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A New Species and New Records of *Cyphostemma* (Vitaceae) from China and Vietnam Based on Morphological and Molecular Evidence

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Abstract—A new species, *Cyphostemma dehongense*, is described from Dehong, Yunnan, China, and *C. auriculatum* is newly recorded from Vietnam. These are the first records of the genus *Cyphostemma* from the two countries, extending both the easternmost and northernmost distribution limit of *Cyphostemma*. Through field explorations over two years, we discovered four populations of *C. dehongense* and collected specimens of the species with both flowers and fruits. *Cyphostemma dehongense* and *C. auriculatum* are morphologically similar, but differ in ovary size, style length, and fruit size. In addition to morphological comparisons, molecular data were used to determine the phylogenetic position of the new species and the newly recorded taxon. Ten nucleotide variation sites were detected between *C. dehongense* and *C. auriculatum* based on five chloroplast loci (*atpB-rbcL*, *rps16*, *trnC-petN*, *trnH-psbA*, and *trnL-F*) and the nuclear ribosomal internal transcribed spacer (ITS). An identification key is provided for the three species of *Cyphostemma* from Asia.

Keywords—*Cyphostemma auriculatum*, *C. dehongense*, distribution range, taxonomy, Yunnan, Son La.

Southern China and the adjacent Southeast Asian countries including Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, and Vietnam, are recognized as a center of diversity for some genera of the grape family (Vitaceae), including *Cayratia* Juss. and *Tetrastigma* (Miq.) Planch. (Chen et al. 2007; Wen 2007). In the last decade, many new species and new records of species and genera of Vitaceae have been discovered from these regions (Trias-Blasi et al. 2010, 2011, 2014, 2015a, b; Wen et al. 2013; Kochaiphat et al. 2014, 2016; Cho et al. 2016; Dang et al. 2016; Lu et al. 2016). Some of these taxa are evolutionarily very unique within the Vitaceae. For instance, the endangered new species *Cayratia cheniana* L. M. Lu & J. Wen, endemic to the limestone mountains of Vietnam, was recognized as the sister species of the rest of the genus *Cayratia* (Lu et al. 2016). Furthermore, new generic records have enhanced our understanding of the biogeographic history of Vitaceae. *Cyphostemma auriculatum* (Roxb.) P. Singh & B. V. Shetty has been recorded from Bangladesh, Bhutan, India, Myanmar, and Sri Lanka (Singh and Shetty 1986; Long and Rae 1991; Shetty and Singh 2000). Discovery of *C. auriculatum* in northern Thailand expanded the easternmost distribution limit of *Cyphostemma* (Planch.) Alston (Trias-Blasi et al. 2015a).

Cyphostemma includes ca. 150 species that are mainly distributed in Africa and Madagascar, with only two species reported from India, Sri Lanka, and Thailand (Shetty and Singh 2000; Wen 2007; Trias-Blasi et al. 2015a; Wen et al. 2015b). *Cyphostemma* was treated as a section of *Cissus* L. (sect. *Cyphostemma* Planch.) by Planchon (1887). It was elevated to the rank of genus by later taxonomists based on its unique floral buds with a constriction near the middle, and nectary disk with four free glands (Alston 1931; Descoings 1960). Species of *Cyphostemma* can be found in diverse habitats including tropical rainforests, savannas, and limestone mountains (Wild and Drummond 1966; Verdcourt 1993; Cattabriga 2007). Generally, species in savannas have developed

succulent stems, fleshy leaves, or tuberous roots to adapt to the seasonally arid conditions (Verdcourt 1993). However, species in rainforest habitats usually possess non-fleshy leaves, strong stems, and robust tendrils by which they can climb to the top of the canopy.

Previous molecular phylogenetic studies for Vitaceae, with limited sampling of *Cyphostemma*, supported it as a monophyletic genus (Ingrouille et al. 2002; Soejima and Wen 2006; Wen et al. 2007b; Ren et al. 2011; Trias-Blasi et al. 2012). By sampling 39 representatives of *Cyphostemma* and five chloroplast DNA regions, Lu et al. (2013) confirmed the monophyly of *Cyphostemma* and recognized two major clades within the genus: one including all species from Madagascar, and the other with species mainly from Africa. The only sample from Asia was nested within the African clade.

