

Three new species of *Boesenbergia* (Zingiberaceae) from Thailand and Lao P.D.R.

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ABSTRACT. *Boesenbergia bella* Mood & L.M.Prince, *B. phengklaii* Mood & Suksathan, and *B. putiana* Mood & L.M.Prince are described with photographs and a comparative table. The description of *Boesenbergia petiolata* Sirirugsa is revised to include morphology not previously noted. Molecular phylogenetic analyses of the relevant taxa using plastid and nuclear DNA sequence data are provided.

Keywords. *Boesenbergia bella*, *B. petiolata*, *B. phengklaii*, *B. putiana*, chromosome counts, nrITS, molecular phylogeny, trnK, Vietnam

Introduction

Beginning in 2010, a taxonomic update of *Boesenbergia* (Zingiberaceae) was initiated for the Flora of Thailand project. The research model was based on a species by species review of those currently recorded in Thailand. The study of each included the protologue, types and all similar specimens, history, field and ex situ data, results of molecular phylogenetic analyses, and photography. The first to be studied was *Boesenbergia longiflora* (Wall.) Kuntze which resulted in the description of five new species (Mood et al., 2013). Since then, several other species have been reviewed and published (Mood et al., 2014a,b, 2016a,b). In this study, *Boesenbergia petiolata* Sirirugsa is reviewed.

The first known specimen of this species, *Pierre s.n. P02203112* (P), was collected in Cochinchine (Vietnam) by the French botanist, Jean Baptiste Louis Pierre (1833–1905), Director of the Jardin Botanique in Saigon from 1865–1877. Sometime during his tenure, this small, petite plant was found and later deposited at the Muséum National d'Histore Naturelle in Paris without annotation or date. In 1917, a similar ginger with well-preserved floral parts was collected in Già Rai, Bạc Liêu Province, Cochinchine, *Chevalier 36.458* (P). The first recorded specimen in Thailand was from

Saraburi Province in 1928, *Phraipurind 2419* (BK), but it was not until 1987 that this and similar specimens were studied in detail and recognised as being of an undescribed species (Sirirugsa, 1987). The specimen selected as the type, *Maxwell 74-631* (holotype BK; isotype L), was collected in 1974 at Sahm Lan (now Namtok Sam Lan National Park), Saraburi Province, central Thailand. In addition to the types, approximately 26 other collections have been found that were either annotated as *Boesenbergia petiolata* or were unidentified but were similar to *B. petiolata*.

Over the course of field work (2013–2015), locations in northern, central and eastern parts of Thailand were visited which corresponded to areas documented on the specimens. Many of the original locations were difficult to locate and some had been lost through development. In total, eight locations were visited that provided sufficient materials for study. In addition to these, two living accessions were added from the Lao P.D.R.

On comparing these ex situ living collections over several years the following observations were made: the first annual growth, flowering cycle, and die-back into dormancy were not calendar concurrent for all nine collections; the vegetative growth habit varied from tightly clumping (tufted) plants to ones with wider-spaced stems; and the leaf and floral characters were not uniform. On the other hand, similarities included: inflorescence shape/size and the same combination of colours on the labellum to include a yellowish tint (especially during senescence).

From the field and ex situ observations, it became clear that six of the ten collections differed considerably from the type of *Boesenbergia petiolata* and were most likely of three distinct, undescribed species. Molecular phylogenetic analyses were carried out to investigate evolutionary relationships among these accessions.

Materials and methods

Herbarium specimens, silica-dried leaf samples, and living plant materials (rhizomes) were collected on site from 2012–2015. Living materials were grown in Hawaii, USA, for comparison and long-term observation. A few collections were also grown at Queen Sirikit Botanic Gardens, Thailand. Ecological data were recorded at each site. Photographic documentation of plants and flowers was accomplished both in the field and ex situ. The *Boesenbergia petiolata* type material along with specimens with similar morphology were studied and compared at BK, BKF, QBG and SING. High resolution digital photos were taken of each specimen for later reference. Photographs from earlier visits to AAU, C, CMU, E, K, L, P, PNU, and VNM were also used for comparison.

Molecular phylogeny

Leaf tissue samples were obtained from living materials in the field, or in the case of *M96P82* and *M3471* from ex situ plants. Selected GenBank sequences deposited as part of previous studies by our research group were combined with data from the current study. A list of included samples (with associated GenBank accession numbers) is provided in Appendix 1.

Methods are consistent with prior publications from our research group. DNA extraction from silica-dried samples was done using the CTAB extraction protocol (Kress et al., 2002). DNA amplification, sequencing, and analytical methods follow Mood et al. (2013). The nuclear ribosomal ITS (nrITS) region was amplified using the plant specific primers developed by Prince (2010): 18S-F (CGATTGAATGGTCCGGTGAAG) and 26S-R (AGGACGCTTCTACAGACTACAA). The plastid *trnK* region was amplified in two parts, the first using either 1F (CTCAACGGTAGAGTACTCG; Manos & Steele, 1997) and m1R (CGTTTCACAAGTACTGAACTA) or 1F and 1235R (TATACTAATACCCACACYCC). The second half was amplified using m1F (GTTTCAGTACTTGTGAAACGTT; Kress et al., 2002) and 2R (AACTAGTCGGATGGAGTAG; Steele & Vilgalys, 1994). Amplicons were bidirectionally sequenced on an ABI Genetic Analyzer at The Field Museum (Chicago, Illinois, USA), and verified and edited in Sequencher v4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA). The resulting contigs were aligned in Se-AL (Rambaut, 1996) and exported as Nexus files.

Phylogenetic analyses were conducted under both maximum parsimony and likelihood criteria in PAUP* (version 4.0b10; Swofford, 2002). Exploratory heuristic searches of the family-wide data matrices were limited to maximum parsimony to assess monophyly of the ingroup. Parameters for these exploratory analyses were 10,000 random addition replicates, saving 10 trees per replicate. Similarly, exploratory bootstrap analyses of the large data matrix were limited to 100,000 fast swap replicates. Alignment of sequences across the family were challenging for the ITS region. No data was excluded and gaps were treated as missing data.

After monophyly was confirmed, most of the outgroup taxa and many of the non-ingroup *Boesenbergia* taxa were removed, the data realigned, and more elaborate heuristic search methods were employed (maximum parsimony with 1000 random addition replicates, saving all shortest trees; maximum likelihood with 10 random addition replicates). No data were excluded and gaps were treated as missing data here as well. Likelihood model selection was conducted in ModelTest 3.7 (Posada & Crandall, 1998). If the Hierarchical Likelihood Ratio Test (hLRT; Felsenstein, 1981) and the Akaike Information Criterion (AIC; Akaike, 1974) differed in the model selected, analyses were run under each model. Branch support was estimated using heuristic search parameters in a parsimony bootstrap (BS; Felsenstein, 1985) in PAUP* and posterior probabilities (PP) via Bayesian analysis in MrBayes 3.2 (Ronquist et al., 2011; conducted via the CIPRES portal, Miller et al., 2010). Bootstrap analyses used 1000 random addition replicates with 100 pseudoreplicates (hold 1 tree, TBR branch swapping, save 10 trees per replicate). Bayesian analyses ran for 5 million generations and were repeated three times, burn-in set to <0.01 standard deviation of the splits frequencies. Results from all three runs were used to generate posterior probabilities. Only posterior probabilities ≥ 0.95 are shown. Parsimony analysis, likelihood model parameter selection and analysis results, and Bayesian run details are provided in Appendix 2.

Chromosome preparations and counting.

Actively growing, young roots were harvested from cultivated plants, and subsequently pre-treated with ice-cold water for 12 hours, fixed in ethanol/acetic acid (3:1, v/v) fixative for 24 hours at 4°C and stored at -20°C until further use. Selected root tips were rinsed in distilled water (twice for 5 minutes) and citrate buffer (10 mM sodium citrate, pH 4.8; twice for 5 minutes), and digested in 0.3% (w/v) cellulase, cytohelicase and pectolyase (all Sigma-Aldrich, St Louis, MO, USA) in citrate buffer at 37°C for 90 minutes. After digestion, individual root tips were dissected on a microscope slide in approximately 10 µl acetic acid and covered with a cover slip. The cell material was then spread evenly using tapping, thumb pressing and gentle flame-heating. Finally, the slide was quick frozen in liquid nitrogen and the cover slip flicked off with a razor blade. Slides were fixed in ethanol-acetic acid (3:1) and air-dried. Chromosomes were counterstained with 2 µg/ml DAPI in Vectashield (Vector Laboratories, Peterborough, UK). Preparations were analysed and photographed using a Zeiss Axioimager Z2 epifluorescence microscope with a CoolCube camera (MetaSystems). At least 20 mitotic chromosome spreads were counted from each accession analysed.

