



Curcuma tuanii (Zingiberaceae) a new species of subgenus *Ecomata* from northern Vietnam based on morphological and molecular evidence

Hoang Tuan Nguyen¹ , Ngọc Anh Nguyen¹ , Leonid Averyanov² , Danh Duc Nguyen³  and Chi Toan Le^{4*} 

Received: February 20, 2023

Accepted: August 22, 2023

ABSTRACT

A new species, *Curcuma tuanii* (Zingiberaceae), is described and illustrated from Hoa Binh Province northern Vietnam based on both the morphological and molecular data. The species is morphologically compared with its closest allies from subgenus *Ecomata*. Additionally, the anatomical structures of the species were provided.

Keywords: Flora of tropical Asia, *Curcuma* subgen. *Ecomata*, medicinal plants, Zingiberaceae, Vietnam.

Introduction

The genus *Curcuma* L. (Zingiberaceae) contains many taxa of economic, medicinal, ornamental, and cultural importance (Záveská *et al.* 2012). This genus comprises approximately 120 currently accepted species (Leong-Škorničková *et al.* 2007; 2015a). However, the exact number of species is still controversial. *Curcuma* is one of the largest genera in the Zingiberaceae family. Its species are widely distributed in South East Asia, with a few species occurring in northern Australia and the islands of the South Pacific (Záveská *et al.* 2012). The highest genus diversity is observed in India and Thailand, with at least 40 species in each country. Then the number of native species follows Myanmar, Bangladesh, Indonesia, and Vietnam (Leong-Škorničková *et al.* 2008).

In the course of the progressing revision of the genus *Curcuma* for the Flora of Cambodia, Laos, and Vietnam, 12 new *Curcuma* species from subgenus *Ecomata* (Záveská *et al.* 2012) were described recently, namely *Curcuma vitellina* Škorničk. & H.Đ. Trần (Leong-Škorničková *et al.* 2010), *C. pambrosima* (Leong-Škorničková & Ly 2010), *C. newmanii*, *C. xanthella* (Leong-Škorničková & Tran 2013), *C. arida*, *C. sahuynhensis* (Leong-Škorničková *et al.* 2015b), *C. cotuana* (Luu *et al.* 2017), *Curcuma sixsensesensis*, *C. vinhlinhensis* (Nguyen *et al.* 2022) from Vietnam; *C. peramoena* (Souvann & Maknoi 2014), *C. corniculata* and *C. flammea* (Leong-Škorničková *et al.* 2014) from Laos. Totally, 13 species were reported in Vietnam after the last work of Nguyen *et al.* (2022). Additionally, Thailand is also recognized as the region with the highest species richness for subgenus *Ecomata* with 29 species (Saensouk *et al.* 2022).

¹ Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, 15 Le Thanh Tong Street, Hoan Kiem, Hanoi, Vietnam.

² Komarov Botanical Institute of the Russian Academy of Sciences, Prof. Popov Street 2, 197376, St. Petersburg, Russia.

³ Institute of Applied Technology, Thu Dau Mot University, no. 06, Tran Van On Street, Phu Hoa, Thu Dau Mot, Binh Duong, Vietnam.

⁴ Hanoi Pedagogical University 2, 32 Nguyen Van Linh Street, Xuan Hoa, Phuc Yen, Vinh Phuc, Vietnam.

* Corresponding author: Lechitoan@hpu2.edu.vn



During floristic conservation in Hoa Binh Province, Vietnam in 2019, the first and the third authors collected a taxon of *Curcuma* subgen. *Ecomata* extremely similar to *C. sahuynhensis* in appearance. After a critical examination of morphology enforced by molecular analysis, we conclude it is a new species for science. Thus, the present work aims to (1) reconstruct the phylogeny of genus *Curcuma* to clarify the phylogenetic position of the new species and its closely related taxa, (2) provide the taxonomic treatment with descriptions, anatomical features of leaves and rhizome, photographs, distribution, ecology, conservation assessment, and morphological comparison of new species with its allies.

Materials and methods

Molecular phylogenetic analyses

We extracted total genomic DNAs from silica gel-dried leaves of the collection and generated 11 new sequences for five *Curcuma* species belonging 'Pierreana' group subgenus *Ecomata*. The 4 primer sequences and protocols of genes ITS, *matK*, *psbA-trnH*, and *trnL-F* used for PCR and sequencing in this study followed the protocols described in Závěská *et al.* (2012). In total, 36 species of *Curcuma* including 2 individuals of the new species *C. tuanii* were sampled for phylogenetic analyses. The *Alpinia conchigera* Griff., *A. galanga* (L.) Willd., *Camptandra parvula* (King ex Baker) Ridl., *Larsenianthus careyanus* (Benth. & Hook.f.) W.J.Kress & Mood were used as outgroups. The sequences used in this study showed in Table S1. Sequences were aligned in Geneious v.8.0.5 using MUSCLE (Kearse *et al.* 2012). The combined dataset consists of 5181 base pairs.

Two methods, maximum likelihood (ML) and Bayesian inference (BI), were used to construct the phylogenetic relationships of *Curcuma* on the CIPRES Science Gateway Portal (Miller *et al.* 2010). Individual analyses of chloroplast and ITS detected no well-supported topological conflicts (i.e., incongruences among individual DNA regions with BS < 70%; Hillis & Bull 1993). We thus conducted further phylogenetic analyses for the combined data set of five DNA regions using both ML and Bayesian inference (BI) methods. The ML analyses were conducted in RAxML 8.2.12 (Stamatakis 2006) using the nucleotide substitution models estimated separately for each gene region under the Akaike Information Criterion (AIC) in jModeltest 2.1.6 (Darriba *et al.* 2012) and applying 1,000 bootstrap replicates. The BI analysis was performed on MrBayes 3.2.6 (Ronquist *et al.* 2012) using the nucleotide substitution models that estimated separately each gene region by jModeltest 2.1.6 (Darriba *et al.* 2012). Two independent runs, each comprising four Markov chain Monte Carlo (MCMC) chains, were conducted. We ran the MCMC for 20 million generations, and trees were sampled every 2000 generations. Tracer v.1.6 was used to check the effective sample sizes

(ESSs) of all relevant parameters (>200) (Rambaut *et al.* 2014). We removed the first 25% of sampled generations as burn-in to obtain the majority-rule consensus tree and Bayesian posterior probabilities (PP). The final result was visualized in FigTree v.1.4.0 (Rambaut 2009).

Morphological analyses

All measurements of morphological characteristics were carried out in the field based on living flowering plants used for the type preparation. Some rhizomes were collected and grown in the garden of the Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy to examine the stability of characteristics. The plant terminology follows Beentje (2016) and the previous work of Nguyen *et al.* (2022). Voucher herbarium specimens are stored at VNMN and HNU herbarium. All the photos were taken with a Nikon D300S fitted with an AF-S 60mm f/2.8 Macro lens.

Conservation status study: The preliminary conservation assessment follows the guidelines of IUCN (2022).

Anatomical method

Anatomical studies were carried out using fresh as well as preserved specimens. The specimens (leave and rhizome) were cut by hand into thin slices (about 10–20 µm). Next, these transverse sections were used for staining. These samples were cleared in 5.0% (w/v) chloramine-T for 10 minutes. Then they are subsequently acidulated in 5.0% (w/v) acetic acid for 15 minutes. Those slices were dyed in safranin and mounted on a clear glass slide using glycerin. The sections were subsequently observed under Leica EZ4 stereoscope (Leica Microsystem, Heerbrugg, Switzerland) with 5X to 100X magnifications.

Results and discussion

Phylogenetic relationships

The topology from ML and BI analyses of the combined matrix were highly congruent, we thus present the BI tree with BS and PP values in Fig. S1. The molecular results indicate four well supported major clades within *Curcuma* representing: '*Curcuma*', '*Hitcheniopsis*', '*Ecomata*' and '*Pierreana*' groups (Fig. 1). These results are in congruence with Závěská *et al.* (2012).

