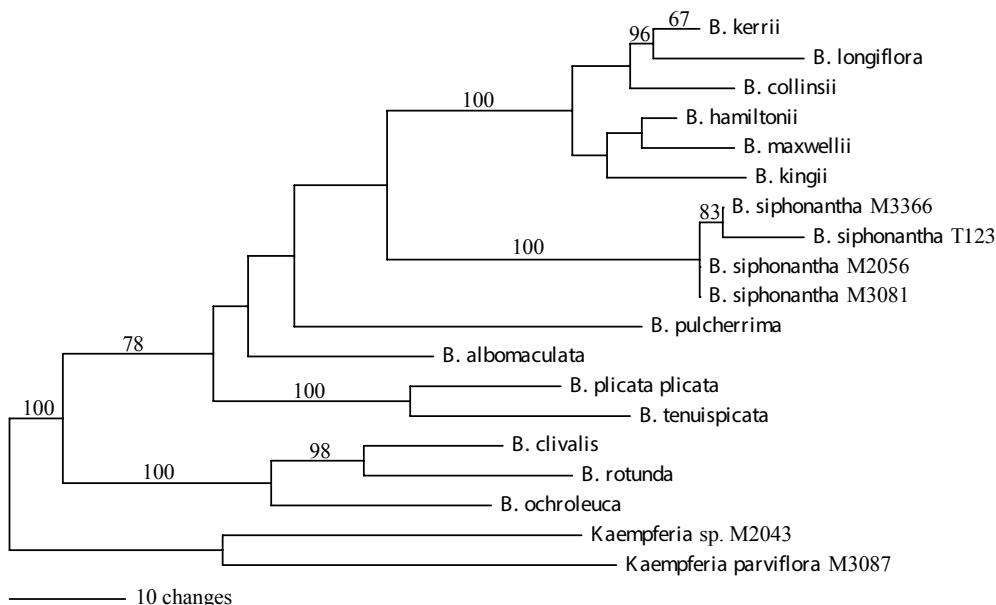


Fig. 1. Combined analysis of *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. using ITS and *trnK* sequence data. Bootstrap values are provided above the branch for those with > 50% support.

Taxonomy

Boesenbergia siphonantha (King ex Baker) M.Sabu, Prasanthk. & Škorničk., Rheedea 14: 55 (2004). — *Kaempferia siphonantha* King ex Baker in Hook.f., Fl. Brit. India 6: 222 (1890). — TYPE: India, Andaman Islands, 1884, *King's Collector* 372 (lectotype CAL [CAL0000000916]; isolectotypes CAL [CAL0000000912, CAL0000000913], K [K000640517]), first step designation by Sabu et al. (2004), second step here. (Figs. 2–6)

Deciduous, perennial herb 20–60 cm tall, tightly clumping. **Rhizome** with multiple elements, development in various directions forming a clonal mat, subterranean, globular, 1.2–1.5 cm diam., cream-yellow inside, faintly aromatic, bitter; primary roots few, fleshy, conical, elongate, to 12 cm long, 1 cm. diam. at the top, white, surface smooth, short root hairs full length, the terminus swollen, spherical to ovoid, 1–2.5 cm long, 0.8–2.2 cm diam., white, short root hairs the full length, many fibrous roots from the rhizome to c. 17 cm long. **Stems** c. 5 cm long, c. 1.1 cm diam., oval in cross section, leafless sheaths 1–2, deeply corrugate, green or purple-red. **Leaves** 4–7 per stem; *leaf sheaths* 10–21 cm long, finely ribbed with white blotches, many scattered hairs, dark green, margin brown; *ligule* bilobed, lobes linear to rounded, 1–6 mm long, light green or translucent turning pale brown, glabrous or with few hairs; *petiole* 3–12 cm, ribbed, green, glabrous; *lamina* oblong-lanceolate or ovate 10–26 × 4–12 cm, base cordate, sometimes oblique, apex acute or acute-acuminate, veins 3–7 mm apart, adaxially dark green, glabrous, abaxially lighter green or purple, glabrous or slightly pubescent. **Inflorescence** radical and terminal, the radical inflorescence attached to a rhizome near the stem base, the terminal inflorescence between the leaf sheaths, sometimes shortly exserted, flowering sequence basipetalous; *peduncle* 1–3 cm long, narrow, slender, branched or unbranched; *spike* cylindrical, 4–6 cm long, c. 0.4 cm diam., base white, apex dark purple-brown, ribbed; *bracts* 2–8, distichous, ovate-lanceolate to linear, 3.5–5 × 0.5–1 cm, white, green or green with maroon tinge at the apex, glabrous, overlapping equally on both sides of the rachis; *bracteole* tubular, 1–2.9 cm long, c. 0.1 cm diam., glabrous or pubescent, white or cream, translucent, apex 2 or 3-toothed, laterally split 1–3.6 mm. **Flowers** 2–8 per inflorescence, one per bract, 7–12 cm long, erect; *calyx* tubular, c. 6 × 1.5 mm, translucent white or white with tiny maroon dots, apex tri-dentate, c. 3 mm longitudinal slit; *floral tube* cylindrical, 6.5–9 cm long, c. 2 mm diam., white, glabrous; *dorsal corolla lobe* triangular to lanceolate, cymbiform, 1.2–1.3 × 0.4–0.6 cm, sometimes mucronate, cream, glabrous; *lateral corolla lobes* linear, c. 1.3 × 0.4 cm, cream, glabrous, veins translucent, margin translucent, all lobes deflexed 180° away from the labellum at maturity; *androecial tube* 4–6 mm long, c. 4 mm wide, throat with few hairs; *labellum* deeply saccate, ovate to obovate (in live plants), 2 × 0.5–1.7 cm, cream-white, yellowing with age, throat and medial line bright red, maculate, colour broadening outward, changing in last third to dark pink, maturing dark purple or distal half violet with reddish bands toward the centre, externally covered in short glandular hairs, margin slightly undulate, sometimes crumpled, apex irregular, emarginate; *lateral staminodes* obovate