Results and discussion

Exploratory genus-wide analyses of ITS sequences identified a modestly-supported *Boesenbergia petiolata sensu lato* clade (73% fast BS; results not shown) containing nine ingroup sequences. The *trnK* data analyses identified a well-supported *Boesenbergia petiolata sensu lato* clade (with 99% fast BS; results not shown), but not all accessions were included in the supported clade. A combined ITS + *trnK* analysis however identified a ten sample, monophyletic *Boesenbergia petiolata sensu lato* clade with modest support (89% fast BS; results not shown).

Parsimony and likelihood analyses of sequences in the target clade (plus a group of place holder samples) resulted in trees of similar in group topology. Phylograms from analyses of the ITS matrix alone (Appendix 3) identified a monophyletic *Boesenbergia petiolata* clade (84% BS, 1.00 PP), with resolution of three internal clades, a *B. petiolata sensu stricto* clade (100% BS, 1.00 PP), a novel clade A (63% BS, 0.78 PP), and a novel clade B (99% BS, 1.00 PP). Results of the *trnK* matrix alone (Appendix 3) identified a monophyletic *Boesenbergia petiolata sensu stricto* clade (99% BS, 1.00 PP) plus a monophyletic clade of morphologically similar accessions, but generally provided lower branch support and less resolution.

Phylogenies from the combined ITS + *trnK* analyses (Fig. 1) were fully resolved and well supported, regardless of analytical method. Topologies within the ingroup were similar across all trees. The best likelihood tree (AIC criterion) is shown in Figure 1 with parsimony BS and Bayesian PP branch values indicated. For marker-specific phylograms of ITS and *trnK* per analysis method see Appendix 3. A well supported (91% BS, 1.00 PP) *Boesenbergia petiolata sensu lato* clade was identified, including a monophyletic *B. petiolata sensu stricto* (100% BS, 1.00 PP), the novel clade A (87% BS, 1.00 PP), and the novel clade B (99% BS, 1.00 PP). At the base of clades A and B (99% BS, 1.00 PP), a clade C, consisting of one accession, is sister to A and B.

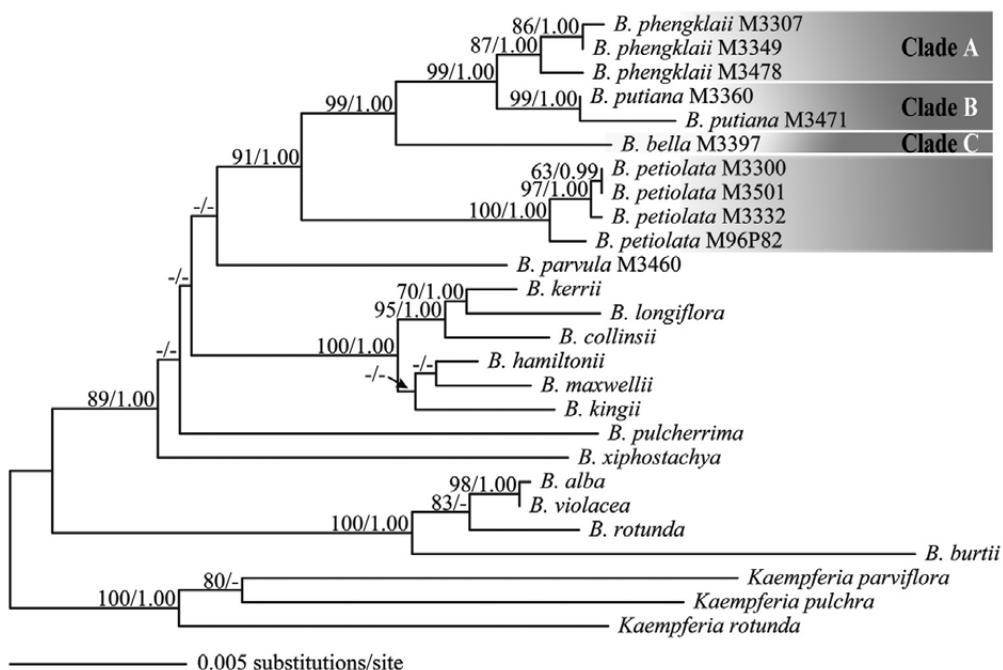


Fig. 1. Maximum likelihood phylogram of combined ITS and *trnK* data analysis for *Boesenbergia petiolata* and closely related taxa based on the General Time Reversible (GTR+I+G) model. Branch support values are BS/PP. See text for details.

Based on the results of the molecular phylogeny a reassessment of the morphological characters of all *Boesenbergia petiolata* collections was made. This study confirmed the presence of a morphologically consistent monophyletic *Boesenbergia petiolata sensu stricto* clade as well as a clade of species related to *Boesenbergia petiolata* that are each morphologically coherent and can be distinguished from other *Boesenbergia* clades by their flower shape and coloration. As a result, three new species are described along with an updated circumscription of *Boesenbergia petiolata*. The new species are *Boesenbergia bella* Mood & L.M.Prince, *B. phengklaii* Mood & Suksathan and *B. putiana* Mood & L.M.Prince. *Boesenbergia phengklaii* Mood & Suksathan corresponds to clade A, *B. putiana* Mood & L.M.Prince to clade B and *B. bella* Mood & L.M.Prince to clade C (Fig. 1).

Taxonomy

Three new species are described along with an updated circumscription of *Boesenbergia petiolata*. A comparison of morphological characters for the four species is presented in Table 1.

Table 1. Comparison of *B. petiolata* to the three newly described species.

Character/ Species	<i>Boesenbergia bella</i>	<i>Boesenbergia petiolata</i>	<i>Boesenbergia phengklaui</i>	<i>Boesenbergia putiana</i>
Type locality	Lao P.D.R.	Thailand	Thailand	Thailand
Soil derivation	Sandstone	Sandstone/Granite	Sandstone/Lime- stone	Shale/Limestone
Forest type	Deciduous	Deciduous	Deciduous	Semi-deciduous
Elevation	c. 250 m	50–700 m	300–700 m	c. 300 m
Growth habit	Clumping	Spreading	Spreading	Spreading
Plant height incl. leaves	to 40 cm	to 50 cm	to 55 cm	to 60 cm
Sheaths	Red, white spotted/ corrugate/ pubescent	Green or reddish/ striate/glabrous	Green, red spotted, or solid red/striate/ glabrous	Light green/ corrugate/ glabrous
Ligule	0.5 cm/apex round	1.5 cm/apex triangular	to 0.8 cm/apex round	to 1 cm/apex triangular
Lamina size	5.5–14 × 13–25 cm	4.5–8 × 18–23 cm	5–8 × 12–22 cm	9–14 × 15–27 cm
Lamina colour Dorsal/ Ventral	Tri-coloured/ wine red	Light to dark green/silvery green or purple	Dark green/ silvery green or purple	Dark green/ silvery green
No. of bracts	14–18	6–14	13–16	c. 12
Labellum shape/ flattened size	2.2 × 2.0 cm	2.5–2.8 × 2.0–2.5 cm	2.8 × 2.5 cm	3.2 × 2.6 cm
Labellum colour & pattern	White, spotted red throat, red streaked margin, apex violet	White, spotted red, throat to apex, margins yellow, apex red spots	White, spotted throat, margins mostly white, apex solid red	White, streaked red throat, margins light yellow, apex striped
Asexual propagules	Bulbils (ex situ)	None observed	Bulbils (ex situ)	None observed

