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Cayratia cheniana (Vitaceae): An Endangered New Species Endemic to the Limestone Mountains of Ninh Thuan Province, Vietnam

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Abstract—Considerable numbers of species have become extinct before being scientifically described, and the loss of natural habitats is known to be one of the major reasons for biodiversity declines. In this study, an endangered new species, ***Cayratia cheniana*** L. M. Lu & J. Wen, endemic to the limestone mountains of Ninh Thuan province of Vietnam is herein described based on both morphological and molecular evidence. With five chloroplast markers, ***Cayratia cheniana*** is placed as the first diverged lineage of *Cayratia* Juss. Morphologically, ***Cayratia cheniana*** shares synapomorphies with all other *Cayratia* s. s. in possessing a membrane enclosing ventral infolds in the seeds and presence of bracts on the lower part of the inflorescence axis. This species can be readily distinguished from other congeners by its tripalmate-trifoliate to bipinnate leaf architecture and stems with shredding bark.

Keywords—Biodiversity conservation, chloroplast DNA, critically endangered species, habitat loss.

Understanding the diversity of life on the planet has been one of the major interests of naturalists since the 1700s (Lomolino et al. 2010; Wen et al. 2013b). Many new life forms continue to be discovered and described (SA2000 1994; Wen et al. 2015a). However, global ecosystems have been substantially disturbed due to impacts of human activities in recent decades. Considerable numbers of species have become extinct or endangered before being described (Wilson 1992; Liu et al. 2013). Loss of natural habitats is one of the major reasons attributed to a decline in biodiversity. *Cayratia* Juss. (Vitaceae) occurs in various habitats including tropical rainforests, temperate deciduous forests, limestone areas, and savannas. Species restricted to limestone or savannah areas are extremely sensitive to environmental changes and are more susceptible to habitat destruction. Here we describe an endangered new species of *Cayratia* endemic to the limestone mountains of Ninh Thuan province, Vietnam based on both morphological and molecular evidence.

The genus *Cayratia* in the traditional sense consists of approximately 60 species, which are widely distributed in the Old World tropics (Galet 1967; Wen 2007). Species of *Cayratia* are usually herbaceous or woody climbers that are hermaphroditic to andromonoecious and sometimes with tuberous underground systems. They possess 4-merous flowers, and axillary, pseudo-axillary or leaf-opposed inflorescences. The generic name *Cayratia* was established by Jussieu (1818) and conserved in favor of an earlier name, *Columella* Lour. (Loureiro 1790). Planchon (1887) later treated *Cayratia* as a section of *Cissus* L., i.e. *Cissus* sect. *Cayratia* (Juss.) Planch. However, Gagnepain (1911) argued for restoring its generic rank based on its compound leaves (vs. mostly simple leaves in *Cissus*), axillary or pseudo-terminal inflorescences (vs. leaf-opposed in *Cissus*), relatively thinner floral disk (vs. much thicker in *Cissus*), and two to four seeds per fruit (vs. 1-seeded fruits in *Cissus*). *Columella* was sometimes adopted as the generic name for taxa of *Cayratia* because of the former's nomenclatural priority over *Cayratia* (e.g. in Merrill 1916). *Cayratia* was conserved as the generic name at the Cambridge International Botanical Congress in 1930 (Merrill 1935). Süssenguth (1953) classified *Cayratia* into two sections: sect. *Koilosperma* Suess. and sect. *Discypharia* Suess., which was followed by Latiff (1981). Li (1998) divided the Chinese *Cayratia* into two subgenera: subg. *Cayratia* and subg. *Discypharia* (Suess.) C. L. Li based on seed morphology and inflorescence structure.