The new species here described from Dehong, Yunnan province, China and the new record of *C. auriculatum* from Mai Son, Son La province, Vietnam are both lianas that grow in tropical rain/monsoon forests. Both species possess conspicuous stipules and flowers constricted in the middle with a floral disk of four separate glands typical of the genus *Cyphostemma*. However, the plants from China and Vietnam differed from each other in ovary size, style length, and fruit size. Variation in molecular data suggested that they represent different species. The discovery of a new species and generic records suggest that there may still be undescribed diversity in the Vitaceae that requires further field surveys in China. New species of *Cayratia* (Lu et al. 2016) as well as new generic records of *Yua* (Dang et al. 2016) and our discoveries of *Cyphostemma* showcase the importance of continued field collection and taxonomic work in poorly explored regions in the new age of discovery (SA2000 1994; Wen et al. 2015a), especially during preparation of the *Flora of Vietnam*, which includes many novelties (e.g. Farjon et al. 2002; Wen et al. 2007a; Do et al. 2015).

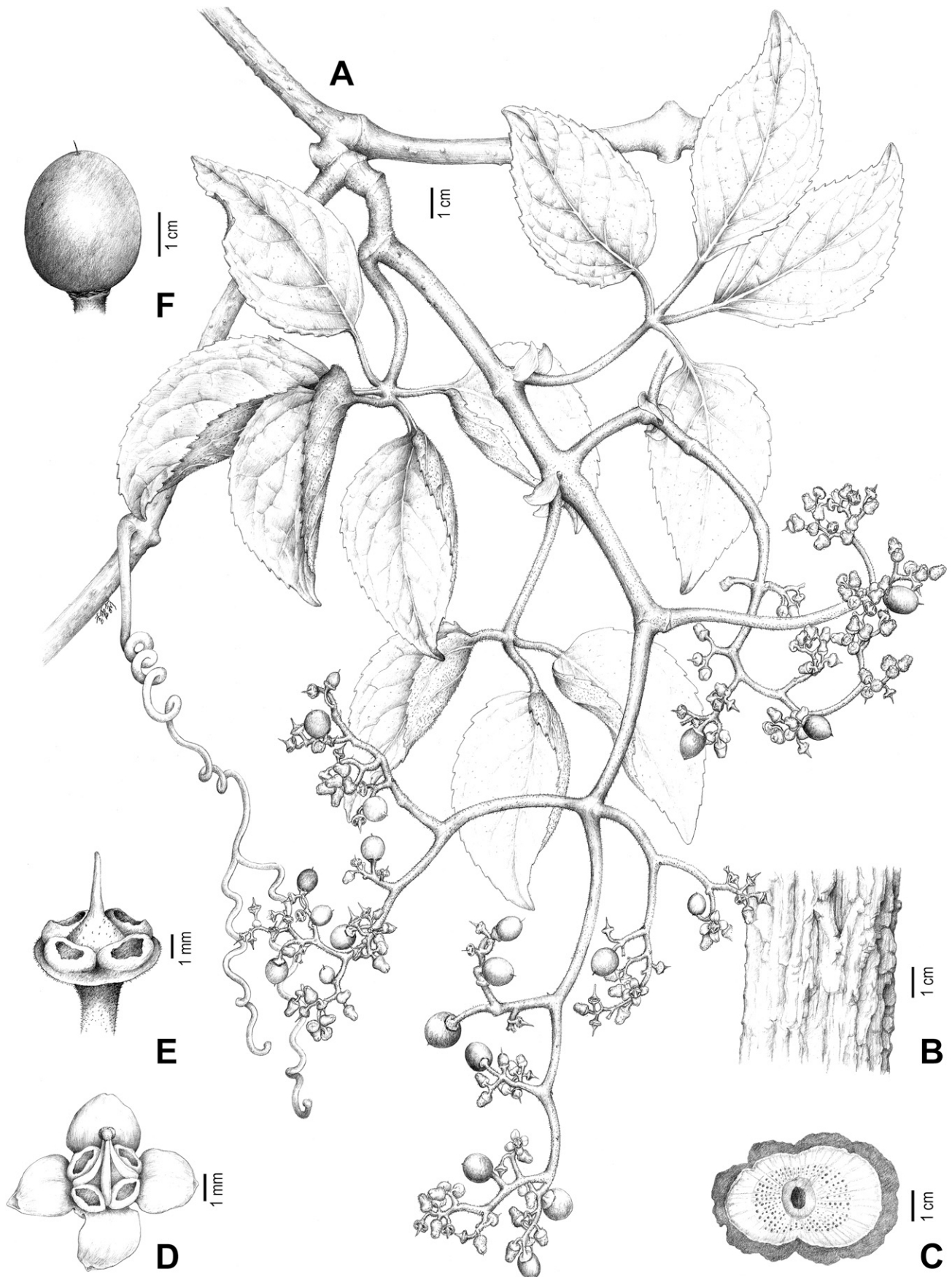


FIG. 1. *Cyphostemma dehongense*. A. Flowering branch. B. Old stem with corky and fissured bark. C. Transverse section of old stem. D. Flower with petals, stamens, and four separate nectary glands. E. Flower with petals and stamens removed to show style and ovary. F. Fruit (Drawn by Ai-Li Li; based on Z. D. Chen YJ28, PE).