Boesenbergia bella Mood & L.M.Prince, **sp. nov.**

Boesenbergiae petiolatae (Wall.) Kuntze *affinis*, *foliis* c. 6 cm *latis multicoloratis*, *labelli apice violaceo bilobato differt*. TYPE: Lao P.D.R., north of Pakse, in hills east of 15°22.236'N 105°48.789'E, c. 248 m asl, collected as *Paprataro s.n.*, cultivated in Hawaii, USA as *J.D. Mood 3397*, vouchered on 1 October 2016 as *J.D. Mood 16P07* and voucher selected as type (holotype BKF; isotype AAU). (Fig. 2, 6–8)

Deciduous, perennial herb up to 40 cm tall, multiple stems, c. 1 cm apart. **Rhizome** branched, length variable to 10 cm, c. 0.5 cm diam., externally orange-brown, internally light orange, tuberous roots numerous, to 10 cm long, c. 0.5 cm diam., externally and internally white, persistent and enlarging over several years, apex tapered, surface with numerous short roots. **Stems** 10–15 per clump, to 10 cm long, c. 1 cm diam., white, few hairs, striate, leafless sheaths 1–2, c. 8 cm long, to 3 cm wide (flattened), light red, greenish toward the apex, mostly glabrous, few hairs on margins, corrugate, green spots between the ridges, apex rounded, minutely unguiculate. **Leaves** 2–3, upper two nearly opposite; *leaf sheaths* 6–8 cm long, 2–3 cm wide at the base (flattened), corrugate, red throughout with white spots, pubescent, short white hairs between the ridges; *ligule* bilobed, auriculate, each lobe to 0.5 cm long, semi-translucent, red spotted, with long white hairs throughout, prominent red veins, apex acute, cucullate, margins white; *petiole* c. 4 cm long, c. 0.5 cm wide, green, red spotted, with scattered few hairs, ridged, channelled; *lamina* ovate to elliptical, 13–25 × 5.5–14 cm, base rounded to acute, oblique, apex shortly acuminate, adaxially tri-coloured, midrib dark red, then a dark green, feathered area either side with two, wide, silver bands, margins dark green, glabrous, primary veins raised, reticulate between the primary and secondary veins, abaxially dark burgundy red, iridescent, glabrous, midrib green, veins sunken. **Inflorescence** terminal on a leafy stem, basal portion clasped between the leaf sheaths, ¼ to ½ exposed; *peduncle* to 0.5 cm long, 0.8 cm diam., white, pubescent, ridged; *spike* to c. 11 cm long, c. 1.2 cm wide; *bracts* 14–18, lanceolate, to 4.5 cm long, to 1 cm wide at the base (flattened), white, numerous long white hairs, apex acute, bracts distichously arranged, compressed together in two ranks on one side, the rachis exposed on the non-flowering side, rachis 0.8 cm wide, light green, glabrous; bracteole ovate, to 4.7 cm long, to 1.5 cm (flattened), larger than the bract, white, glabrous, apex falsely pungent. **Flowers** c. 4.5 cm long, all facing outward from the bract; *calyx* tubular, c. 0.8 cm long, c. 0.3 cm diam., translucent white, sparse hairs, apex bifid, with unilateral incision; floral tube c. 2.5 cm long, 0.2 cm diam., white, glabrous; *dorsal corolla lobe* oblong, c. 1.6 × 0.5 cm, white, glabrous, three-veined, apex slightly cucullate, sometimes revolute; *lateral corolla lobes* oblong, c. 1.4 × 0.4 cm, white, glabrous, three-veined, apex slightly cucullate; androecial cup c. 0.2 × 0.2 cm, initially oriented c. 45° to the floral tube on opening, throat with few hairs either side; *labellum* saccate, nearly orbicular (flattened), 2.2 × 2.0 cm, white, midrib marked with red spots to ½ the length, then darker red bands spreading outward ¼ length, then a broad violet band to the apex, margin white or light yellow, turning darker yellow toward maturity, crisped, crenulate, exterior glabrous; *lateral staminodes* ovate, c. 1 × 0.5 cm, white, turning slightly yellow at maturity, internally and externally glabrous, apices acute, slightly

revolute. **Stamen** c. 1 cm long, filament c. 0.3×0.1 cm, channelled, white, anther c. 0.8 cm, white, thecae c. 1 mm wide when mature, dehiscent the full length, connective tissue with glandular hairs, apex truncate; pollen white. **Ovary** trilocular, style filiform, c. 4 cm long, white, stigma funnel-shaped, white, ostiole rounded, margin tri-lobed, non-ciliate; epigynous glands 2×1 mm, yellowish, segmented in two sections, top half diameter smaller than lower half. **Fruit** not seen. **Bulbils** cylindrical, c. 2×0.5 cm, green, sericeous, occurring at the base of the inflorescence.

Distribution. Known only from Champasak Province, Lao P.D.R.

Ecology. Found in lightly shaded locations in deciduous forest on sandstone derived soils rich in organic matter. Associated species: *Boesenbergia rotunda* (L.) Mansf., *Gagnepainia* sp., *Kaempferia champasakensis* Pichens. & Koonterm, *K. gigantiphylla* Pichens. & Koonterm, and *Zingiber pellitum* Gagnep.

Phenology. In cultivation, flowering from July – mid-October.

Etymology. Named for its beauty.

Chromosome count. $2n=18$.

Notes. This species came to the first author's attention at Chatuchak Market, Bangkok, Thailand where it was sold by a local Thai vendor. The small, tufted plant presented one of the most striking examples of a *Boesenbergia* ever seen. Later it was found to have been collected in the Lao P.D.R.

Boesenbergia petiolata Siriruga, Nordic J. Bot. 7: 423 (1987). TYPE: Thailand, Saraburi Province, Nam Tok Sam Lan National Park, Maxwell 74-631, 29 June 1974 (holotype BK; isotypes AAU, L). (Fig. 3, 6–8)

Deciduous, perennial herb to 50 cm tall, multiple stems, 1–5 cm apart. **Perennating organ** a rhizome, branched, expanding linearly, length variable, c. 1 cm diam., externally orange-brown, internally yellowish, tuberous roots numerous, persistent and enlarging over several years, 3–12 cm long, 0.5–0.8 cm diam., externally and internally white, apex tapered, surface with numerous short roots. **Stems** 10–15 per clump, to 25 cm long, 0.8–1.2 cm diam., light green to dark green, glabrous, leafless sheaths 0–1, c. 4 cm long, 1 cm wide (flattened), striate, green, glabrous, apex acute to rounded. **Leaves** 4–5, alternate, upper two nearly opposite; leaf sheaths 7–13 cm long, striate, green, glabrous; **ligule** bilobed, each lobe to 1.5 cm long, translucent white or pink, glabrous, veined, apex obtuse; **petiole** 2.5–8 cm long, 0.2–0.4 cm wide, green, glabrous; **lamina** ovate to lanceolate, asymmetrical, 8–23 \times 4.5–8 cm, base cordate, sometimes oblique, apex acuminate, adaxially light green to dark green, glossy, glabrous, primary veins raised, lightly reticulate, abaxially light silvery green, glabrous, veins sunken.