Fig. 2. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. **A.** Flowering plant. **B.** Flower. **C.** Flower dissection. **D.** Seed with aril. (Photos: J. Leong-Škorničkova of an ex situ plant from the Andaman Islands)

to oblong, $0.8\text{--}3 \times 0.4\text{--}1$ cm, closely appressed with the labellum towards the base, cream-white, dorsally covered with short, glandular hairs or glabrous, apex revolute. **Stamen** c. 7 mm long; filament 1–4 mm long, white, glabrous; anther elliptical, pale yellow, darker towards the tip, glabrous, thecae $5\text{--}6 \times 3\text{--}3.7$ mm (each) at opening, $5\text{--}6 \times 4\text{--}4.7$ mm at maturity, rotating away from labellum 90° , dehiscent full length,



Fig. 3. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. from Kanchanaburi, Thailand. **A.** Plants in situ. **B.** Flower on a radical inflorescence. **C.** First day flower. **D.** New flower on a terminal inflorescence. From J. Mood & P. Chalermglin M2056. (Photos: J. Mood)

white, light brown at senescence, connective white, anther crest absent, pollen white. *Ovary* trilocular, 3–6 × 2 mm, placentation axile, white, glabrous, shiny; *style* filiform, to 7.5 cm long, white; *stigma* white, ostiole ciliate; *epigynous glands* linear, two, 4–4.5 mm long, yellowish or pale cream, apex acute. *Fruit* a capsule, elliptical, c. 1 cm long, trilocular, white with red spots throughout, darker on the interior, dehiscence

loculicidal, valves rolling outward into coils; seed ellipsoidal, brown, hirsute, apex yellowish, funicus present, yellowish, aril medusa-form, yellow-white, translucent. (Note: This description is based on the protologue and collections from Thailand and Vietnam.)

Distribution & Ecology. Andaman Islands: Moist, deciduous and inland evergreen forests on humus rich soil, 5–45 m elevation. Thailand: On and around limestone outcrops in deciduous, secondary forest with bamboo, medium to heavy shade at c. 70–800 m elevation. Vietnam: Deciduous *Lagerstroemia* L. forest on lateritic rocks.

Phenology. See Table 1.

Additional specimens examined. THAILAND: **Mae Hong Son:** Baan Bo Khai, 800 m, 12 Sep 1999, Srisanga, P. & Puff, C. 1067 (QBG); Baan Bo Khai, 800 m, 12 Sep 1999, Srisanga, P. & Puff, C. 1069 (QBG); **Tak:** N. of Ban Ta Song Yang, 17°32.691' N 97°56.291' E, 180 m, 28 Jul

Table 1. Comparison of *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. from three geographical areas