Generic circumscription and concepts of character evolution in Vitaceae have changed substantially with recent progress in understanding the molecular phylogenetics and character analyses of the family in a phylogenetic framework (Jackes and Rossetto 2006; Wen et al. 2013a, 2013c, 2014, 2015b; Ickert-Bond et al. 2015; Zhang et al. 2015). Previous phylogenetic studies of Vitaceae revealed that *Cayratia* was not monophyletic and that at least some members have a close relationship with *Cyphostemma* (Planch.) Alston and *Tetrastigma* (Miq.) Planch. (Soejima and Wen 2006; Rossetto et al. 2007; Wen et al. 2007; Chen et al. 2011; Ren et al. 2011; Trias-Blasi et al. 2012). With the expanded taxon sampling of *Cayratia* and its allied genera, Lu et al. (2013) resolved *Cayratia* into three lineages (the African clade of *Cayratia*, subg. *Cayratia*, and subg. *Discypharia*) and recommended splitting *Cayratia* s. l. into three genera. Recently, Wen et al. (2013a) treated taxa in *Cayratia* subg. *Discypharia* (seeds without a membrane covering the ventral infolds) as members of a segregate genus, *Causonis* Raf., and made two new combinations of the genus distributed in the Philippines: *Causonis corniculata* (Benth.) J. Wen & L. M. Lu and *C. pterita* (Merr.) J. Wen & L. M. Lu. At the recent Botany 2015 meetings held in Edmonton, Alberta, Canada, Wen et al. (2015b) proposed a revised classification of Vitaceae splitting the traditional concept of *Cayratia* s. l. into three genera: the new genus consisting of the African species of *Cayratia*, the resurrected *Causonis* Raf. and *Cayratia* s. s. (also see Moore and Wen, in press).

The new species described here is endemic to the limestone mountains of Ninh Thuan province of Vietnam, and possesses leaf-opposed tendrils, axillary inflorescences, 4-merous flowers with well-developed disks, and undivided stigmas. Furthermore, this species has seeds with a membrane enclosing

the ventral infolds and has bracts on the lower part of the inflorescence axis. These characteristics firmly place the new species in *Cayratia* s. s. following the recently reduced circumscription of the genus (Wen et al. 2013a, 2015b; Moore and Wen, in press).

Materials and Methods

Morphological Observation—Specimens of *Cayratia* from several major herbaria in China (KUN, PE) and abroad (E, HN, K, MO, P, and US) were examined. Terminology used to describe the unusual leaf division in this species follows Jiménez-Saa (2011). Seed morphology was