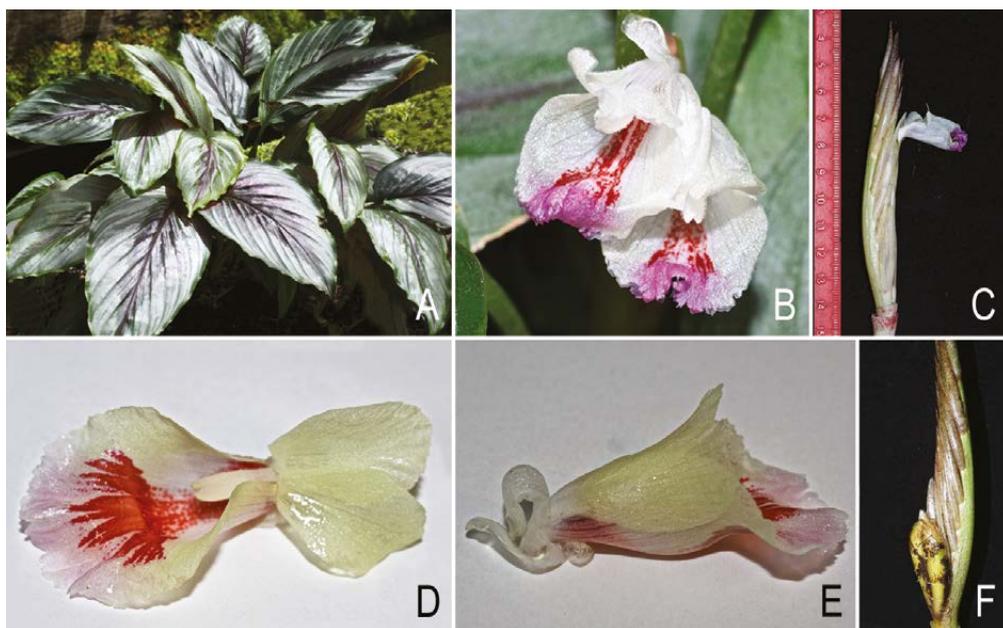


Fig. 2. *Boesenbergia bella* Mood & L.M.Prince. **A.** Plant ex situ. **B.** Flowers. **C.** Inflorescence. **D.** Opened young flower. **E.** Flower in profile. **F.** Bulbils at the inflorescence base. All from M3397. (Photos: J. Mood).

Inflorescence terminal, distichous, basal portion clasped between the leaf sheaths, $\frac{1}{4}$ to $\frac{1}{2}$ exposed; *peduncle* c. 1 cm long, 0.5 cm diam., green, glabrous; *spike* c. 12 cm long, c. 1 cm wide; *bracts* 6–14, lanceolate, to 4 cm long, to 0.8 cm wide at the base (flattened), white with light green in upper half, glabrous, apex falsely pungent, bracts compressed together in two ranks on one side, the rachis exposed on the non-flowering side; *bracteole* lanceolate, to 4 cm long, to 0.8 cm wide (flattened), identical to the bract, white with green toward apex, glabrous, apex falsely pungent. **Flowers** c. 4 cm long, all facing outward from the bract, tilted slightly downward; *calyx* tubular, c. 0.5 cm long, c. 0.3 cm diam., translucent white, glabrous, apex trifold; *floral tube* 2–2.8 cm long, 0.1 cm diam., white, glabrous; *dorsal corolla lobe* oblong to obovate, c. 1.3×0.5 cm, white, glabrous, three-veined, apex slightly cucullate, sometimes revolute; *lateral corolla lobes* oblong, c. 1×0.3 cm, white, glabrous, three-veined, apex slightly cucullate; *androecial cup* c. 0.2×0.2 cm, orientated c. 90° to the floral tube, throat glabrous; *labellum* deeply saccate, nearly orbicular (flattened), $2.5\text{--}2.8 \times 2.0\text{--}2.5$ cm, white, throat marked with two red spots, more red spots continue along all the veins outward, short of the apex, there longitudinal red stripes or spots in a broad patch reaching almost to the margins, margins slightly revolute, light yellow, becoming darker with age, slightly crisped, apex yellow, crisped, slightly crenulate, externally covered with a few, short, glandular hairs; *lateral staminodes* obovate, c. 1.3×0.9 cm, white, internally and externally glabrous, apices slightly revolute. **Stamen** c. 1 cm long, filament c. 4×0.2 cm, channelled, yellowish white, anther c. 0.8 cm long, white,

thecae c. 1 mm wide when mature, dehiscent the full length, dorsally covered with glandular hairs, apex slightly indented; pollen white. **Ovary** trilocular, style filiform, c. 3.8 cm long, white, stigma funnel-shaped, white, ostiole round, few cilia; epigynous glands 2 × 1 mm, yellowish. **Fruit** not seen.

Distribution. Central and eastern Thailand, Lao P.D.R., Vietnam.

Ecology. Semi-deciduous forest, normally on sandstone derived soils.

Phenology. In cultivation, flowering from July – mid-October.

Etymology. Named for its long petiole.

Chromosome count. 2n=18.

Additional specimens examined. LAO P.D.R. **Champasak:** exact location data lost, *Mood M96P82* (AAU, BKF).

THAILAND. **Uttaradit:** Nam Pat, Phu Soi Dao National Park, 680 m, 7 Aug 2013, *Newman 2620* (QBG). **Sukhothai:** Khao Luang, 13 Sep 1994, *Niyomdham 3850* (BKF); Ram Kham Haeng National Park, Khiri Mat, Khao Luang, 700 m, 11 Aug 2012, *Middleton et al. 5563* (BKF). **Phitsanulok:** Thung Salaeng Luang, 500 m, 19 Jul 1966, *Larsen 463* (AAU, BKF); Thung Salaeng Luang, 400 m, 24 Jul 1966, *Larsen 837* (AAU, BKF); Thung Salaeng Luang National Park, Bahn Mung, 100 m, 9 Aug 2008, *Sreepoowiang K44* (BKF, PNU); Thung Salaeng Luang, c. 250 m asl, Aug 2012, *Vatcharakorn s.n., M3300* (BKF); Thung Salaeng Luang, 245 m asl, 24 Jul 2013, *Mood & Vatcharakorn 3332* (BKF); Ban Chom Phu, Noen Maprang, 28 Jul 2001, *Maknoi 158* (QBG); Tham Pha Tha Phon Non-hunting Area, Noen Maprang, 7 Sep 2011, *Norsaengsri & Insea 8149* (QBG). **Khon Kaen:** Paphuang Cave, Phu Pha Man National Park, 460 m, 14 Aug 2012, *Norsaengsri 9883* (QBG); Phu Pha Man National Park, 488 m, 16 Aug 2012, *Norsaengsri 9971* (QBG). **Kamphaeng Phet:** Klong Lan Waterfall, 6 Aug 2003, *Maknoi 452* (QBG). **Sakon Nakhon:** Phu Phan National Park, 290 m, 31 Jul 1999, *Newman 950* (E). **Saraburi:** Hin Lap, 20 Aug 1929, *Put 2419* (BK); Kao Ngob, 21 Aug 1945, *Din 10* (BKF); Nam Tok Sam Lan, 76 m asl, 9 Aug 2015, *Mood & Harwood 3501* (BKF). **Nakhon Nayok:** Eastern part, Khao Yai National Park, 300 m, 10 Aug 1968, *Larsen 3252* (AAU, BKF). **Surat Thani:** Ban Thakhun Dist., Khlong Saeng Wildlife Sanctuary, 90 m, 6 Sept 2008, *Middleton et al. 4306* (E).

VIETNAM. No location or date, *Pierre s.n.* [P02203112] (P). **Bạc Liêu:** Giá Rai District, Sep 1917, *Chevalier 36458* (P). **Dak Nong:** Draysap Waterfall, 380 m asl, 27 Jul 2009, *Trần 161* (E, SING, VNMD).

Notes. The description above is based on *Boesenbergia petiolata sensu stricto* with thin, light green foliage, cordate leaf bases, and yellowish flowers with light red markings. The measurements are based on living material of *M3501* (type locality) and cultivated material of *M96P82* (Lao P.D.R.). It should be recognised that at several locations cited, especially in Phitsanulok Province (Chat Trakan, Noen Maprang, and Thung Salaeng Luang), the populations have slightly different character states. In these populations, the leaves are more robust with mostly rounded leaf bases, the abaxial leaf surfaces