The new species of *Curcuma* was placed together with some native Vietnamese species classified in 'Pierreana' group belonging to subgen. *Ecomata* (Fig. 1). In this clade, *C. xanthella* and *C. newmanii* were weakly supported as sisters to the remaining species. While, four species including *C. vitellina*, *C. sahuynhensis*, *C. pambrosima* and the new species *C. tuanii* formed a subclade with supported by the BI analysis (Fig. 1). *Curcuma tuanii* is recovered with strong support, the two individuals of the new species formed a separate clade.



Curcuma tuanii (Zingiberaceae) a new species of subgenus *Ecomata* from northern Vietnam
based on morphological and molecular evidence

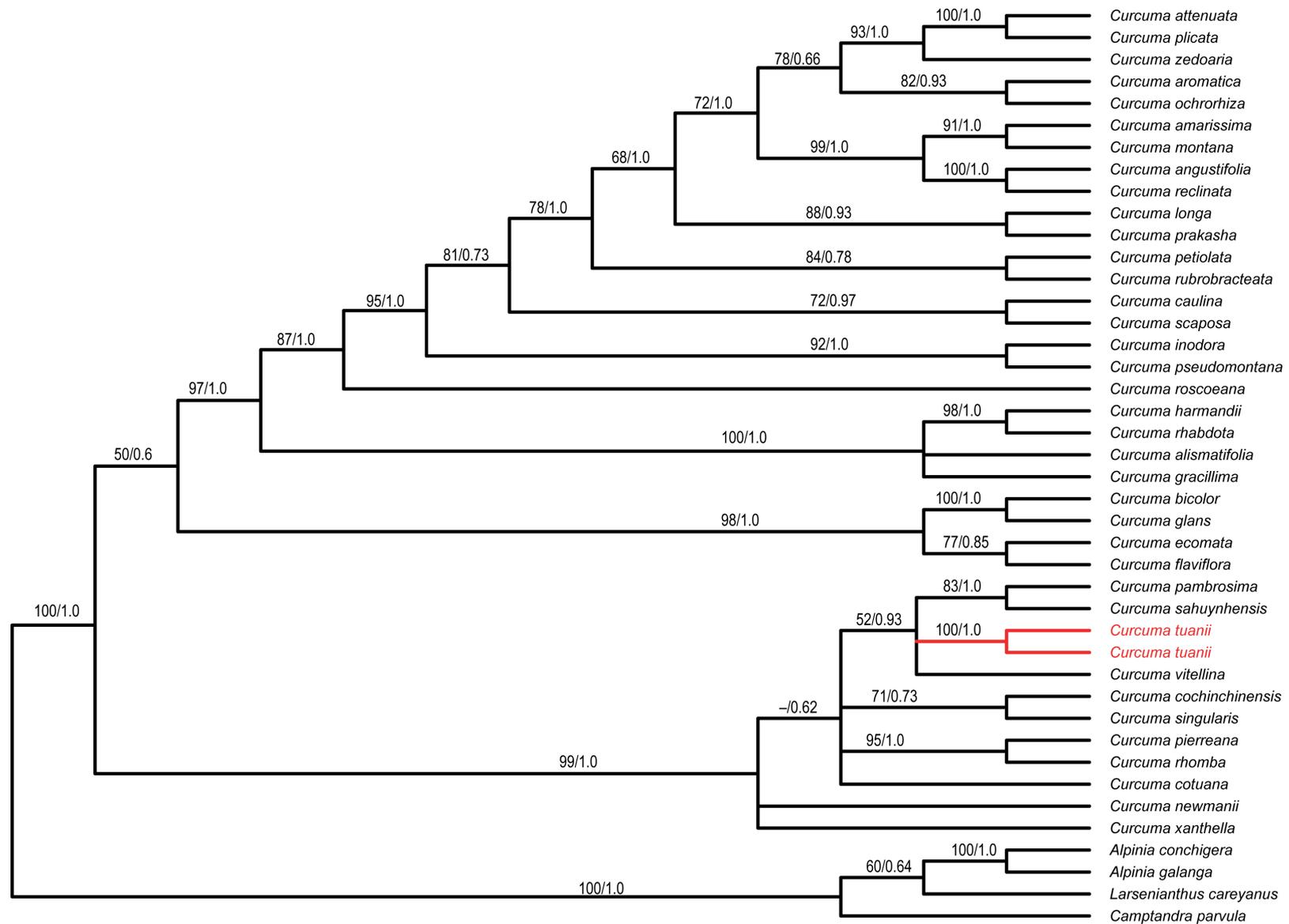


Figure 1. Majority rule consensus tree from the Bayesian inference based on the combined datasets of four DNA regions (ITS, *matK*, *psbA-trnH*, and *trnL-F*). ML bootstrap values and posterior probabilities (PP) of the BI analysis are presented above the branches. Support values less than 50% are indicated with “-”.



On the other hand, *C. sahuynhensis* and *C. pambrosima* were recovered as closely related species by molecular data (Fig. 1). By comparing specimens in the herbaria, the description in literature (Leong-Škorničková *et al.* 2015b), and our observation in the field, the two species *C. tuanii* and *C. sahuynhensis* share some morphological characters such as color and shape of bracts, rich yellow staminodes as long as labellum, and L-shaped anther. However, the molecular data indicated that *C. sahuynhensis* is most closely to *C. pambrosima* than *C. tuanii* and *C. vitellina*. In addition, *C. tuanii* and *C. cotuana* share several morphological characters, but the molecular data reveals that these two species are not so closely related (Fig. 1). Thus, the morphological differences between *C. tuanii* and closest relatives (*C. sahuynhensis*, *C. vitellina* and *C. pambrosima*) are discussed below and presented in Table 1.

Morphology

The morphological comparison between the five *Curcuma* species studied here strongly supports *C. tuanii* as new species. *Curcuma tuanii* is most similar to *C. sahuynhensis* and *C. cotuana* in its terminal inflorescence, yellow to orange flowers, as well as L-shaped anthers that are unusual in the representatives of the *C.* subgen. *Ecomata*. The new species is clearly distinguished from all remaining members of this subgenus by its (1) larger habit, particularly oblong (vs. ovate to elliptic) leaf blade up to 40 (vs. 20–25) cm long; (2) leafy shoot 5–20 per plant forming larger clump (vs. leafy shoot 1–3 per plant); (3) longer spike composed densely of bracts 20–50 per spike (vs. shorter spike or spike composed from fewer bracts 5–20, rarely to 24).

Table 1. Comparison of the morphologically vegetative and reproductive characteristics of *Curcuma tuanii* with its closest relatives: *C. sahuynhensis*, *C. vitellina*, *C. pambrosima* and *C. cotuana*.