Character	India (Sabu et al. 2004)	Thailand (M2056)	Vietnam (Trần 123)
Plant height	20–30 cm	50–60 cm	c. 30 cm
Petiole length	3–6 cm	10–12 cm	4–6 cm
Lamina dimensions	10–15 × 4–7 cm	23–26 × 10–12 cm	12–15 × 8 cm
Inflorescence (radical)	Branched	Unbranched	Unbranched
Inflorescence (terminal)	Unbranched	Unbranched	Unbranched
Flowers per inflorescence	4–8	2–5	2 or 3
Flower length	10.5–12 cm	7–10.5 cm	c. 9.5 cm
Labellum	c. 20 × 5–17 mm	c. 21 × 11 mm	c. 20 × 14 mm
Flowers per day	Several	One	One
Floral display position	Above the lamina	Below the lamina	Intermediate
Phenology	February–August	July–August	July–August

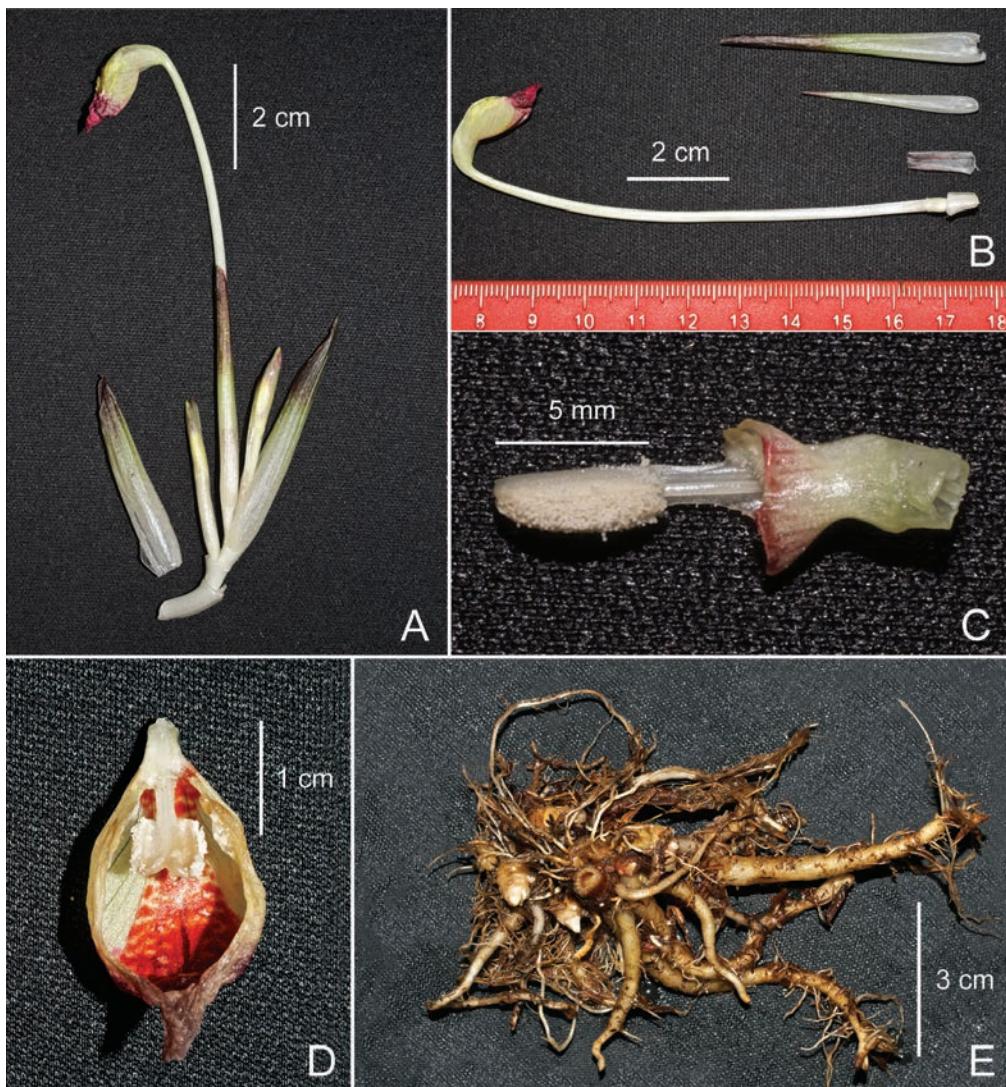


Fig. 4. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. from Kanchanaburi, Thailand. **A.** Inflorescence opened. **B.** Flower with calyx, bracteole and bract removed. **C.** Stamen with staminodal cup at base. **D.** Senesced flower with thecae rotated 180°. **E.** Rhizome with storage roots. From J. Mood & P. Chalermglin M2056. (Photos: J. Mood)

2013, Mood, J. & Vatcharakorn, P. 3366 (BKF). **Kanchanaburi:** Sai Yok, Wangkamen, 200 m, 29 Jan 2000, Phengklai et al. 14098 (BKF); W of Chong Sadao 20 km, 14° 12.626' N, 99° 02.925' E, 70 m, 21 Aug, 2011, Mood, J. & Vatcharakorn, P. 3081 (BKF).