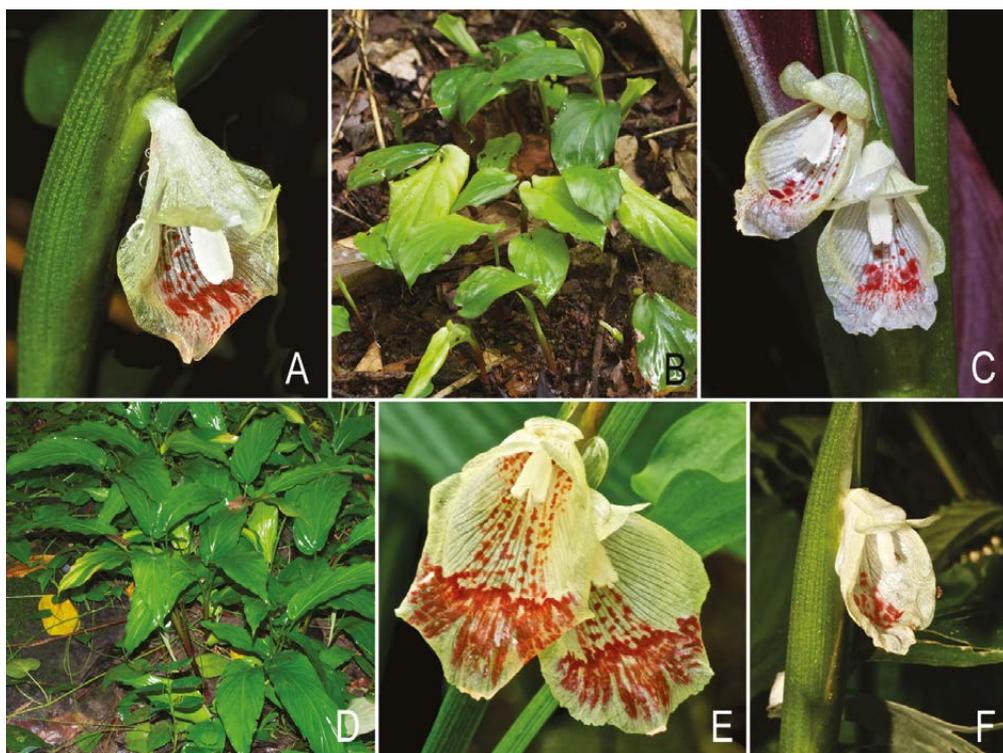


Fig. 3. *Boesenbergia petiolata* Sirirugsa. **A.** Flower at type locality. **B.** Young plants at type locality. **C.** Old and young flower showing abaxial leaf colour. **D.** Plants of *M3300*. **E.** Old and young flowers of *M96P82* (Lao P.D.R.) **F.** Flower of *Trần 161* (Vietnam). B from *M3501*; C & D from *M3300*; E from *M96P82*; F from *Trần 161*. (Photos: A, R. Pooma, BKF; B–E, J. Mood; F, H.Đ. Trần.)

have red pigmentation, the flowers have less yellow tinting overall, the labellum has a darker red centre, and the labellum apex has violet colouring (Fig. 2 C–D). Nonetheless, based on the molecular phylogenetic results, these samples (*M3300*, *M3332*) show a very close affinity to plants from the type locality as represented by *M3501*.

***Boesenbergia phengklaii* Mood, sp. nov.**

Boesenbergiae petiolatae (Wall.) Kuntze affinis, caulibus vaginisque maculose ad solide rubris, labello atrorubro apice violaceo differt. TYPE: Thailand, Chiang Mai Province, Doi Suthep foothills, 18°48.063'N 98°56.026'E, c. 635 m asl, collected as *Mood & Vatcharakorn 3349*, cultivated in Hawaii, USA as *M3349*, vouchered on 1 October 2016 as *Mood 16P08* and voucher selected as type (holotype BKF; isotype AAU). (Fig. 4, 6–8)

Deciduous, perennial herb to 55 cm tall, multiple stems, c. 1 cm apart. **Perennating organ** a rhizome, expanding linearly, length variable, 0.5–1.5 cm diam., externally

brown, internally white, tuberous roots few, persistent, to 14 cm long, c. 0.3 cm diam., externally and internally white, apex spheroid, c. 3 cm long, 1 cm diam., surface with few roots. **Stems** c. 15 per clump, to 30 cm long, c. 0.8 cm diam., green, glabrous, leafless sheaths 1–2, c. 7 cm long, striate, green spotted with red to a darker and solid red, glabrous, apex rounded to 0.8 cm wide. **Leaves** 4–5, alternate, upper two nearly opposite; *leaf sheaths* 14–15 cm long, striate, green or spotted red to solid red, glabrous; *ligule bilobed*, each lobe 0.2–0.8 cm long, translucent green or spotted red, glabrous, apex rounded; petiole 3–6 cm long, 0.2–0.3 cm wide, green or red spotted, glabrous; *lamina* ovate, asymmetrical, 12–22 × 5–8 cm, base slightly cordate, or rounded to attenuate, sometimes oblique, apex acuminate, adaxially dark, glossy green, glabrous, primary and secondary veins prominently raised, reticulate, abaxially light silvery green, sometimes dark reddish-purple, glabrous, completely covered with minute, round bumps, silky to touch. **Inflorescence** terminal on a leafy shoot, basal portion clasped between the leaf sheaths, $\frac{1}{4}$ to $\frac{1}{2}$ exposed; *peduncle* c. 1 cm long, 0.4 cm diam., white, glabrous; *spike* c. 9 cm long, c. 1 cm wide; *bracts* 13–16, lanceolate, 2.5–3.5 cm long, 0.3–0.4 cm wide at the base (flattened), white with light green in upper half, glabrous, covered with small gland-like markings, apex falsely pungent, bracts compressed together in two ranks on one side, the rachis exposed on the non-flowering side; *bracteole* lanceolate, 2.5–3.2 cm (flattened), slightly smaller than the bract, light green, glabrous, apex falsely pungent. **Flowers** c. 4.5 cm long, variable in size based on plant age, all facing outward from the bract, tilted slightly downward; *calyx* tubular, c. 0.6 cm long, translucent white, glabrous, apex bifid; floral tube c. 3.8 cm long, 0.2 cm diam., white, glabrous; *dorsal corolla lobe* ovate, c. 1.5 × 0.4 cm, white, glabrous, three-veined, apex slightly cucullate, sometimes revolute; *lateral corolla lobes* ovate, c. 1.7 × 0.3 cm, white, glabrous, three-veined, apex slightly cucullate, quickly revolute; *androecial cup* c. 0.2 × 0.2 cm, orientated c. 90° to the floral tube, throat glabrous; *labellum* deeply saccate, broadly elliptical (flattened), c. 2.8 × 2.5 cm, white, throat marked with red, maculate red pattern extending outward on either side of the centre, ending at $\frac{3}{4}$ the length, then forming a broad rectangular area, white in the centre with a red margin, then red stripes to near the apex, apex interspersed with violet, margins crenulate, revolute c. 2 mm wide near the androecial tube, broadening to c. 1 cm at the apex, as the flower matures, the white colour yellows, especially along the margins, externally covered with short, glandular hairs; *lateral staminodes* obovate, c. 1.5 × 1 cm, white, internally glabrous, externally with short, glandular hairs, apices slightly revolute. **Stamen** c. 1.5 cm long, white, glandular hairs along the backside, full length, filament c. 4 × 1.5 mm, channelled, white, anther c. 0.8 cm, white, thecae c. 1 mm wide when mature, dehiscent the full length, apex slightly indented; pollen white. **Ovary** trilocular, style filiform, c. 5.3 cm long, white, stigma rounded, white, ostiole round, non-ciliate; epigynous glands 2 × 1 mm, yellowish. **Fruit** not seen.

Distribution. Currently known only from locations in Chiang Mai, Lampang and Phrae Provinces, northern Thailand.