Characters	<i>C. tuanii</i>	<i>C. sahuynhensis</i> (Leong-Škorničková <i>et al.</i> 2015b)	<i>C. vitellina</i> (Leong-Škorničková <i>et al.</i> 2010)	<i>C. pambrosima</i> (Leong-Škorničková & Ly 2010)	<i>C. cotuana</i> (Luu <i>et al.</i> 2017)
Habit	Up to 100 cm tall	Up to 60 cm tall	70 cm	80 cm	Up to 50 cm tall
Petiole	Up to 40 cm long	Up to 24 cm long	5–20 cm	5–25 cm	Up to 20 cm
Lamina	Oblong	Ovate to elliptic	elliptic to elliptic-ovate	elliptic-lanceolate	Ovate to elliptic
Spike (cm long, number of bracts)	13–17 cm long, composed of 25–50 bracts	6–15 cm long, composed of 10–23 bracts	8–15 cm long, 15–60 bracts	10–25 cm, 7–34 bracts	5–9 cm long, composed of 16–24 bracts
The bract color	Orange-red	Orange-red	Cream white	pinkish	Orange-red
The number of bracts: the length of the spike	1.92–2.94	1.53–1.67	1.88–4	0.7–1.36	2.66–3.20
corolla lobe	Orange-red	Orange-red	Cream white	pinkish	Orange-red
Lateral staminodes	18–21 × 11–13 mm	15–22 × 15–18 mm	18 × 11 mm	12–15 × 8–10 mm	20–22 × 5–6 mm
Labellum	incision extending more than 1/2 of the length of the labellum	incision extending less than 1/3 of the length of the labellum	incision extending less than 1/3 of the length of the labellum	incision extending 1/3 to 1/2 of the length of the labellum	incision extending less than 1/3 of the length of the labellum
Anther	10–11 mm long, yellow, L-shaped	7–8 mm long, yellow, L-shaped	4–6 mm long, yellow, oblong	8–9 mm, cream white, oblong	ca. 12.5 mm long, yellow, weak L-shaped
Anther spurs	3–3.5 mm long, parallel with apex outward-facing	3.5–5.0 mm long, parallel with apex outward-facing	0.5 mm long, filamentous	1.5 mm long, curved inwards	Each with a blunt knob positioned below thecae and extending into the acute apex
Anther crest	1.0–1.5 mm long, entire rounded apex	Reduced 0.3–1.0 mm long, emarginate apex,	1–1.5 mm long, Rounded apex	1.5 mm long, rounded	1–1.2 mm long, emarginate apex



Curcuma tuanii (Zingiberaceae) a new species of subgenus *Ecomata* from northern Vietnam based on morphological and molecular evidence

The differences between *C. sahuynhensis* and *C. tuanii* are argued in the diagnosis below and presented clearly in Table 1 and Fig. 2. *Curcuma cotuana* is also similar to *C. tuanii*, but easily distinguished from the latter by its smaller habit as the argument above, and besides (1) shorter inflorescence 5–9 (vs. 13–17) mm long; (2) labellum with incision extending less than 1/3 (vs. more than 1/2) of the length of the labellum and (3) a weakly (vs. strongly L-shaped) anther with a bi-lobed (vs. entire round) apex crest, and present (vs. absent) two blunt knobs positioned below the thecae. *Curcuma tuanii* is similar to *C. vitellina* in long terminal inflorescence composed densely of bracts, yellow to orange flowers but differ in other characters like (1) green bracts tinged red (vs. pure white bracts); (2) corolla lobes orange-red (vs. pure white); (3) incision extending more than 1/2 (vs. less than 1/3 of the) of the length of the labellum; (4) longer anther 10–11 mm long (vs. 4–6 mm long); (5) anther L-shaped (vs. oblong) in side view with anther spurs 3–3.5 mm long and cylindrical (vs. 0.5 mm and filamentous) (also see Table 1). *Curcuma tuanii* is also similar to *C. pambrosima* in long inflorescence and yellow flower but easily distinguished from the latter by presenting a terminal inflorescence (vs. lateral inflorescence), spike composed densely of orange-red bracts (vs. spike composed of fewer pinkish bracts), warm yellow (vs. white) anther with longer spurs 3–3.5 mm long (vs. 1.5 mm long) and apex outward-facing (vs. apex curved inwards).

Taxonomic treatment

Curcuma tuanii H. T. Nguyen, D. D. Nguyen & N. A. Nguyen, *sp. nov.* Figs. 3 and 4

Diagnosis:—The species is similar to *Curcuma sahuynhensis* (*C.* subgenus *Ecomata*) in color and shape of bracts, rich yellow staminodes as long as labellum, and L-shaped anther, but readily distinguished in (1) leafy shoot 5–15 (vs. 1–3) per plant forming a larger clump; (2) oblong (vs. ovate to elliptic) leaf lamina 40–80 cm (vs. 20–38 cm) long, prominent petiole up to 40 cm (vs. 24 cm) long; (3) terminal only (vs. terminal or lateral) inflorescence; (4) spike composed of more dense bracts with 25–45 bracts, the ratio of the number of bracts to the length of the spike is 2.50–3.3 (vs. 1.53–1.67); (5) narrower staminodes 18–21 × 11–13 (vs. 15–22 × 15–18) mm; (6) labellum with incision extending more than 1/2 (vs. less than 1/3) of the length of the labellum and (7) longer anther 10–11 (vs. 7–8) mm long with longer anther crest 1.0–1.5 (vs. 0.3–1.0) mm, and entire rounded (vs. emarginate) apex.

Etymology:—This species is named in honour of Mr. Tuan who has the first observation of this species in situ.

Type:—Collected from plants in the anthesis cultivated in the garden of the Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, 15 August 2019, HNU 22972 – *Nguyen Hoang Tuan* № *NHTuan* 30 (holotype HNU, isotypes VNMN).

Originally collected by *Nguyen Hoang Tuan* from VIETNAM, Hoa Binh Province, Da Bac District, Hien Luong Commune, 20°51'30.6" N 105°15'11.3" E, elevation 150–200 m a.s.l., 15 June 2017.

Description:—Rhizomatous terrestrial herb to 0.8–1.0 m tall. **Rhizome** narrowly ovoid to ovoid, 0.5–1.5 × 3.0–5.0 cm, with thin branches pointing downwards, outside light brown, white to pale yellow inside (on cross-section), with light fragrance; **rhizome scales** triangular, brown, glabrous; **root tubers** fusiform, 2.0–3.5 × 0.7–1.2 cm, light brown, white with translucent white center inside, distant to 8 cm from the rhizome. **Leafy shoot** 5–20 per plant with 2–5 leaves during anthesis; **pseudostem** 15–25 cm long, pale green composed of leaf sheaths and sheathing bracts; **sheathing bracts** 3–5, glabrous, becoming papery and decaying with age; **leaf sheaths** green, glabrous; **ligule** 2–5 mm long, bilobed, hyaline, greenish, glabrous, with sparsely hairy margin; **petiole** (5–)15–40 cm (petioles of basal leaves much shorter than the others of apical), canaliculate, green, glabrous; **leaf lamina** 40–80 × 8–17 cm, oblong, obtuse to rounded and slightly unequal at the base, attenuate to acuminate apex, plicate, puberulent, adaxially bright green, glabrous, abaxially light green, glabrous; **median vein** green, glabrous on both sides, adaxially slightly canaliculate. **Inflorescence** apical, with peduncle wrapped by pseudostem; **peduncle** 10–35 cm, light green; **spike** 13–17 cm long, 5–7 cm in diameter at the middle, with no obvious coma, composed of 25–50 bracts; **bracts** 3.0–5.5 × 1.5–4.0 cm, broadly ovate to rhombic (broader at the base, narrowed to the apex), white to pale green at the base with pink-red tinge towards the apex and margins, glabrous on both sides, connate in lower 1/4–1/3 portion, cute, reflexed; **cincinni** with 2–3 flowers at the base of the inflorescence, with 1–2 flowers near the apex; **bracteoles** subulate, 2–5 × 0.5–1.0 mm, narrowly triangular, white or translucent, glabrous, often completely reduced. **Flowers** 4.5–5.5 cm long, exerted from bracts; **calyx** 20–22 mm long, 3-toothed, with unilateral incision ca. 3–5 mm long, pale pink to semi-translucent, glabrous; **floral tube** ca. 2.5–3.0 cm long, narrowly cylindrical at base for 1.5–2.0 cm above the ovary, funnel-shaped at apex, externally white or white with pink tint, puberulent at funnel-shaped part, internally white, glabrous at base, hairy towards the throat, with dorsally placed loose groove holding the style; **dorsal corolla lobes** 20–22 × 6–11 mm, triangular ovate, concave, with sides slightly incurved, apex mucronate, mucro ca. 2–3 mm, externally pale orange-red, internally white to pale yellow; **lateral corolla lobes** 15–18 × 6–8 mm, triangular, apex broadly acute to obtuse, externally semi-translucent or pale orange red, internally with a pale yellow band running through the center; **labellum** 20–23 × 15–16 mm, obovate, with an incision 10–14 mm long, white at base, rich yellow