VIETNAM: Đồng Nai: Cát Tiên National Park, August 2008, Trần et al. 123.

Note: A Huk, A. s.n. collection from northern Myanmar, Jul 1892 (CAL) could also be this species.

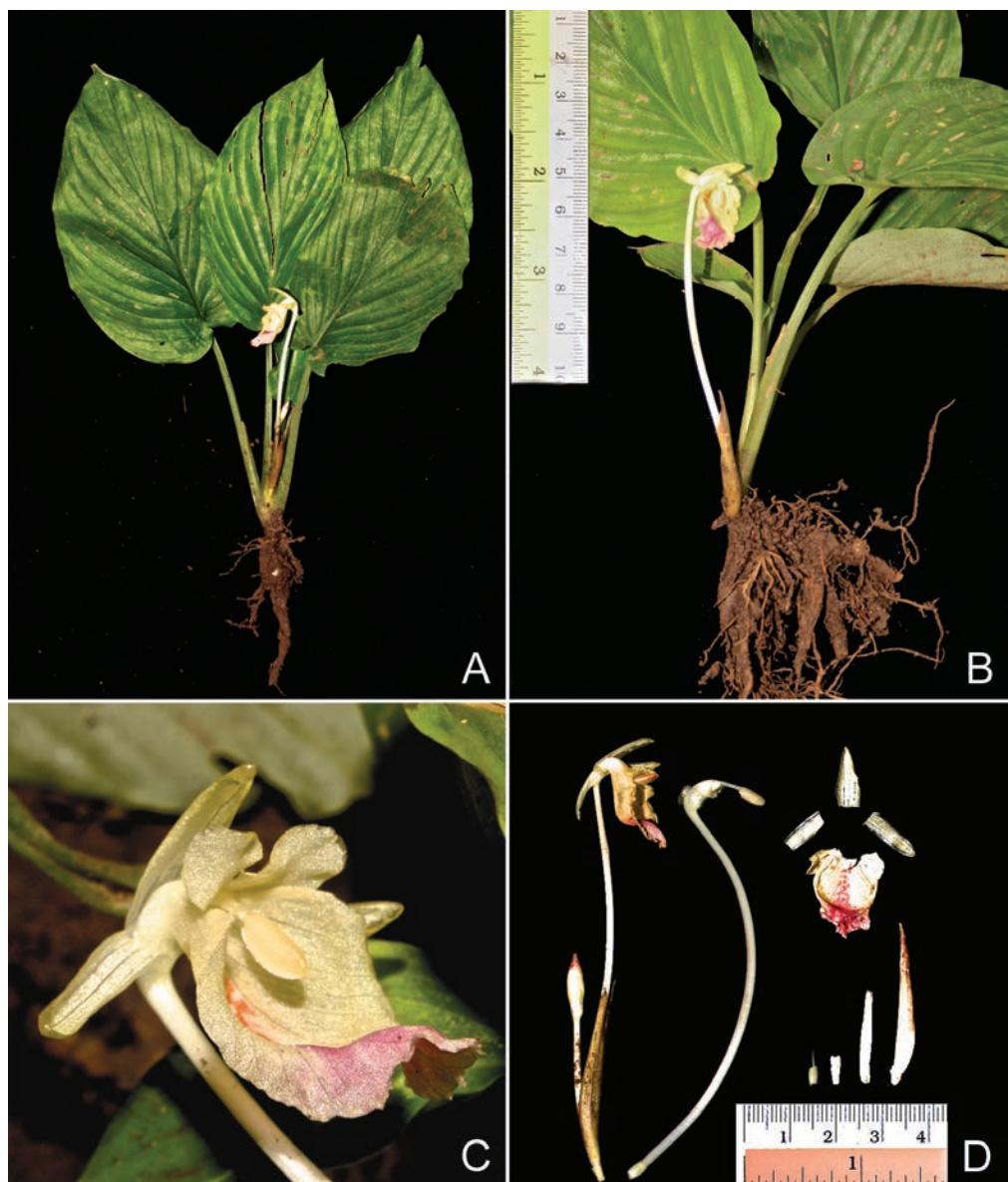


Fig. 5. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. from Đồng Nai Province, Vietnam. **A.** Mature plant with terminal inflorescence. **B.** Mature plant with radical inflorescence. **C.** First day flower. **D.** Dissected inflorescence and flower (Scale in cm). From Trần et al. 123. (Photos: H.D. Trần)