MATERIALS AND METHODS

Morphological Observations—Specimens of *Cyphostemma* in several major herbaria in China (PE) and elsewhere (E, HN, K, MO, P, US, and VNM) were examined. Seed morphology was 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, Chinese Academy of Sciences. Terminology for describing leaves and seeds follows Jiménez-Saa (2011) and Chen and Manchester (2007), respectively.

Phylogenetic Analyses—The phylogenetic position of both the new species and the new record was inferred in a global sampling scheme from Lu et al. (2013) using the nuclear ribosomal ITS and five chloroplast DNA

regions (*atpB-rbcL*, *rps16*, *trnC-petN*, *trnH-psbA*, and *trnL-F*). Thirty-six new sequences from six newly collected samples of *Cyphostemma* were generated for this study with the other sequences from Lu et al. (2013, 2017) and Liu et al. (2016) downloaded from GenBank (Appendix 1). Sequence assembly and alignment followed the protocols described in Lu et al. (2013, 2017). Single gene trees were constructed to check whether well-supported incongruences (bootstrap > 70%) exist between the chloroplast and ITS topologies (Hillis and Bull 1993) prior to combining all individual data sets.

The combined dataset was analyzed with the maximum likelihood (ML) and Bayesian inference (BI) methods. The ML analyses were conducted in RAxML 8.1.11 (Stamatakis 2006), applying 1,000 bootstrap replicates. A partitioned Bayesian analysis was conducted in