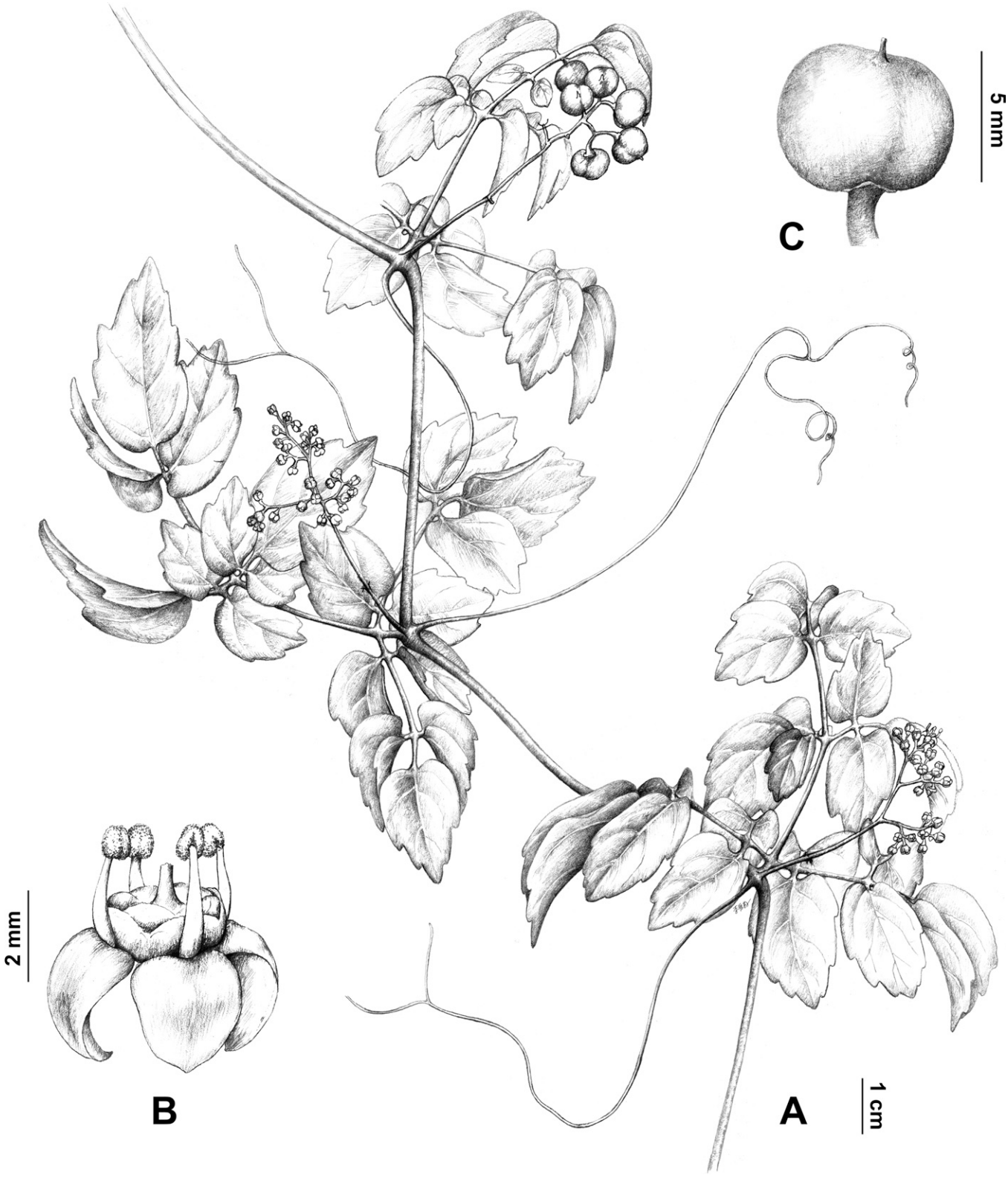

Fig. 1. *Cayratia cheniana* L. M. Lu & J. Wen. A. Flowering branch. B. Flower. C. Fruit (drawn by Ai-Li Li; Based on *Z. D. Chen, J. B. Zhang* & *L. M. Lu 565*, PE).