Discussion

The Andaman Islands were physically connected to the Southeast Asian mainland along the Arakan Yoma ridge in southwestern Myanmar at the end of the Pleistocene



Fig. 6. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk. Ink line drawing with watercolour by Linda Ann Vorobik (2015).

c. 10,000 years ago (www.worldwildlife.org/ecoregions/im0101). This land bridge occurred due to ocean levels being c. 100 m lower than at present. Although the duration of the connection is unknown, it was long enough to allow dispersal of species to and from the islands, to the point that the present-day flora of the Andamans has 72% of its species in common with the mainland. Most of the remaining species are also found in the adjacent Nicobar Islands which have a greater species similarity to Peninsular Malaysia and Sumatra. (www.worldwildlife.org/ecoregions/im0101). It is not known which direction *Boesenbergia siphonantha* spread during this era of low sea levels but, based on its close phylogenetic relationship to the *B. longiflora* clade of six continental species, most likely its origin is also continental. The morphological variations observed among the country populations show considerable plasticity in vegetative form, floristic habit and ecology.

Vegetatively, these differences are not unlike other widely distributed *Boesenbergia* species such as *B. kingii* Mood & L.M.Prince. Floristically, the Andaman populations are very showy with multiple flowers displayed well above the leaves. In contrast, the continental plants normally only produce one flower at a time, hidden down amongst the leaves. This

significant variation suggests very different pollination strategies which are yet to be investigated. Finally, although the ecology among country populations varies considerably, it has been shown from ex situ cultivation experiments in India using Andaman plants (Kumar et al., 2010) and in Thailand with Thai plants (pers. obs.), that this species is quite adaptable to a variety of environmental regimes.

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Appendix 1: GenBank accession numbers for Zingiberaceae used in this study. Identification: plant sample number [for newly sequenced samples] (collector and voucher number, herbarium), ITS GenBank number/trnK GenBank number.

Boesenbergia albomaculata S.Q. Tong: M11C83 (Murata, J. et al. 20050595K, MBK) KU159317/KU159408. *Boesenbergia clivalis* (Ridl.) Schltr.: M11C127 (Lim, C.K. s.n., UPM) KU159398/KU159409. *Boesenbergia collinsii* Mood & L.M.Prince: Mood, J. 12P171 (BK) JX992751/JX992812. *Boesenbergia kerrii* Mood, L.M.Prince & Triboun: Mood, J. & Triboun, P. 12P170 (BK) JX992756/JX992817. *Boesenbergia hamiltonii* Mood, S. Dey, & L.M. Prince: Dey NU53 (CAL) JX992754/JX992815. *Boesenbergia kingii* Mood & L.M.Prince: Mood, J. & Vatcharakorn, P. 12P173 (BK) c2-JX992789, c3-JX992790, c4-JX992791, cJX992792, c8-JX992793/JX992829. *Boesenbergia maxwellii* Mood, L.M.Prince & Triboun: Mood, J. & Triboun, P. 12P172 (BK) JX992800, JX992833. *Boesenbergia ochroleuca* (Ridl.) Schltr.: M3116 (Mood, J. & Vatcharakorn, P. 3116, BKF) KU159399/KU159410. *Boesenbergia plicata* var. *plicata* (Ridl.) Holttum: Mood, J. & Vatcharakorn, P. 3177 (BKF) JX992807/JX992840. *Boesenbergia pulcherrima* (Wall.) Kuntze: Mood, J. 08P276 (BKF) JX992748/JX992809. *Boesenbergia rotunda* (L.) Mansf.: M11P27 (Mood, J. 11P27, BKF) KU159400/KU159411. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk.: M2056 (Mood, J. & Chalermglin, P. 2056, BKF) KU159401/KU159412. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk.: M3081 (Mood, J. & Vatcharakorn, P. 3081, BKF) KU159402/KU159413. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk.: M3366 (Mood, J. & Vatcharakorn, P. 3366, BKF) KU159403/KU159414. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk.: Trân & al. 123 (SING, VNM) KU159404/KU159415. *Boesenbergia siphonantha* (King ex Baker) M.Sabu, Prasanthk. & Škorničk.: Sabu, M. 73-1(CALI) Not registered in GenBank. *Boesenbergia tenuispicata* K. Larsen: M3292 (Mood, J. 3292, BKF) KU159405/KU159416. *Kaempferia* sp.: M2043 (Mood, J. 2043, BKF) KU159395/KU159406. *Kaempferia parviflora* Wall. ex Baker: M3087 (Mood, J. 3087, BKF) KU159396/KU159407.