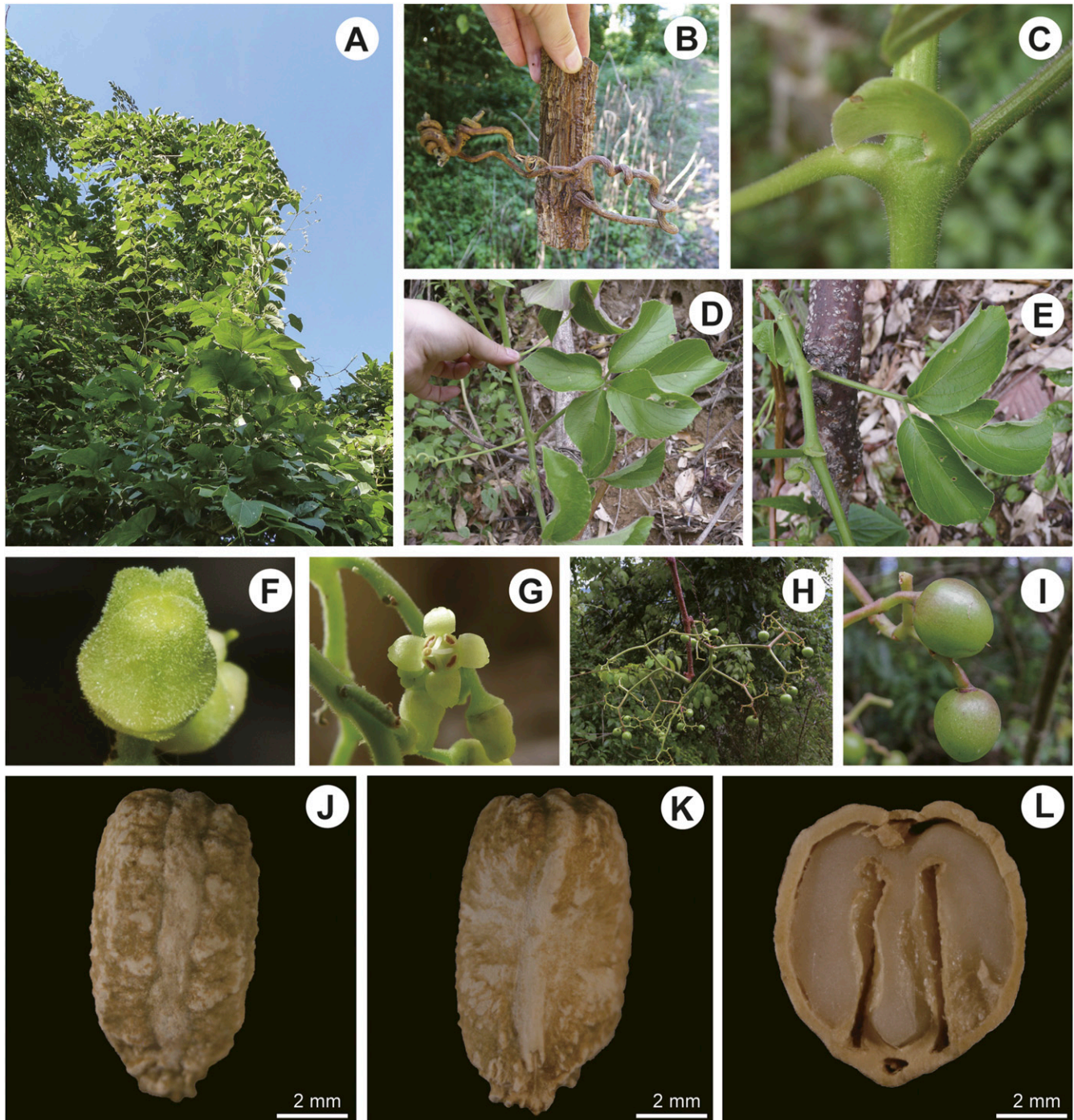


FIG. 2. *Cyphostemma dehongense*. A. Habitat. B. Old stem and tendril. C. Stipule. D. Leaves, 5-foliolate. E. Leaves, 3-foliolate. F. Floral buds. G. Flower. H. Infructescence. I. Fruits. J. Seed, dorsal view. K. Seed, ventral view. L. Seed, transverse section.