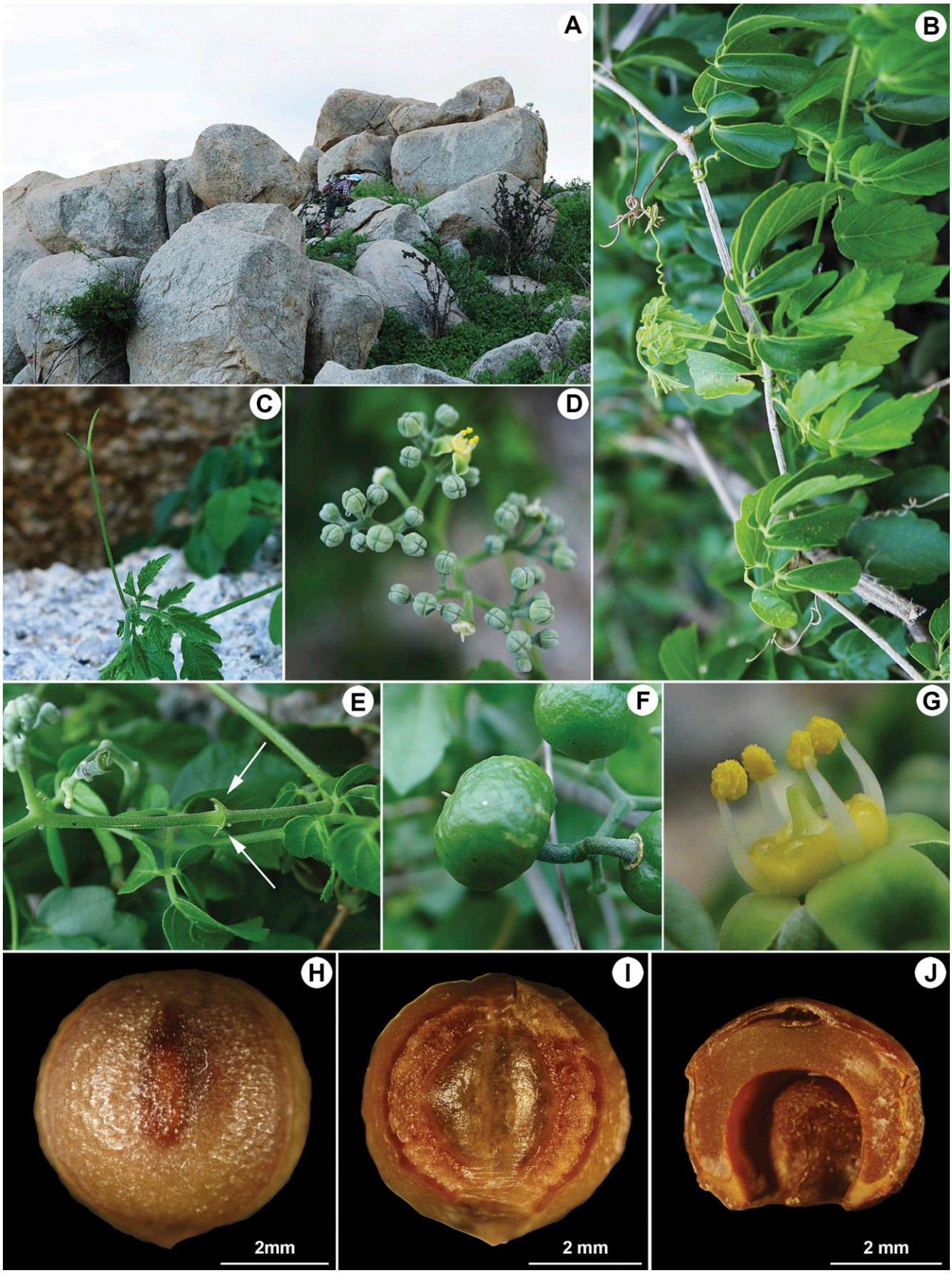

FIG. 2. *Cayratia cheniana* L. M. Lu & J. Wen. A. Limestone habitat. B. Leaves and stem. C. Bifid tendril. D. Inflorescence. E. Presence of bracts on inflorescence axis. F. Fruit. G. Flower. H–J. Dorsal (H) and ventral (I) views and transverse section (J) of seed.

observed and photographed with a stereomicroscope (a Nikon SMZ1000 with a Nikon DXM 1200F digital camera) in the State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences.

Phylogenetic Analyses—Five new sequences (*atpB-rbcL*: KU167491, *rps16*: KU167492, *trnC-petN*: KU167493, *trnH-psbA*: KU167494, and *trnL-F*: KU167495) were generated and the phylogenetic position of the new species was examined in a global sampling scheme from Lu et al. (2013). Sequence assemblage and alignment followed the protocols described in Lu et al. (2013).

The combined chloroplast markers were analyzed with the maximum likelihood (ML) and Bayesian inference (BI) methods. The ML analyses were conducted in RAxML 8.1.11 (Stamatakis 2006) and GARLI 2.1 (Zwickl 2006), applying 1,000 bootstrap replicates with the substitution model selected in MrModeltest 2.3 (Nylander 2004). A partitioned Bayesian analysis was conducted in MrBayes 3.2.6 (Ronquist et al. 2012) as implemented on the CIPRES Science Gateway Portal (Miller et al. 2010). Parameter configuration and convergence estimation followed Lu et al. (2013).

Results

Phylogenetic Position—The new species was identified as a *Cayratia* in the field based on its leaf-opposed tendrils, axillary inflorescences, 4-merous flowers, and undivided stigmas (Fig. 1). In addition, the species has a pair of bracts on the lower part of the inflorescence axis (indicated by arrows in Fig. 2E) and seeds with a membrane enclosing the ventral infolds (Fig. 2I). These characteristics supported it as a member of the newly circumscribed genus *Cayratia* s. s. Based on molecular data, both the ML and BI analyses