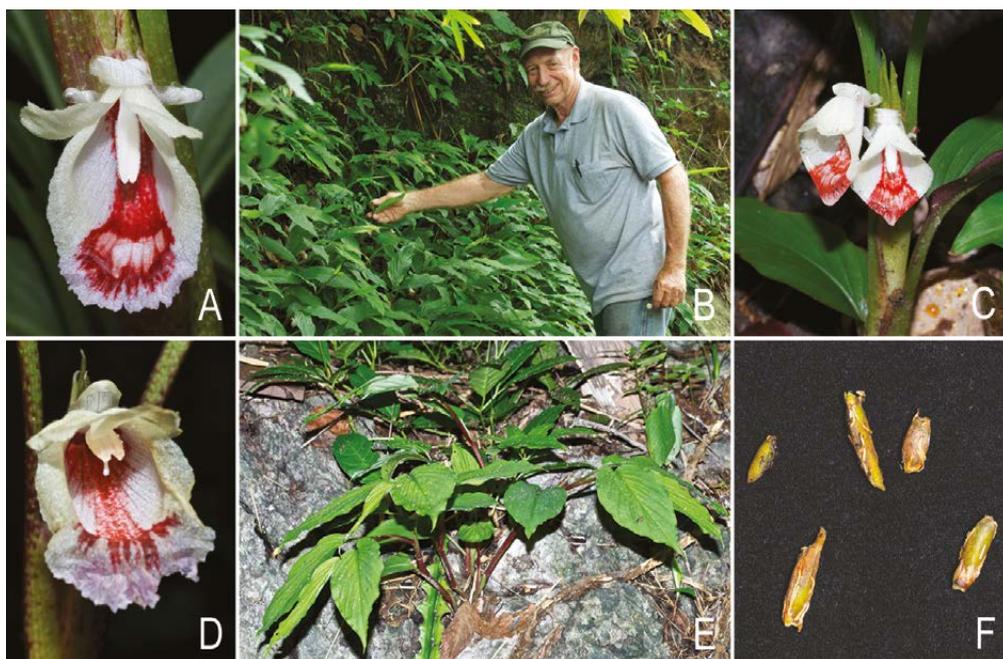


Fig. 4. *Boesenbergia phengklaii* Mood & Suksathan. **A.** Young flower. **B.** First author with plants at Doi Suthep. **C.** Flowers at mid-day with margins turned under. **D.** Old flower with thecae spread and style released. **E.** Plants showing long, semi-succulent stems. **F.** Bulbils (c. 1 cm long) found on old plants going dormant. A–C from M3349; D–F from M3478. (Photos: J. Mood)

Ecology. Found in semi-deciduous forest on well-drained, sloped areas with light shade, often on rock surfaces. The Chiang Mai populations grow on fairly deep soil with high organic matter, while the Phrae plants grow in crevices with humus on large limestone outcrops.

Phenology. In cultivation, flowering occurs July through to early October.

Etymology. Named for Dr Chamlong Phengklai (BKF), one of the first collectors of this new species.

Chromosome count. $2n=18$.

Additional specimens examined. THAILAND: **Chiang Mai:** Doi Inthanon National Park, c. 550 m, 20 Jul 1988, *Phengklai et al.* 6631 (BKF); Doi Inthanon National Park, c. 650 m, 21 Jul 1988, *Phengklai et al.* 6725 (BKF); Doi Sutep, 850 m, 3 Jul 1958, *Larsen* 3866 (C); Muang, Doi Suthep National Park, Palaht Temple area, 740 m, 7 Aug 1993, *Phuakam* 45 (CMU, K); Unknown location/collector, 1 Jun 1932, *BKF091304* (BKF); Muang, Doi Suthep National Park, east side, 500 m, 18 Jul 1987, *Maxwell* 87-690 (BKF, CMU); Doi Inthanon National Park, along Mae Klang River (RS-4), 570 m, 3 Aug 1988, *Tamura* T-60386 (BK); Doi Suthep

National Park, 18°38.063'N 98°56.026'E, 633 m asl, 25 Jul 2013, *Mood & Vatcharakorn 3307* (BKF). **Lampang:** Rahng Chat, Doi Kuhn Dahn National Park, 400 m, 29 Jul 1994, *Maxwell 94-818* (BKF). **Phrae:** Phukao Hin Pakarang, Pha Klong cave, near 18°09.153'N 99°59.079'E, c. 305 m asl, Aug 2015, *Mood & Suksathan 3478* (BKF, QBG).

Notes. The measurements given in the description are based on the living, cultivated material of *M3349*, the parent plant from which the type specimen was collected. Although all specimens of *Mood & Suksathan 3478* are similar to the type, this collection sports several differences. The plants of this population have long, semi-succulent stems with widely-spaced leaves, while the type tends to have shorter internodes with fewer leaves which occur closer to the terminus. Also, the flowers of the variant have a slightly different red colour pattern on the labellum with more intense violet at the margins and apex. Bulbils were observed on ex situ plants of this collection. They are cylindrical, c. 1.8 × 0.4 cm, light yellowish green or dark green, covered with a few, short, and white roots. They are produced under the leaf sheaths near the nodes, breaking through as they mature (Fig. 4F). Bulbils were not observed in the wild. Whether they form in the natural environment has yet to be determined.

***Boesenbergia putiana* Mood & L.M.Prince, sp. nov.**

Boesenbergiae petiolatae affinis, planta maiore ad 60 cm longa caespitosa, labello atrorubre striato, maiore ad 3.2 cm lato differt. TYPE: Thailand, Mae Hong Son Province, Mueang District, near Tam Pla Pha Suea cave, 19°25.915'N 97°59.871'E, c. 275 m asl, collected as *Mood & Vatcharakorn 3360*, cultivated in Hawaii, USA as *M3360*, vouchered on 1 October 2016 as *Mood 16P09* and voucher selected as type (holotype BKF; isotype AAU). (Fig. 5–8)

Deciduous, perennial herb to 60 cm tall, robust, multiple stems, c. 1 cm apart. **Perennating organ** a rhizome expanding linearly, length variable, 0.5–1.5 cm diam., externally orange-brown, internally white, tuberous roots numerous, persistent and enlarging over several years, to 16 cm long, c. 1 cm diam., externally and internally white, apex sometimes a spheroid, surface with few short roots. **Stems** 15–20 or more per clump, to 20 cm long, c. 2 cm diam., light green, glabrous, **leafless sheaths** 1–2, broadly lanceolate, to 11 × 3 cm, corrugate, light green, glabrous, margins slightly revolute in upper half, apex deflexed, acute. **Leaves** 4–5, alternate, upper two nearly opposite; **leaf sheaths** 12–14 cm long, corrugate, light green, glabrous, margins deflexed outward; **ligule** bilobed, to 1 cm long, triangular, green, glabrous; **petiole** 4–6 cm long, 0.4 cm wide, deeply channelled, green, glabrous; **lamina** ovate, c. 15 × 9 cm (lower), c. 27 × 14 cm (upper), base truncate, apex acuminate, adaxially dark, glossy green, glabrous, primary veins prominently raised, abaxially light silvery green, red tinged on lower two leaves, glabrous, completely covered with minute, round bumps, silky to the touch. **Inflorescence** terminal on a leafy shoot, basal portion clasped between the leaf sheaths, ¼ exposed; **peduncle** c. 1 cm long, to 0.8 cm diam., white, glabrous; **spike** to 13 cm long, 1–2.2 cm wide; **bracts** distichous, lanceolate, c. 12, to 6 cm long, c. 1 cm wide at the base (flattened), white with dark green in

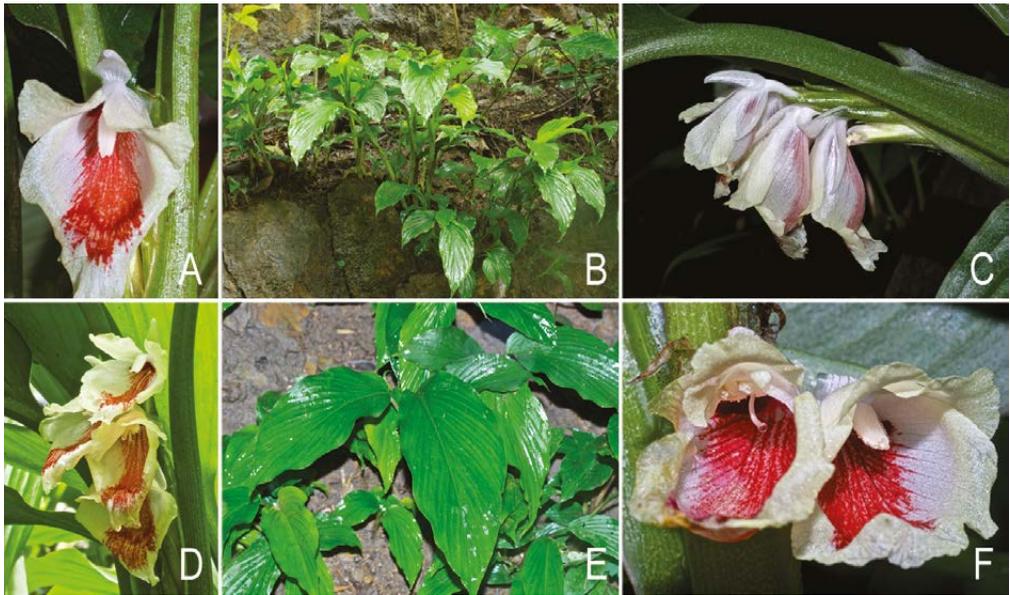


Fig. 5. *Boesenbergia putiana* Mood & L.M.Prince. **A.** Young flower. **B.** Plants. **C.** Flowers in profile. **D.** Flowers showing new and old. **E.** Leaf close-up. **F.** Old flower with thecae rotated and nectar drop on stigma (left), young flower with thecae closed and stigma not released. All from M3360. (Photos: J. Mood)