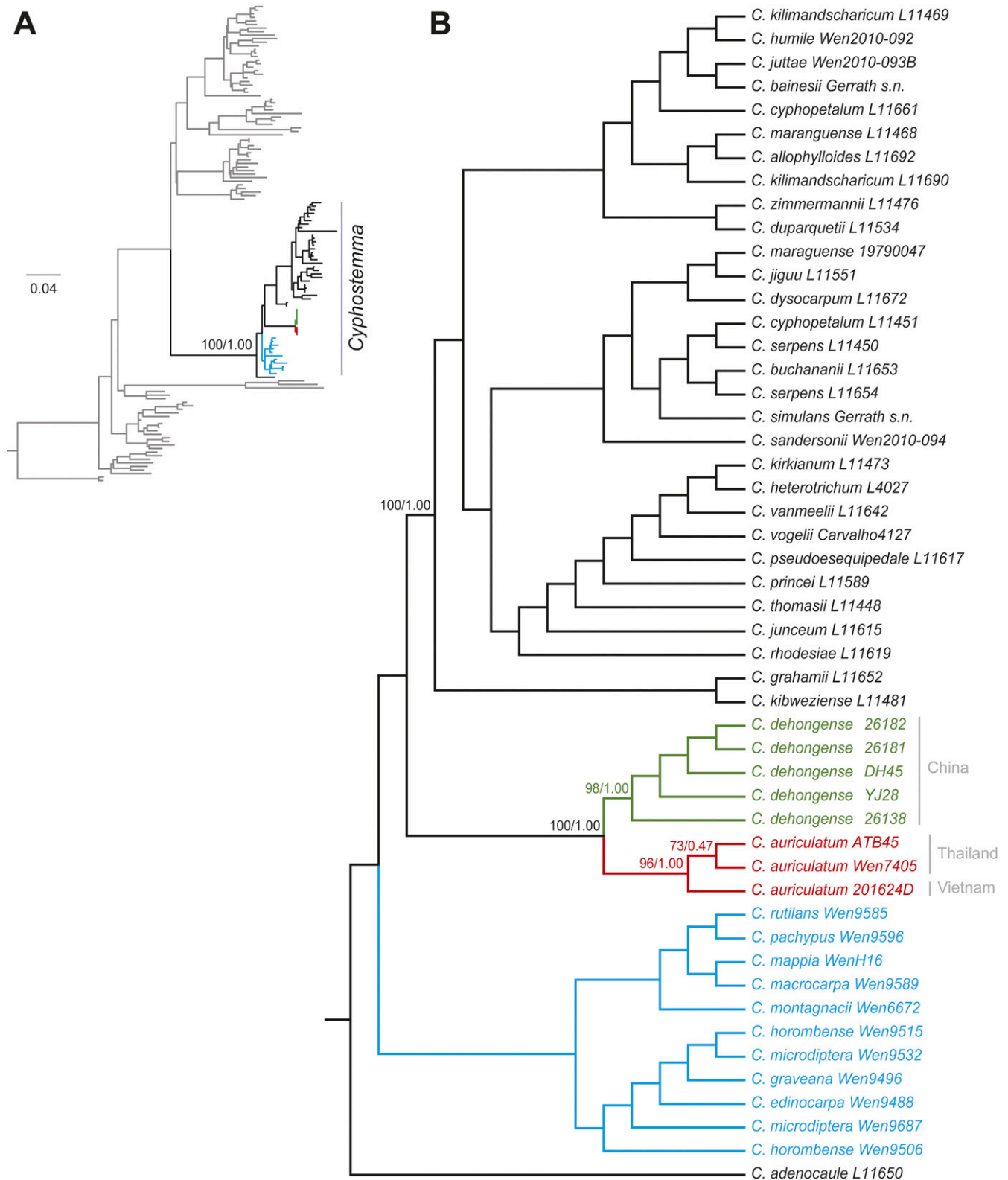


FIG. 3. Maximum likelihood tree for Vitaceae based on the combined data set (*atpB-rbcL*, *rps16*, *trnC-petN*, *trnH-psbA*, *trnL-F*, and ITS). A. A phylogram overview is shown in the upper left-hand corner. B. Phylogenetic relationships within *Cyphostemma*. Species of *Cyphostemma* from Africa and Madagascar are colored with black and blue, respectively. The newly described species *C. dehongense* and its close relative *C. auriculatum* are highlighted in green and red, respectively. Bootstrap values (RAXML) and Bayesian posterior probability values of key nodes are indicated above branches.

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). Best-fitting model for each of the single partition was calculated using MrModeltest 2.3

(Nylander 2004) under the Akaike information criterion (AIC). GTR + G was found to be the most appropriate nucleotide substitution model for *atpB-rbcL*, *rps16*, *trnC-petN*, *trnH-psbA*, and *trnL-F*; GTR + I + G for ITS.

RESULTS

The newly collected specimens from China (illustrated in Fig. 1) and Vietnam were identified as species of *Cyphostemma* in the field based on their conspicuous stipules (Fig. 2C), leaf-opposed tendrils (Fig. 2D), and floral disk with four separate glands (Figs. 1D–E, 2G). The new record from Vietnam was determined as *C. auriculatum* based on its digitately 5-foliolate leaves, smaller ovary, shorter styles, and smaller berries, while the taxon from China was recognized as a new species because it differed from *C. auriculatum* in its 3–5-foliolate leaves (Figs. 1A, 2D–E), larger ovary (Fig. 1E), longer styles (Fig. 1E), and larger berries (Fig. 1F).