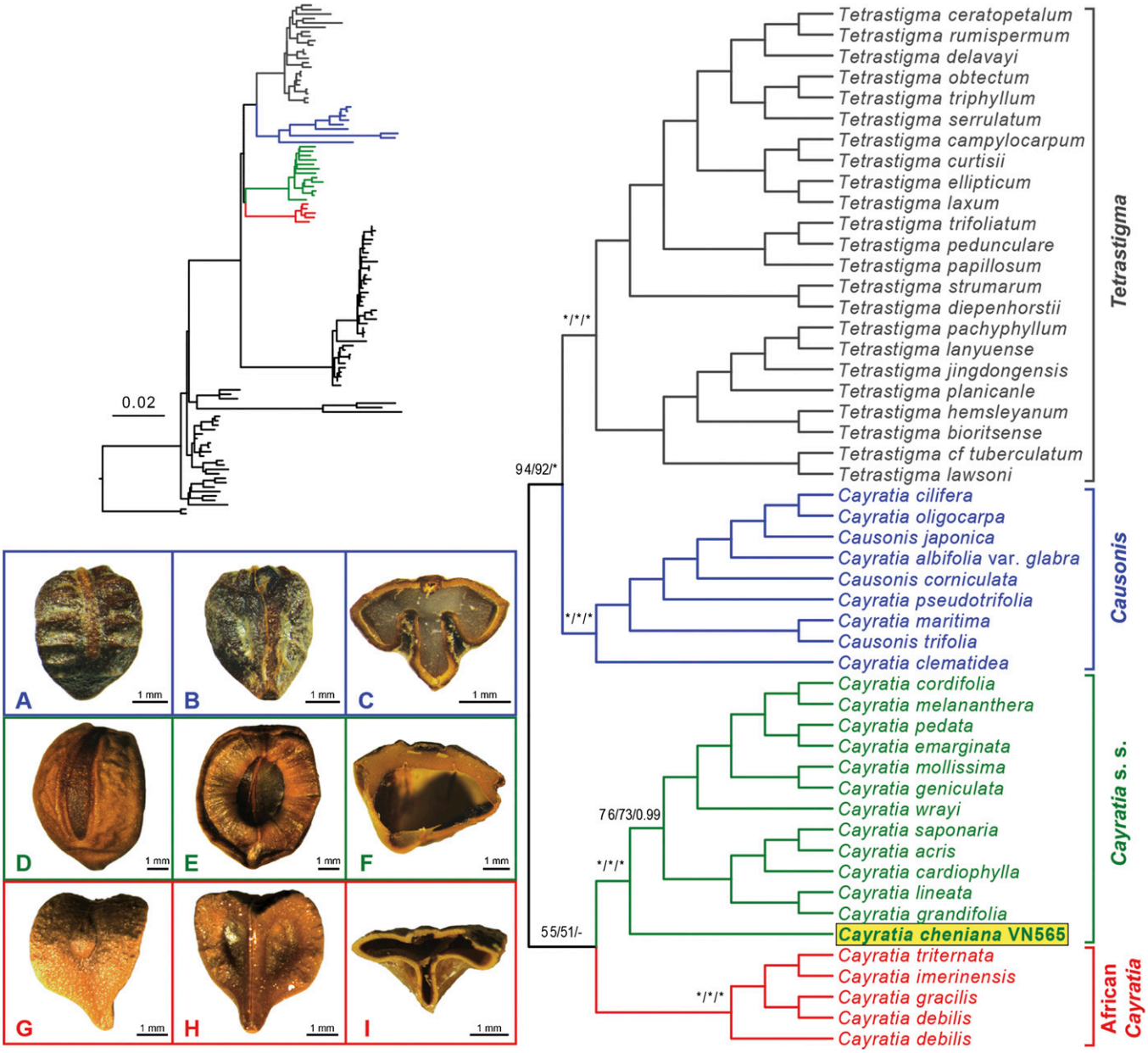

Fig. 3. Maximum likelihood tree for Vitaceae based on the combined chloroplast data sets (*atpB-rbcL*, *rps16*, *trnC-petN*, *trnH-psbA* and *trnL-F*). A phylogram overview is shown in the upper left-hand corner. Three major clades of *Cayratia* s. l. in the traditional sense are colored with blue (*Causonis*), green (*Cayratia* s. s.), and red (the *African Cayratia*), respectively. The newly described species *Cayratia cheniana* is highlighted in yellow. Bootstrap values (RAxML and GARLI) and Bayesian posterior probability values of major clades and key nodes are indicated above branches. Dorsal, ventral views and transverse sections of seeds of *Causonis* (A–C), *Cayratia* s. s. (D–F), and the *African Cayratia* (G–I) are provided in the bottom left-hand corner.