at apex, with two darker and thicker bands along the median vein; **lateral staminodes** ca. 18–21 × 11–13 mm, unequally narrowly ovate to ovate, rich yellow throughout, pale yellow at base, adaxially with short glandular hairs. **Stamen** 14–15 mm long; **filament** 6–7 mm long, 4–5 mm in diameter at the base, 2–3 mm in diameter at the apex (the point of attachment to the connective), pale yellow to orange-yellow, puberulent with glandular hairs; **anther** 10–11 mm long, L-shaped, spurred; **connective** yellow to orange-yellow throughout,

lighter at back near filament, darker at spurs and crest, densely puberulent with glandular hairs; **anther spurs** 3–3.5 mm long, stout, acute apices, yellow; **anther crest** 1–1.5 mm long, stout, apex rounded; **thecae** 6–8 × 0.5–1 mm, narrowly obovate, dehiscent along their entire length; **pollen** white. **Epigynous glands** two, 5–6 mm long, 0.6 mm in diameter, white, with blunt apices. **Style** white, glabrous, basally placed in a dorsal groove of the floral tube; **stigma** capitate, ca. 1.1 cm in diameter, pale yellow, with **ostiole** ciliate,

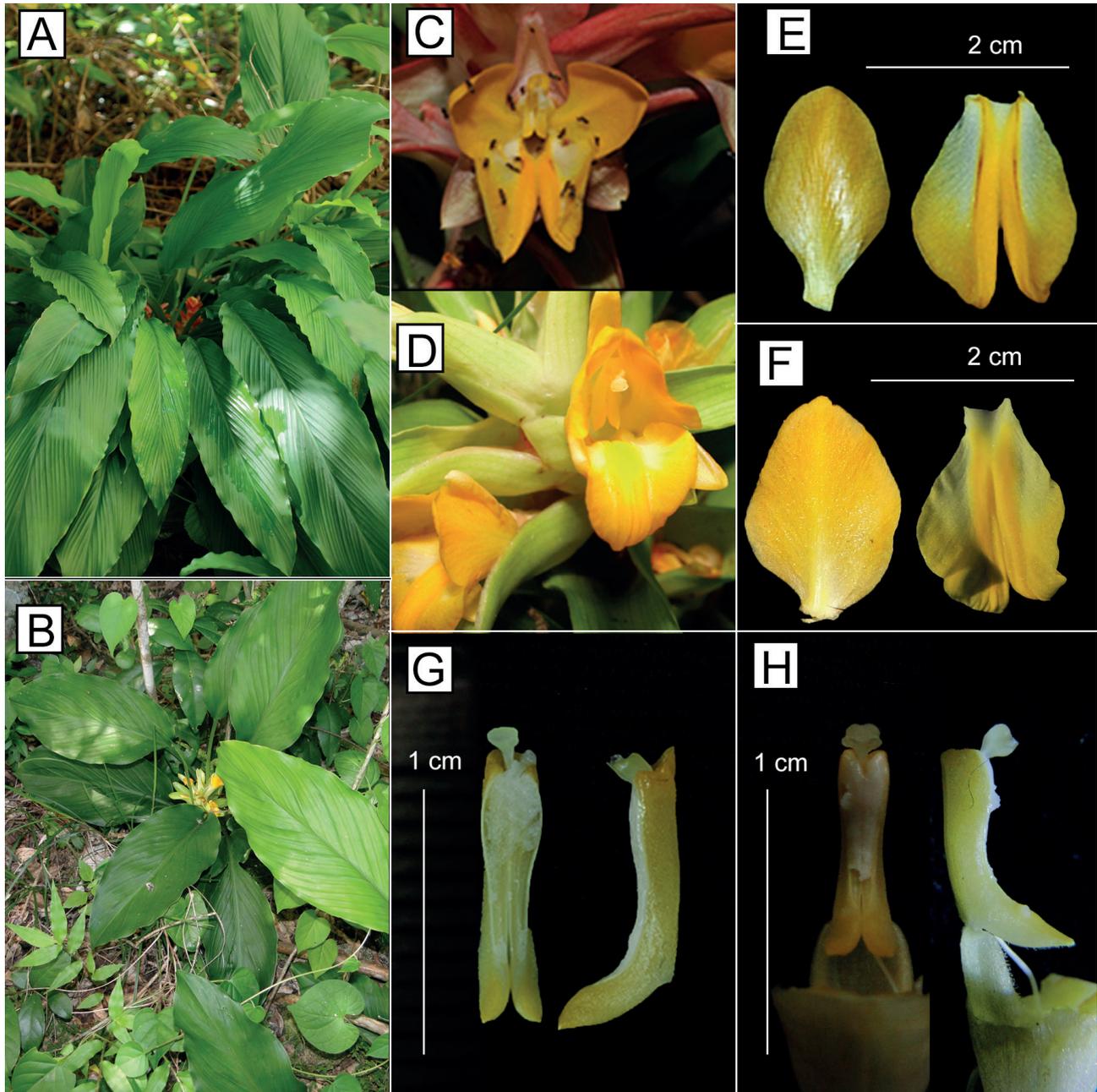


Figure 2. Comparison of *Curcuma tuanii* sp. nov. and its closest species. **A.** Habit. **C.** Flower. **E.** Lateral staminode and labellum. **G.** Anthers. Vs. *Curcuma sahuynhensis*. **B.** Habit of an adult with terminal inflorescence. **D.** Flower. **F.** Lateral staminode and labellum. **H.** Anthers. Photos (A), (C), (E), (G) by Hoang Tuan Nguyen from the type HNU 022972, and photos (B), (D), (F), (H) by Danh Duc Nguyen from specimens NDD-278 (collected from the type locality, Sa Huynh town in November 2016).

Curcuma tuanii (Zingiberaceae) a new species of subgenus *Ecomata* from northern Vietnam
based on morphological and molecular evidence