The ML and Bayesian analyses of the combined data set of five chloroplast loci and the ITS sequences generated congruent topologies. The ML tree with ML bootstrap (BS) and Bayesian posterior probability (PP) values is shown in Fig. 3. As in previous studies, the monophyly of *Cyphostemma* was strongly supported (BS = 100%, PP = 1.00; Fig. 3A). The new species *Cyphostemma dehongense* from China and *C. auriculatum* from Vietnam formed a strongly supported clade with *C. auriculatum* from Thailand (BS = 100%, PP = 1.00; Fig. 3B). Five samples of *C. dehongense* grouped together (BS = 98%, PP = 1.00), sister to a clade of *C. auriculatum* from Thailand and Vietnam (BS = 96%, PP = 1.00). Of the 6,065 bp aligned sequences, ten nucleotide variation sites were detected that distinguish *C. dehongense* from *C. auriculatum*.

TAXONOMIC TREATMENT

***Cyphostemma dehongense* L. M. Lu & V. C. Dang, sp. nov.—**

TYPE: CHINA. Yunnan province: Dehong, Yingjiang, Tongbiguan, near King of *Ficus*, W slope, road side, 877 m, 97°35'43.3"E, 24°40'00.2"N, 30 Apr 2016, Z. D. Chen YJ28 (holotype: PE!; isotype: PE!).

Cyphostemma dehongense is morphologically similar to *C. auriculatum*, but differs from it by having a larger ovary (2.7–3.2 mm in diam in *C. dehongense* vs. 1.5–2 mm in diam in *C. auriculatum*; Fig. 1E), longer styles (2.9–3.1 mm in *C. dehongense* vs. 0.8–1.2 mm in *C. auriculatum*; Fig. 1E), and larger berries (2–3 cm in diam in *C. dehongense* vs. 1–1.7 cm in diam in *C. auriculatum*; Fig. 1F) (Table 1).

Large woody climber. Older stems cylindrical, growing to 14 cm in diam, bark corky and deeply fissured; younger stems ridged, yellow-brownish, sometimes with lenticels. Tendrils leaf-opposed, bifurcate, robust, and pubescent in younger branches. Leaves digitately compound, 5-foliolate, or usually 3- or 4-foliolate on the reproductive branches; petiole 5–15 cm × 3–5 mm, petiolule of central leaflet 1.5–4 cm long, petiolules of lateral leaflets 1–3 cm long, pilose; central leaflet ovate-elliptic, 10–15 × 6–9 cm, lateral leaflets 7.5–12 × 5–7 cm, leaflet blade abaxially light green or pink, adaxially dark green, pubescent on both surfaces, apex acute or shortly

TABLE 1. Morphological comparisons between *Cyphostemma dehongense* and its closest relative *C. auriculatum*. Descriptions of *C. auriculatum* were based on Shetty and Singh (2000) and Trias-Blasi et al. (2015a).

Character	<i>C. dehongense</i>	<i>C. auriculatum</i>
Leaves	3–5-foliolate	5-foliolate
Ovary	2.7–3.2 mm in diameter	1.5–2 mm in diameter
Style	2.9–3.1 mm long	0.8–1.2 mm long
Fruit size	2–3 cm in diameter	1–1.7 cm in diameter