Fig. 6. Left to right in each picture—*Boesenbergia petiolata* Sirirugsa (M96P82), *B. petiolata* Sirirugsa (M3300), *B. putiana* Mood & L.M.Prince (M3360), *B. phengklaii* Mood & Suksathan (M3349), *B. bella* Mood & L.M.Prince (M3397). **A.** Leaf adaxial surface. **B.** Leaf abaxial surface. **C.** Plant with inflorescence and flower. **D.** Inflorescence (unshathed) and flower. (Photos: J. Mood)

upper third, glabrous, apex falsely pungent, bracts tightly compressed together in two ranks on the flowering side, angled at 45° to the rachis, rachis exposed on the non-flowering side, c. 1 cm diam. at the base, light green, glabrous; *bracteole* lanceolate, to 6 cm long, c. 1 cm wide at the base, light green, glabrous, apex falsely pungent, completely clasping the floral tube. **Flowers** c. 4–5 cm long, variable in size based on plant age, all facing outward from the bract, tilted slightly downward; *calyx* tubular, c. 1 cm long, translucent white, glabrous, apex bifid; *floral tube* c. 3 cm long, 0.2 cm diam., white, glabrous; *dorsal corolla lobe* oblong, c. 1.8 × 0.6 cm, white, glabrous, five-veined, apex slightly cucullate; *lateral corolla lobes* lanceolate, c. 1.7 × 0.5 cm, white, glabrous, three-veined, apex slightly cucullate; *androecial cup* c. 0.3 × 0.3 cm, orientated c. 90° to the floral tube, throat glabrous; *labellum* deeply saccate, broadly elliptical (flattened), c. 3.2 × 2.6 cm, white, throat marked with red lines, broadening outward, fan-like onto the deflexed portion, ending at $\frac{3}{4}$ the length, from there to the apex white, margins mostly entire, wavy, sometimes notched, apex crinkled, as the flower matures the margins turn yellowish, externally covered with short, glandular hairs; *lateral staminodes* obovate, c. 2.0 × 1.1 cm, white, internally glabrous, externally with short, glandular hairs, apices slightly revolute. **Stamen** c. 1.2 cm long, white, few glandular hairs along the backside, filament c. 4 × 2 mm, deeply channelled, white, *anther* c. 8 mm long, 4 mm wide, white, thecae c. 1 mm wide when mature, dehiscent the full length, apex slightly indented; pollen white. **Ovary** trilocular, style filiform, c. 1.5 cm long, white, stigma rounded, white, ostiole rounded, non-ciliate; *epigynous glands* c. 2 × 1 mm, yellowish. **Fruit** not seen.

Distribution. Known only from Mae Hong Son Province, Thailand.

Ecology. Found growing in small populations under medium shade in evergreen forests on (what appears to be) calcareous shale or limestone of the Thong Pha Phum group.

Phenology. In cultivation, flowering from July – September.

Etymology. This species is named for Put Phraisurind, the Thai botanical assistant of A.F.G. Kerr. During his tenure (1920–1932), Put collected 4548 numbers of Thai plants which were deposited at ABD, BK, BKF, BM, E, K, L, MO, NY, and perhaps other herbaria.

Chromosome count. 2n=18.

Additional specimens examined: THAILAND: **Mae Hong Son:** No exact locality, *Wongnak s.n.* [QBG 20130908] (QBG); Pang Mapha, *Panyachan s.n.* [QBG 201406854] (QBG); Mueang, Ban Huay Pha, Tham Pha Daeng, 260 m, 1 Aug 2013, *Newman et al.* 2583 (QBG); Na Pla Chad, Tham Pla-Namtok Pha Suea National Park, 322 m, 11 Sep 2013, *Lakoet 0394* (QBG).

Notes. The measurements in the description are based on the living, cultivated material of M3360, the parent plant from which the type specimen was collected.

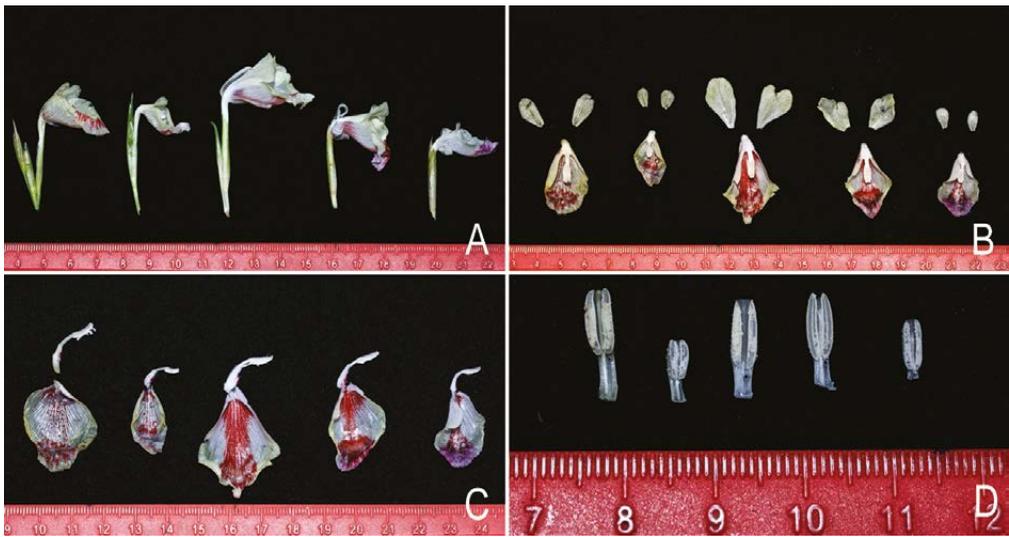


Fig. 7. Left to right in each picture—*Boesenbergia petiolata* Sirirugsa (M96P82), *B. petiolata* Sirirugsa (M3300), *B. putiana* Mood & L.M.Prince (M3360), *B. phengklaii* Mood & Suksathan (M3349), *B. bella* Mood & L.M.Prince (M3397). **A.** Flower and bract in profile. **B.** Labellum and lateral staminodes. **C.** Labellum with anther. **D.** Anther. (Photos: J. Mood)