generated congruent topology and the ML tree with bootstrap and Bayesian posterior probability values is shown in Fig. 3. Similar to our previous analyses, *Cayratia* s. l. was not monophyletic but three well-supported clades were detected: the African *Cayratia*, subg. *Cayratia*, and subg. *Discypharia*, corresponding to the three genera proposed by Wen et al. (2015b). The new species, *Cayratia cheniana*, is resolved as sister to the clade of the remaining species of *Cayratia* s. s. (RAxML BS = 76%, GARLI BS = 73%, PP = 0.99). Leaf architecture has been highlighted in traditional infrageneric classifications of Vitaceae (Li 1998; Chen et al. 2007). *Cayratia* s. l. in the traditional sense has trifoliate, digitate, and pedate leaf architecture throughout its distribution range. However, species with pinnate leaves are restricted to Madagascar,

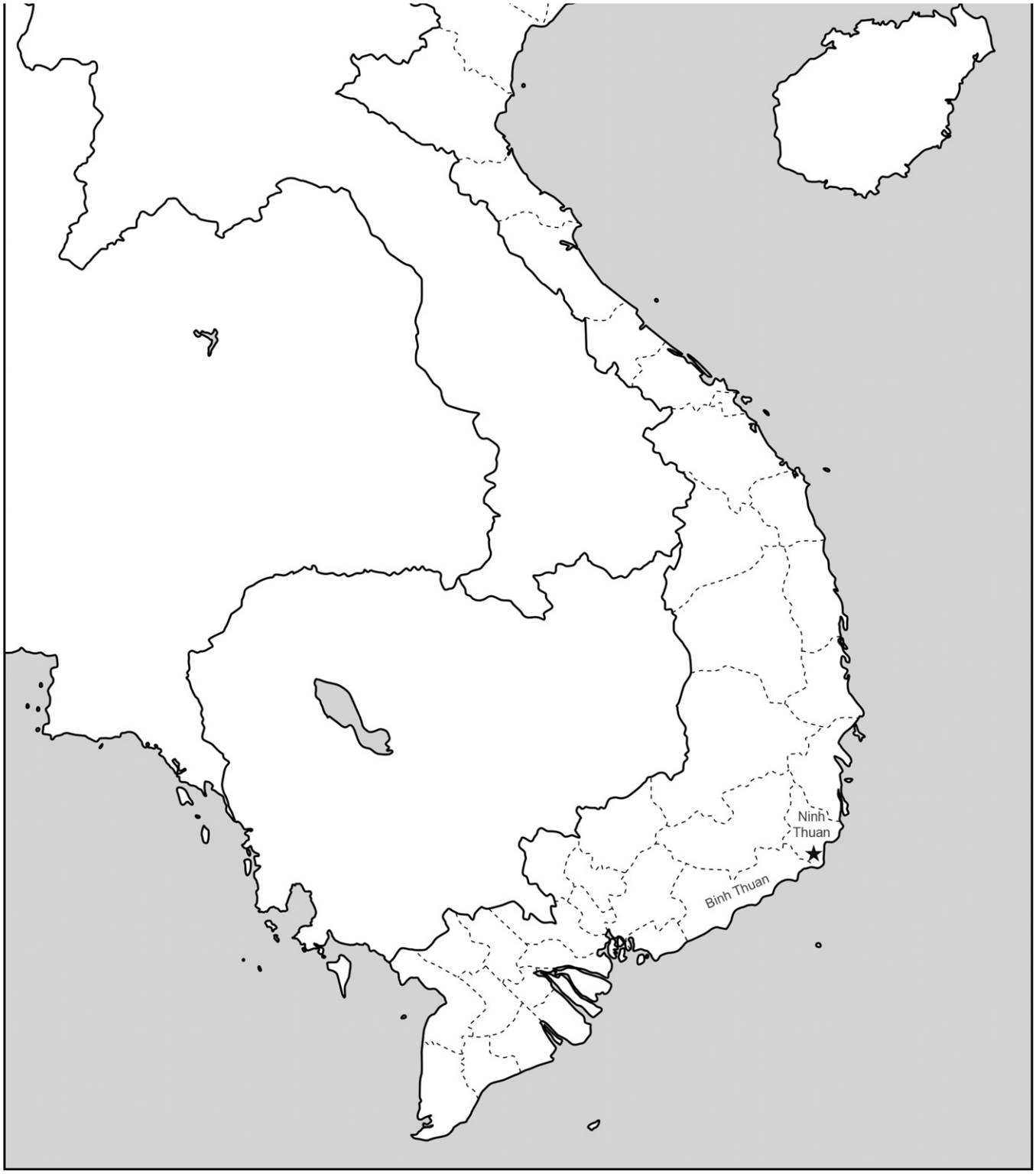

Fig. 4. Distribution of *Cayratia cheniana* L. M. Lu & J. Wen in Vietnam, indicated by a black asterisk.

which were recognized as belonging to a distinct clade of the newly proposed segregate genus (Lu et al. 2013; Wen et al. 2015b). The new species, endemic to the limestone mountains of Vietnam, has a tripalmate-trifoliate to bipinnate leaf architecture. As the first diverged lineage of *Cayratia* s. s., the new species plays an important role in understanding the morphological evolution and biogeographic dispersal of *Cayratia* s. l.

Taxonomic Treatment

Cayratia cheniana L. M. Lu & J. Wen, sp. nov.—TYPE: VIETNAM. Ninh Thuan province: Cana, limestone mountains, alt. 120 m, 11°20′27.74″N, 108°52′20.97″E, 18 Aug 2013, *Z. D. Chen, J. B. Zhang & L. M. Lu 565* (holotype: PE!; isotypes: PE!, US!).

The new species differs from other known *Cayratia* in its tripalmate-trifoliate to bipinnate leaf architecture and old stems with shredding bark.