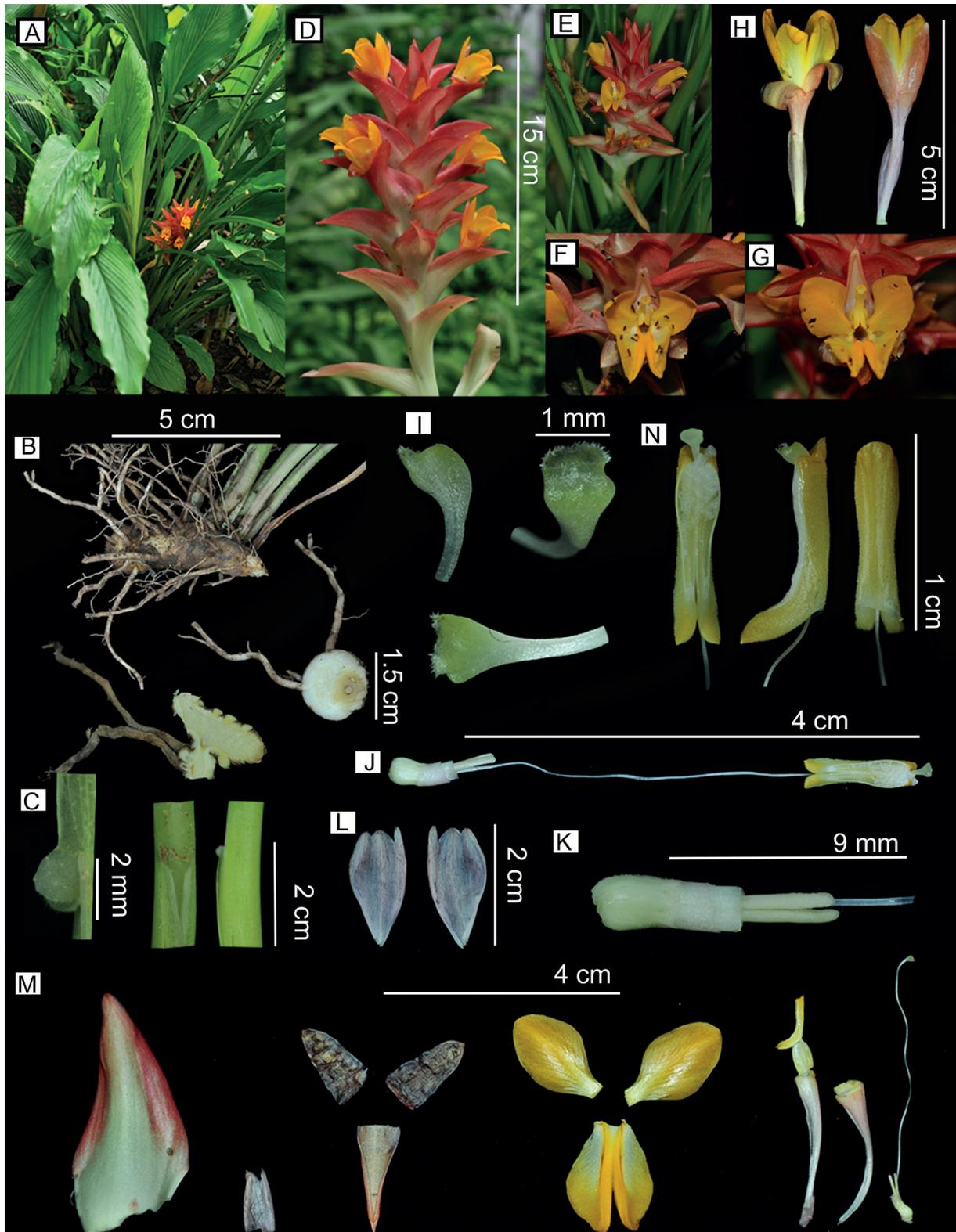


Figure 3. *Curcuma tuanii* H.T. Nguyen, D.D. Nguyen & N.A. Nguyen. **A.** Flowering habit. **B.** Intact rhizome (cross and longitudinal section). **C.** Ligule, frontal and side section. **D, E.** Inflorescences. **F, G.** Intact flower, frontal view. **H.** Flattened flowers, side view. **I.** Detail of stigma. **J.** Ovary, epigynous glands, style binded terminally by stamen. **K.** Ovary with epigynous glands. **L.** Calyx. **M.** Flower dissection: bract, calyx, dorsal and lateral corolla lobes, labellum and lateral staminodes, floral tube with ovary and stamen attached, floral tube, ovary with style and epigynous glands attached (from left to right). **N.** Detail of stamen with filament removed, frontal, back, and side views. All photos were made from the type HNU 022972, and taken by Hoang Tuan Nguyen, designed by Hoang Tuan Nguyen, Duc Danh Nguyen and L. Averyanov.

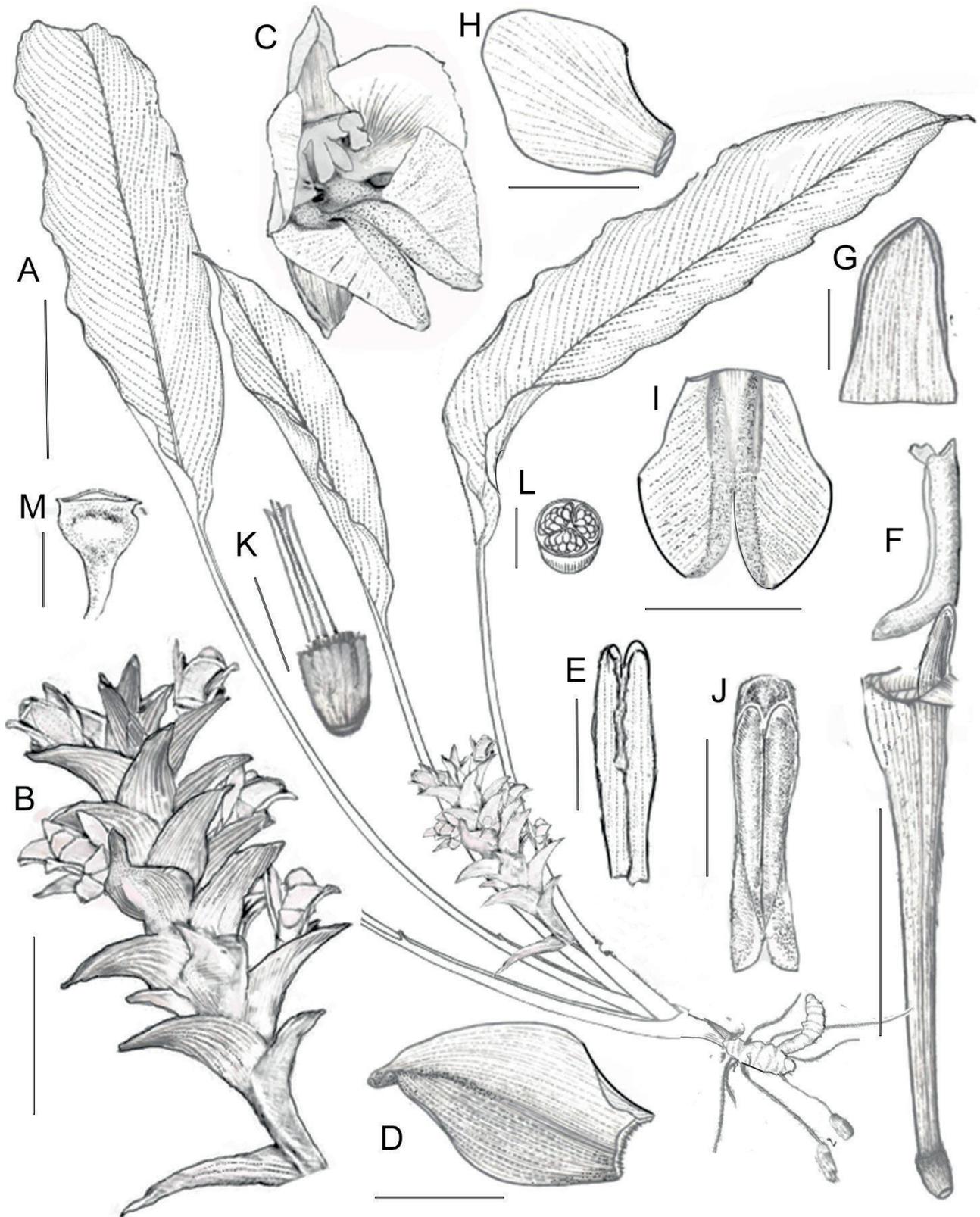


Figure 4. The drawing of *Curcuma tuanii*. **A.** Flowering habit with rhizomes and leaves. **B.** Inflorescent with opening flowers. **C.** Flower in frontal view. **D.** Bract. **E.** Calyx. **F.** Anther in side view attached with filamen, floral tube and ovary. **G.** Corolla dorsal lobes. **H.** Lateral staminode. **I.** Labellum. **J.** Anther in frontal view. **K.** Epigynous glands and ovary. **L.** Ovary (cross section). **M.** Stigma. A: 10 cm, B = 5 cm, D = 2 cm, E = 1 cm, F = 1.5 cm, G = 1 cm, I = 1 cm, J = 5 mm, K = 5 mm, L = 3 mm, M = 1.5 mm. Drawn by: Danh Duc Nguyen.

Curcuma tuanii (Zingiberaceae) a new species of subgenus *Ecomata* from northern Vietnam based on morphological and molecular evidence

facing forward; **ovary** ca. 3–5 × 2–4 mm, 3-locular, white, densely pilose. **Fruits** not seen.

Habitat, ecology, and phenology:—Shady lowland forests at elevations 150–200 m, usually along stream valleys. Flowering in June – October.