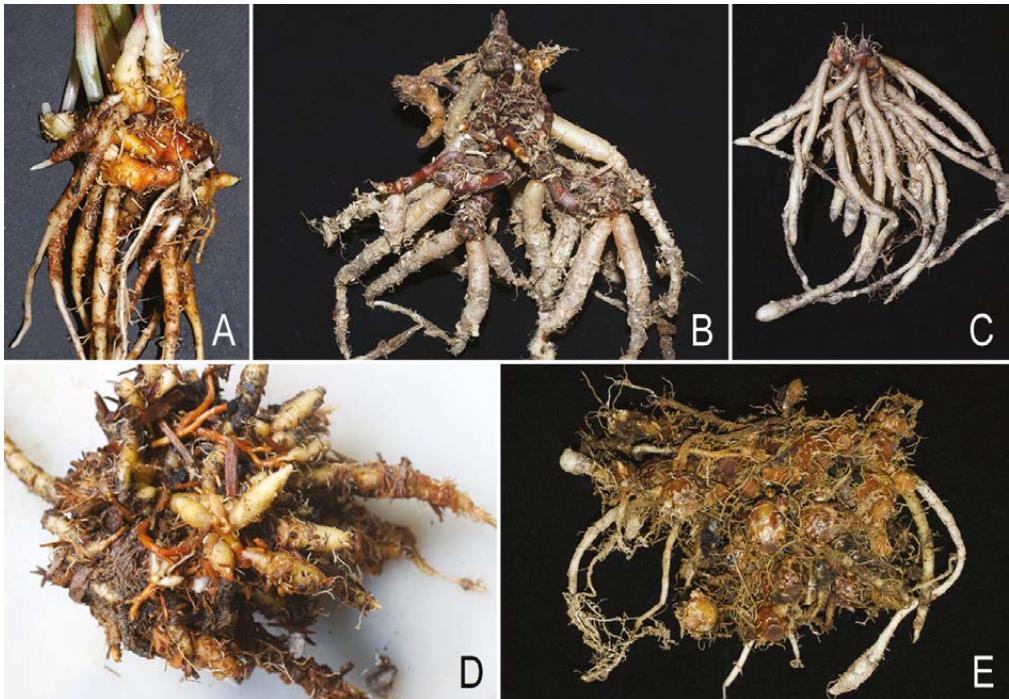


Fig. 8. Rhizomes. **A.** *Boesenbergia petiolata* Sirirugsa. **B.** *B. petiolata* Sirirugsa. **C.** *B. putiana* Mood & L.M.Prince. **D.** *B. bella* Mood & L.M.Prince. **E.** *B. phengklaii* Mood & Suksathan. A from M96P82; B from M3300; C from M3360; D from M3397; E from M3349. (Photos: J. Mood)

Conclusions

As a result of the morphological and molecular phylogenetic findings presented here, three new species are described as distinct from *Boesenbergia petiolata*. All four species form a clade with close affinity based on a common ancestor. The chromosome number of $2n=18$ is unique to the clade, whereas the sister species, *Boesenbergia parvula* (Wall.) Kuntze and numerous other Thai species are $2n=20$.

ACKNOWLEDGEMENTS. We would like to especially thank P. Vatcharakorn (Chanthaburi), R. Harwood (BKF), and staff of the Queen Sirikit Botanical Garden for field assistance and donations of their collections of ginger rhizomes. We also thank the Plant Quarantine office in Bangkok for phytosanitary certificates and the USDA APHIS in Honolulu, Hi. for import; The Field Museum, Chicago, Il USA (F) for use of Pritzker DNA Laboratory facilities; and Linda Ann Vorobik (USA) for the plate compositions.

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Appendix 1. GenBank accession numbers for Zingiberaceae used in this study. Format: identification, plant sample number (collector and voucher number, herbarium), ITS GenBank number/trnK GenBank number.

- Boesenbergia alba* (K.Larsen & R.M.Sm.) Mood & L.M.Prince: M3253 (*Mood 3253*, BISH) KF982792/KF982801
- Boesenbergia bella* Mood & L.M.Prince: M3397 (*Mood 16P07*, BKF) KY701326/ KY701339
- Boesenbergia burtii* (K.Larsen & Jenjitt.) Mood & L.M.Prince: M12C32 (*Funakoshi, H. s.n.*, MBK) KF982794/KF982803
- Boesenbergia collinsii* Mood & L.M.Prince: M06P14 (*Mood 12P171*, holotype, BK) JX992751/JX992812
- Boesenbergia hamiltonii* Mood et al.: M3017 (*Dey, S. NU 53*, holotype, CAL) JX992754/JX992815
- Boesenbergia kerrii* Mood et al.: M2044 (*Mood & Triboun, P. 12P170*, holotype, BK) JX992756/JX992817
- Boesenbergia kingii* Mood & L.M.Prince: M3074 (*Mood & Vatcharakorn, P. 12P173*, holotype, BK) JX992792/JX992829
- Boesenbergia longiflora* (Wall.) Kuntze: M11P48 (live material of *Kress, W.J. 03-7305*, US) JX992797/JX992831
- Boesenbergia maxwellii* Mood et al.: M2032 (*Mood & Triboun, P. 12P172*, holotype, BK) JX992800/JX992833
- Boesenbergia petiolata* Sirirugsa: M3300 (*Vatcharakorn, P. M3300*, BKF) KY701321/ KY701334
M3332 (*Mood & Vatcharakorn, P. 3332*, BKF) KY701323/ KY701336
M3501 (*Mood & Harwood, R. 3501*, BKF) KY701329/ KY701342
M96P82 (*Mood 96P82*, BKF) KY701330/ KY701343
- Boesenbergia phengklaii* Mood & Suksathan: M3307 (*Mood 16P08*, BKF) KY701322/ KY701335
M3349 (*Mood & Vatcharakorn, P. 3349*, BKF) KY701324/ KY701337
M3478 (*Mood & Suksathan, P. 3478*, BKF) KY701328/ KY701341
- Boesenbergia pulcherrima* (Wall.) Kuntze: M08P276 (*Mood 08P276*, BISH) JX992748/JX992748
- Boesenbergia putiana* Mood & L.M.Prince: M3360 (*Mood 16P09*, BKF) KY701325/ KY701338
M3471 (*Panyachan, P. s.n. QBG 20140685A*, QBG) KY701327/ KY701340
- Boesenbergia xiphostachya* (Gagnep.) Loes.: M11C86 (*Mood 11C86*, BKF) KX425643/KX432277
- Boesenbergia violacea* (K.Larsen & Triboun) Mood & L.M.Prince: M12C31 (*Funakoshi, H. s.n.*, MBK) KF982796/KF982805

Appendix 2. Molecular data analysis details for *Boesenbergia petiolata*. *excludes parsimony uninformative characters.

Matrix	Maximum Parsimony	Maximum Likelihood Parameters and tree characteristics	Bayesian Inference Burn-In
ITS	110 PIC # trees=60 (1 island) Length*=235 CI=0.6128 RI=0.7964 RC=0.4880	hLRT: 1 tree, L=3021.305 K80+G: Base=equal Nst=2 TRatio=2.7960 Rates=gamma Shape=0.3332 Pinvar=0 AIC: 1 tree, L=3018.237 TIMef+G: Base=equal Nst=6 Rmat=(1.0000 6.4272 1.5891 1.5891 8.0956) Rates=gamma Shape=0.3397 Pinvar=0	Run 1: 120,000 Run 2: 110,000 Run 3: 120,000
trnK	48 PIC # trees=4050 (1 island) Length*=61 CI=0.8361 RI=0.9270 RC=0.7750	hLRT: 3 trees, 2 islands, L= 4907.954 HKY+I+G: Base=(0.3138 0.1693 0.1729) Nst=2 TRatio=2.3264 Rates=gamma Shape=0.6880 Pinvar=0.6383 AIC: 3 trees, 2 islands, L=4904.326 GTR+I+G: Base=(0.3183 0.1642 0.1732) Nst=6 Rmat=(1.2974 4.8484 0.7847 1.6791 6.3931) Rates=gamma Shape=0.6758 Pinvar=0.6226	2 partitions: trnK intergenic spacers, matK Run 1: 105,000 Run 2: 130,000 Run 3: 120,000
Combined	158 PIC # trees=24 Length*=304 CI=0.6414 RI=0.8134 RC=0.5217	hLRT: 1 tree, L=8249.150 HKY+I+G: Base=(0.3138 0.1693 0.1729) Nst=2 TRatio=2.3264 Rates=gamma Shape=0.6880 Pinvar=0.6383 AIC: 1 tree, L=8241.758 GTR+I+G: Base=(0.3183 0.1642 0.1732) Nst=6 Rmat=(1.2974 4.8484 0.7847 1.6791 6.3931) Rates=gamma Shape=0.6758 Pinvar=0.6226	3 partitions: ITS, trnK intergenic spacers, and matK Run 1: 100,000 Run 2: 110,000 Run 3: 30,000

Appendix 3. Maximum likelihood phylograms of separate ITS and *trnK* data analyses for *Boesenbergia petiolata* and closely related taxa based on the General Time Reversible (GTR+I+G) model. Branch support values are BS/PP. Taxon labels based on the recircumscribed clade including the new taxa. Top. ITS. Bottom. *trnK*.