acuminate, base obtuse, margin serrate with 15–20 rounded teeth on each side, veins prominent on the abaxial side, 1 main basal vein, 5–7 pairs of secondary veins. Stipules falcate, 1–1.5 × 0.3–0.7 cm, conspicuous. Inflorescence a compound cyme, dichotomously branched, axillary or pseudo-terminal, 10–26 cm long; peduncles 10–14 cm × 3.5–6 mm, densely pubescent. Flowers 4–6 mm long; pedicel 4–9 mm long, densely pubescent. Calyx cupular, entire, pubescent. Petals 4, ovate-elliptic to oblong, constricted at the middle, 3–5 × 1.5–2 mm, tomentose outside. Stamens 4, filaments filiform, 2.7–3.2 mm long, anthers yellow, ovate. Ovary 2.7–3.2 mm in diam, hairy, style 2.9–3.1 mm long, stigmas inconspicuous. Floral disk with four separate glands, almost covering the ovary, yellow. Fruit a 1-seeded berry, globose, 2–3 cm in diam, glabrous, base attenuate. Seed oblong-obovoid, 9–10 × 5.5–6.5 mm, rugose on adaxial side with linear groove, abaxial side with a crest. Endosperm M-shaped.

Phenology—Flowering from February to March; fruiting from March to May.

Distribution and Habitat—The new species is only known from the vicinity of the type locality over a range of 75 km, in the tropical monsoon forest at an altitude of 700 to 1,000 m in Dehong, Yunnan, China (Fig. 4).

Etymology—The specific epithet “*dehongense*” refers to the type locality, Dehong prefecture in Yunnan Province.

Conservation Status—Four populations of the new species have been discovered in the protected areas so far. Its area of occupancy (AOO) is less than 20 km². Although individuals grow well, the new species is suffering from loss of natural habitats due to the ongoing deforestation in its distribution range. If an IUCN Red List evaluation was conducted, the species would be classified as vulnerable (VU D2).

Representative Specimens Examined—CHINA. Yunnan, Dehong, Yingjiang, Tongbiguan, near King of *Ficus*, W slope, road side, 877 m, 97°35'43.3"E, 24°40'00.2"N, 9 Nov 2014, Z. D. Chen et al. DH45 (PE); Yunnan, Dehong, Yingjiang, near King of *Ficus*, 918 m, 97°45'32.4"E, 24°46'09.9"N, 1 May 2016, B. Liu et al. 26138 (PE); Yunnan, Dehong, Yingjiang, Taiping Town, Xueli Village, 751 m, 97°57'55.3"E, 24°45'04.4"N, 2 May 2016, B. Liu et al. 26181 (PE); Yunnan, Dehong, Yingjiang, Taiping Town, Xueli Village, 755 m, 98°01'28.4"E, 24°45'48.1"N, 2 May 2016, B. Liu et al. 26182 (PE).

NEW RECORD FROM VIETNAM—CYPHOSTEMMA AURICULATUM (Roxb.) P. Singh & B. V. Shetty, Taxon 35(3): 596. 1986. *Cissus auriculata* Roxb., Fl. Ind. 1: 430. 1820. *Vitis auriculata* (Roxb.) Wall., Numer. List [Wallich] 206. 1828–1849. *Cayratia auriculata* (Roxb.) Gamble, Fl. Madras 2: 237. 1918.—TYPE: Roxburgh Flora Indica illustration in Kew, No. 1788 (lectotype: K!, designated by Trias-Blasi et al. 2015a: 2).

Phenology—Flowering from February to March; fruiting from March to May.

Distribution and Habitat—In mixed deciduous forests and degraded, fire-prone areas of Bangladesh, Bhutan, Laos, India, Myanmar, Sri Lanka, Thailand, and Vietnam, 600–1,300 m (Fig. 4).

Conservation Status—Trias-Blasi et al. (2015a) recommended to treat this species as data deficient (DD) due to insufficient specimen data from other Asian countries. It is known from four populations in Thailand and only two populations were found in the non-protected forest areas of northern Vietnam. If an IUCN Red List evaluation was conducted (IUCN, 2012), the conservation status of *C.*