Distribution and IUCN preliminary assessment:—*Curcuma tuanii* has only been recorded in north Vietnam. The distribution range of *C. tuanii* is located in northern Vietnam and well isolated from populations of relative species in Central and South Vietnam (Fig. 5). The distribution ranges of these four relative taxa are nearby but do not overlap. *Curcuma sahuynhensis* and *C. pambrosima* distributions are limited to the eastern coast of South Central. Whereas, *C. cotuana* and *C. vitellina* occur in the western montane forest of Central and South Vietnam, respectively.

According to the data obtained from local people, at the type locality in Hoa Binh Province where we have counted about 500 adult individuals, this new species forms several subpopulations within the area of Hien Luong Commune (Hoa Binh Province, Da Bac District) with no imminent threat. The forest, where this species grows, is protected by local community. Two paratype specimens of this species were collected from plants cultivated in Nghe An and Ha Tinh Provinces by local people from the wild population in Can Loc District, Ha Tinh Province, near border to Nghe An Province. We propose conservation status for the new species as Data Deficient (DD) following the IUCN criteria (2022) due to the lack of verified data on its current distribution and population size.

Additional specimens examined (Paratypes): VIETNAM. Nghe An Province: Vinh City, Ha Huy Tap War, elevation 50 m, cultivated and collected by Oanh Trần, 10 July 2021, NHTuan 056 (HNU!). Ha Tinh Province, Can Loc District, Truong Loc community, collected from plants in the anthesis cultivated by Le Viet Hung in the private garden, 15 September 2022, NDD 308 (VNMN!).

Anatomical feature of *Curcuma tuanii*

Anatomical characters of leaf:—Transverse sections of *C. tuanii* leaf are symmetrical, passing through a midrib and lamina are shown in Fig. 6.

Midrib and vascular bundles: **The epidermis** is a single-layer tissue consisting of polygon-shaped cells, whose walls vary in size and are totally soaked with cellulose. **Short unicellular trichomes** are observed sparsely. **The upper collenchyma** consists of polygon-shaped and diverse-magnitude cells, which are arranged tightly into 5–15 layers. In the leaf midrib, ground tissue is made up of parenchyma cells which are thin-walled and polygonal. Some cells contain oil globules or oleoresin. The upper parenchyma arranged tightly into 5–15 layers. Whereas the lower one was 3–5. **Vascular bundles** are the closed type with 1–3 metaxylem vessels

and phloem forming a cap-like structure. 3–5 layers of fibrous sclerenchymatous cells are observed below large veins. In this each vascular bundle, the xylem is located in the superior position while the phloem is located in the inferior site. It is covered by a ring of the irregularly-sized sclerenchymatous sheath, which included 3–5 layers of lignified polygonal cells. **Xylem** included 1–3 primary veins and 2–4 secondary veins, which are nearly round-shaped, lignified, and randomly distributed. **Phloem** is larger than xylems in every bundle and consists of cellulose-covered polygonal cells, which are made up of 7–10 layers. There are two types of vascular bundles that differ in size and position: 10–13 larger vascular bundles formed a single conspicuous abaxial arc toward the lower epidermis, alternating with air chambers and embedded in collenchyma observed. Smaller vascular bundles are scattered in ground tissue. Where as, the central midrib has 4–6 smaller phloem-xylem bundles scattered in the upper parenchyma. *The air chamber* is a kind of aerenchyma formed through a tight arrangement of about 30–40 circular-shaped and unequal-size cells. Leaf blade: A transverse section through the leaf blade is composed of 5–7 layers of **collenchymatous cells**. The adaxial and abaxial **epidermis** consisted of a single layer, polygonal-slightly elongated cells. It is covered with a moderately thin cuticle. Below both of the epidermal layers, there are thin-walled, large, irregular polygonal cells of single layer **hypodermis**. The layers of **palisade cells** are observed on the upper surface continuously in leaf lamina but observed on the ventral side where the main vascular bundle, towards ventral side. Beneath the palisade mesophyll is the spongy mesophyll cells consisting of 3–5 layers, with irregular shapes. Oleoresin cells are scattered in layers of mesophyll cells.

Anatomical characters of the rhizome:—Transverse section of the rhizome is circular in outline. The outermost layer is the periderm which consists of more layers of rectangular and tangentially elongated cells. The outer layer is a slightly cutinized epidermis, which is built by a single rectangular or polyhedral cell layer; the exodermis (suberized tissue) is located under the epidermis, consisting of 1–3 layers of polygonal cells, scramble; the third layer is a multilayered hypodermis consisting of 4–6 rectangular or polygonal layers. Followed to these is a wide cortex with irregularly scattered vascular bundles. Each vascular bundle is nearly circular and composed of groups of xylem entirely surrounded by phloem. **Xylem** consists of parenchyma, vessels, tracheids, and fibers. Metaxylem consists of 1–2(–4) lignified polygonal vessels and their magnitudes are 1–2 times larger than protoxylem. Where as, the protoxylem consists of 2–3(–5) lignified polygonal vessels. **Phloem** is seen surrounding the xylem and consists of polyhedral cells with curved cellulose walls arranged into clusters. The sclerenchymatous sheath