Lianas, woody. Branchlets terete; stems green with dense hairs when young, later turning to brownish or gray-brownish, with conspicuous lenticels, bark eventually shredding. Tendrils leaf-opposed, bifurcate and lacking adhesive discs. Leaves mostly tripalmate-trifoliolate, sometimes bipinnate; petiole 5–8 cm; central petiolule 0.5–1 cm, pilose, petiolules of lateral leaflet 0.1–0.3 cm; leaflet oblong or ovate-elliptic, 0.8–2.8 × 0.4–1.6 cm, leaf blade abaxially light green, adaxially dark green, pubescent on both surfaces, lateral veins 3–5 pairs, veinlets slightly raised, base slightly cordate or subtruncate, apex acute, margin undulate with 2–5 rounded teeth on each side. Inflorescence a compound cyme, axillary; peduncle 4–6 cm, densely pubescent; bracts present on lower part of inflorescence axis; pedicel 3–5 mm, pubescent. Calyx cupular, entire, pubescent. Petals 4, oval, 1.5–2 mm, with papillose hairs. Stamens 4, anthers yellow, oval; floral disk well developed, margin undulate. Ovary with its lower part adnate to disk; style short, 1–1.5 mm long at anthesis. Fruit a 2–3-seeded berry, globose, 4–7 mm in diam. Seeds semi-globose, ca. 1.7–4.7 mm in diam., chalaza linear, with a membrane covering the pair of ventral infolds.

Distribution and Habitat—The new species is only known from its type locality in the limestone mountains of Ninh Thuan, Vietnam (Fig. 4).

Etymology—The epithet "*cheniana*" is to honor the first author Limin Lu's supervisor, Zhiduan Chen, who first discovered the new species and has made significant contributions to Vitaceae collections all over the world.

Conservation Status—*Cayratia cheniana* is only known from its type locality and is critically endangered (CR) according to the IUCN red list criteria (IUCN 2012).

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