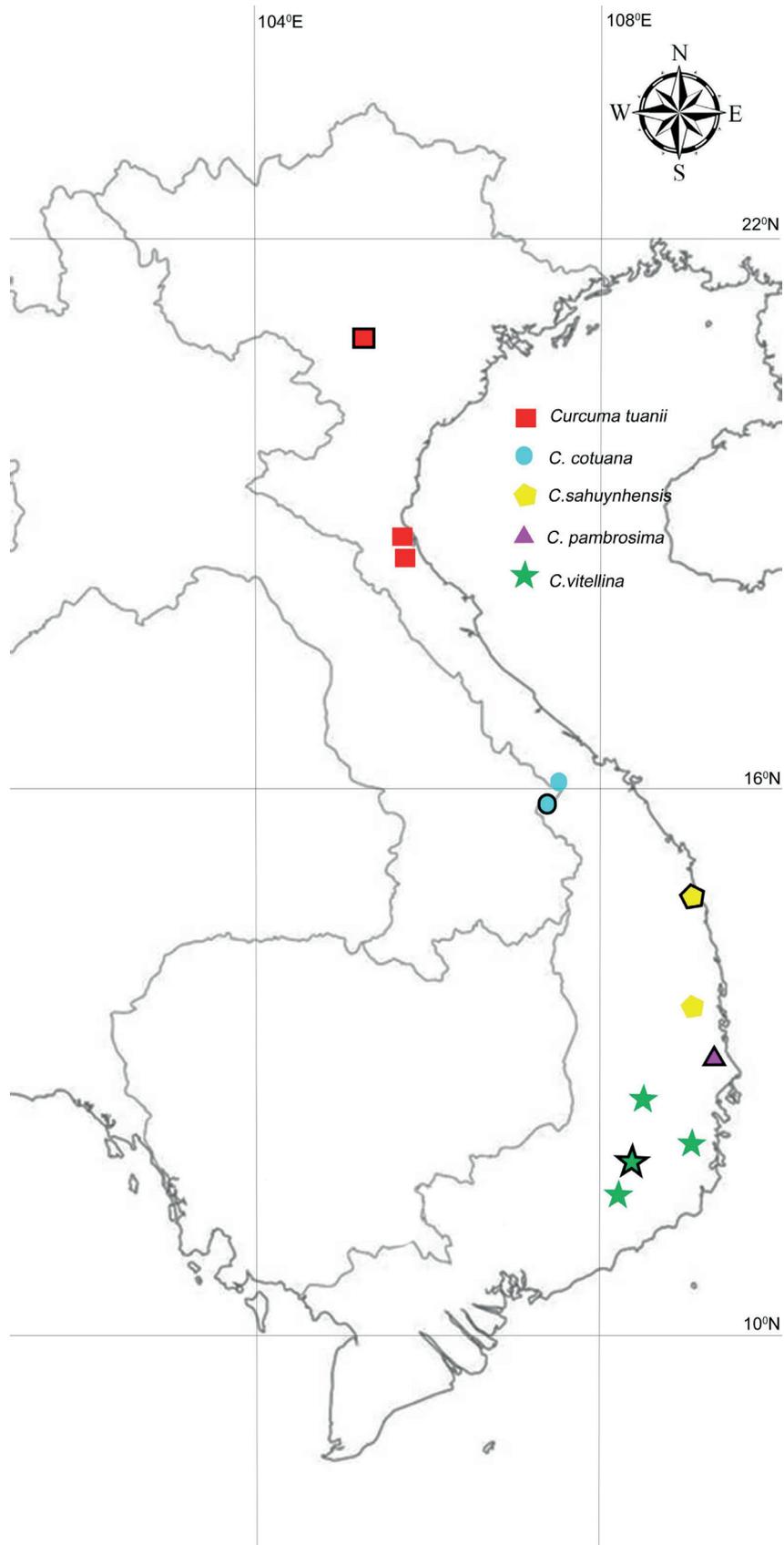


Figure 5. Distributions of *Curcuma tuanii* sp. nov. and its closest allies based on study of herbarium and field records of Vietnamese botanists. *Curcuma tuanii* (red square), *C. sahuynhensis* (yellow pentagon), *C. cotuana* (blue circle), *C. vitellina* (green star), *C. pambrosima* (purple triangle). The type locality marked by black border.

Curcuma tuanii (Zingiberaceae) a new species of subgenus *Ecomata* from northern Vietnam based on morphological and molecular evidence

around the vascular bundles is absent. There is a single-layered endodermis called **Casparian strips** composed of thin-walled rectangular cells separating the cortical region and stellar region. In the **stellar region**, inner vascular bundles are similar to those in the cortex and also present scattered inside. **Parenchymatous**

cells in both regions are thin-walled, polygonal to circular, and also filled with starch grains. Some of these reproductive cells contain deposition of orange-red substance oleoresin. Numerous **oleoresin cells** are scattered throughout the rhizome. Some other cells contain pale yellow **oil globules** (Fig. 7).

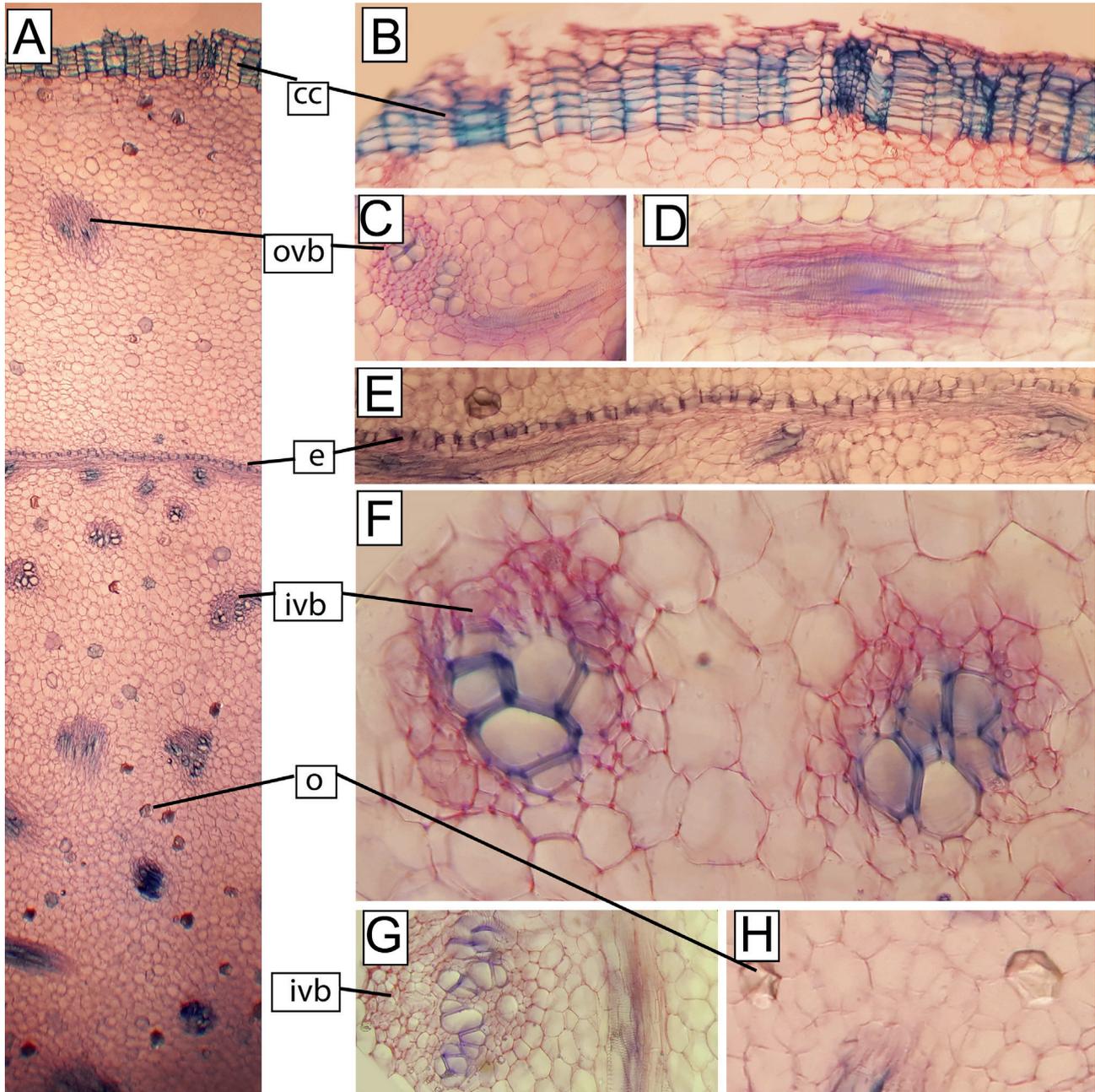


Figure 6. Transverse section of midrib: **A.** overall view, **B.** upper epidermis layer, **C.** a unit of the single conspicuous abaxial arc including vascular bundle, lignified pericyclic fiber, and air chamber, **D.** detail of a vascular bundle scattered in ground tissue, **E.** detail of a main vascular bundle in the single conspicuous abaxial arc. Transverse section of leaf passing through lamina: **F.** with lower epidermis & **G.** with upper epidermis. Note: e: epidermis, t: trichomes, x: xylem, p: phloem, vb: vascular bundle, lpf: Lignified pericyclic fiber (along with vascular bundle/ sclerenchymatous cells), pc: parenchymatous cells, h: hypodermis, pm: palisade mesophyll, o: oleoresin/oil globules, sm: spongy mesophyll, ac: air chamber. Photos by Nguyen Ngoc Anh, correction and design by Hoang Tuan Nguyen & Danh Duc Nguyen.



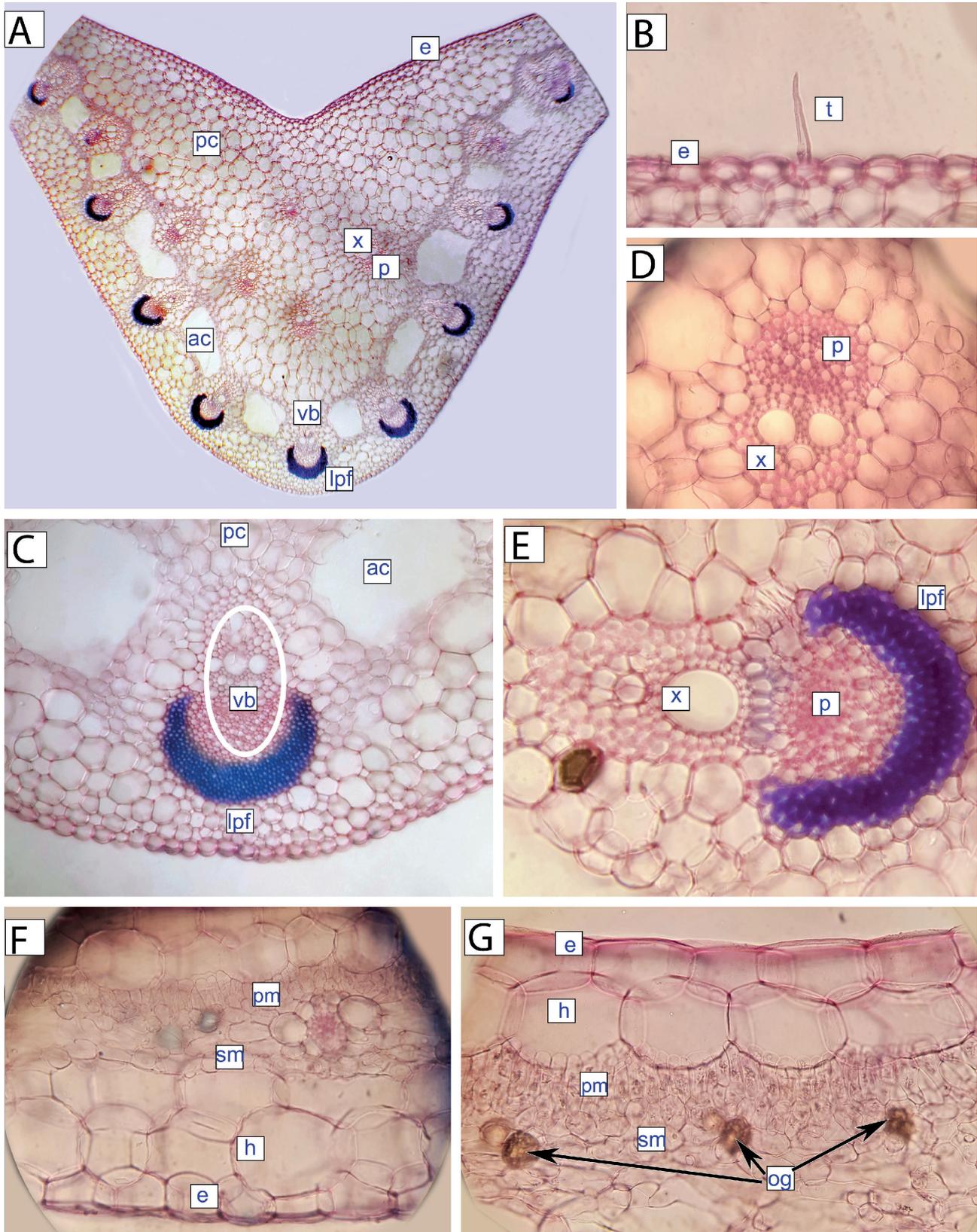


Figure 7. Transverse section of rhizome. **A.** overall view, **B.** detail of the outermost layer, **C, D.** detail of vascular bundles in the outer cortical region, **E.** Detail of endodermis layer, **F, G.** detail of vascular bundles in the stellar region, **H.** oleoresin in stellar region. Notes: cc: cork cell, ovb: outer vascular bundle, Ca: Casparian strip, ivb: inner vascular bundle, o: oleoresin. Photos by Nguyen Ngoc Anh, correction and design by Hoang Tuan Nguyen & Danh Duc Nguyen.

Supplementary material

The following online material are available for this article: Table S1. Voucher information and GenBank accession numbers for DNA sequences generated or used in this study. “–” indicates missing data, and the sequences generated in this study begin with OR.

Figure S1. Majority rule consensus tree with branch lengths from the Bayesian inference based on the combined datasets of four DNA regions (ITS, *matK*, *psbA-trnH*, and *trnL-F*). Posterior probabilities (PP) of the BI analysis are presented at the nodes.

References

- Beentje H. 2016. The Kew plant glossary: An illustrated dictionary of plant terms. 2nd. edn. Kew, Royal Botanic Gardens Kew.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9: 772.
- Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182-192.
- IUCN – International Union for Conservation of Nature’s. 2022. Guidelines for using the IUCN red list categories and criteria, version 15. Standards and Petitions Committee. <https://www.iucnredlist.org/documents/RedListGuidelines.pdf>. 17 May 2022.
- Kearse M, Moir R, Wilson A *et al.* 2012. Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.
- Leong-Škorničková J, Ly NS, Nguyen QB. 2015b. *Curcuma arida* and *C. sahuynhensis*, two new species from subgenus *Ecomata* (Zingiberaceae) from Vietnam. *Phytotaxa* 192: 181-189.
- Leong-Škorničková J, Ly NS. 2010. *Curcuma pambrosima* sp. nov. (Zingiberaceae) from central Vietnam. *Nordic Journal of Botany* 28: 652-655.
- Leong-Škorničková J, Šída O, Bouamanivong S, Souvannakhoummane K, Phathavong K. 2014. Three new ginger species (Zingiberaceae) from Laos. *Blumea* 59: 106-112.
- Leong-Škorničková J, Šída O, Jarolímová V *et al.* 2007. Chromosome numbers and genome size variation in Indian species of *Curcuma* (Zingiberaceae). *Annals of Botany* 100: 505-526.
- Leong-Škorničková J, Šída O, Závěská E, Marhold K. 2008. Taxonomic and nomenclatural puzzles in Indian *Curcuma*: The identity and nomenclatural history of *C. zedoaria* (Christm.) Roscoe and *C. zerumbet* Roxb. (Zingiberaceae). *Taxon* 57: 949-962.
- Leong-Škorničková J, Šída O, Závěská E, Marhold K. 2015a. History of infrageneric classification, typification of supraspecific names, and outstanding transfers in *Curcuma* (Zingiberaceae). *Taxon* 64: 362-373.
- Leong-Škorničková J, Tran HD, Newman MF. 2010. *Curcuma vitellina* (Zingiberaceae), a new species from Vietnam. *The Gardens’ Bulletin, Singapore* 62: 111-117.
- Leong-Škorničková J, Tran HD. 2013. Two new species of *Curcuma* subgen. *Ecomata* (Zingiberaceae) from southern Vietnam. *The Gardens’ Bulletin, Singapore* 65: 169-180.
- Luu HT, Tran HD, Nguyen TQT, Leong-Škorničková J. 2017. *Curcuma cotuana* sp. nov. (Zingiberaceae: Zingibereae) from central Vietnam. *Nordic Journal of Botany* 35: 552-556.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments workshop (GCE). New Orleans, USA, Institute of Electrical and Electronics Engineers (IEEE). p. 1-8.
- Nguyen DD, Le TA, Hoang QH, Le QT, Nguyen E. 2022. Two new taxa of *Curcuma* sect. *Ecomata* (Zingiberaceae: Zingibereae), from coastal Central Vietnam. *Biodiversitas* 23: 2512-2519.
- Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v1.6. <http://tree.bio.ed.ac.uk/software/tracer/>. 2 May 2014.
- Rambaut A. 2009. FigTree v.1.4. <http://tree.bio.ed.ac.uk/software/figtree/>. 21 Dec. 2009.
- Ronquist F, Teslenko M, Mark PVD *et al.* 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542.
- Saensouk S, Boonma T, Saensouk P. 2022. *Curcuma pulcherrima* (Zingiberaceae), a new rare species of *Curcuma* subgen. *Ecomata* from eastern Thailand. *Biodiversitas* 23: 6635-6644.
- Souvann KK, Maknoi C. 2014. *Curcuma peramoena* Souvann & Maknoi (Zingiberaceae): A new species from Lao PDR. *Thai Journal of Botany* 6: 125-130.
- Stamatakis A. 2006. RAxML-VI-HPC, maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.
- Závěská E, Fér T, Šída O, Krak K, Marhold K, Leong-Škorničková J. 2012. Phylogeny of *Curcuma* (Zingiberaceae) based on plastid and nuclear sequences: Proposal of the new subgenus *Ecomata*. *Taxon* 61: 747-763.