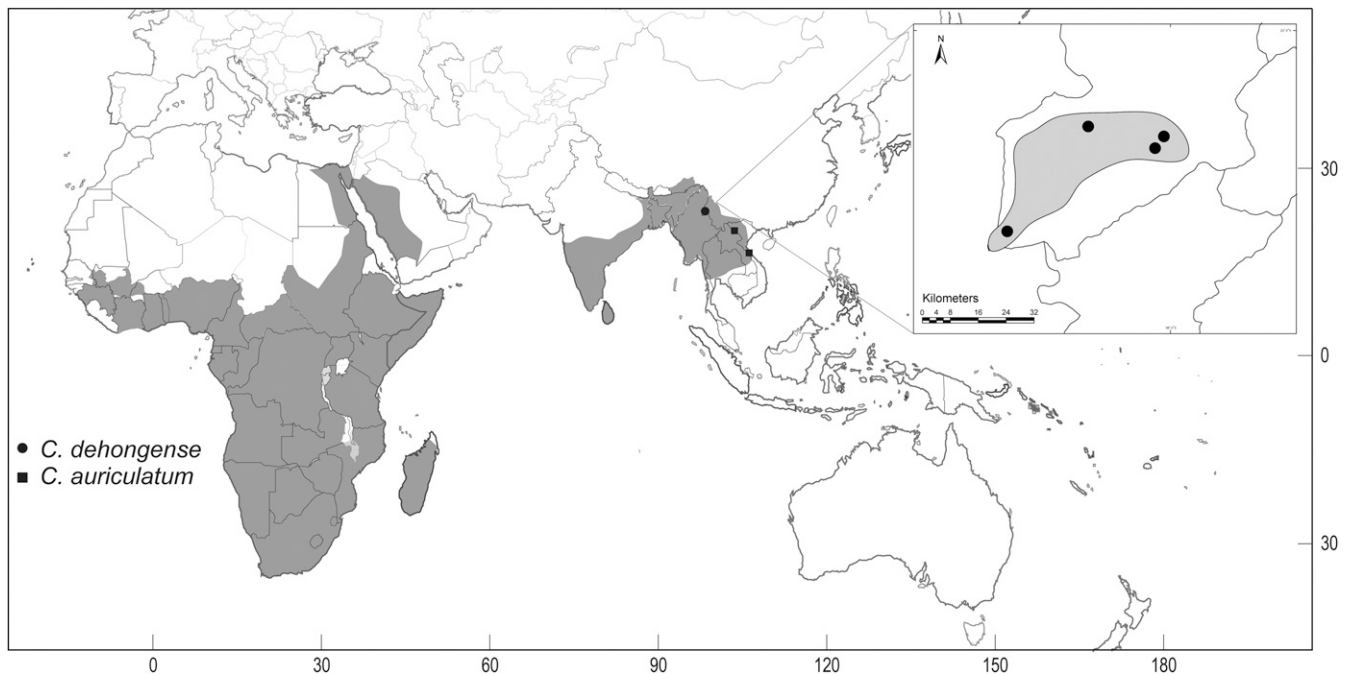


FIG. 4. Distribution map of *Cyphostemma* (gray shade), *Cyphostemma dehongense*, and *C. auriculatum*. Inset: enlarged map showing the four populations of *C. dehongense* in Dehong, Yunnan, China.

auriculatum would be Data Deficient (DD), because thorough field investigations should be conducted to determine the distribution and abundance of the species.

Representative Specimens Examined—INDIA. Rajmahal, Ganges R., 1820, *Wallich 6031a* (K); Irrawaddy R. [Ayeyarwady R.], Yenangheum [Yenangyaung], 1825, *Wallich 6031b* (K); Prome [Pyay],

1826, *Wallich 6031c* (K). LAOS. Haut Laos, 15 km de Phong Saly sur piste de Bun Tai, 1200 m, 8 May 1936, *E. Poilane 26021* (P). THAILAND. Mae Hong Son, Mae Sariang Dist., 44–45 km markers ca. 44.5 km W of Hot, 5 Nov 2003, *J. Wen 7405* (US). VIETNAM. Quang Tri, Laobao, 256.2 km de la route du Mékong, 8 Apr 1920, *E. Poilane 1358* (P); Son La, Mai Son, Phieng Cam, 930 m, 103°49'37.1"E, 21°05'49.6"N, 2 Aug 2016, *V. C. Dang 201624D* (PE).

KEY TO THE SPECIES OF *CYPHOSTEMMA* IN ASIA

1. Leaves trifoliolate; subsessile; berries glandular-hispid *C. setosum* (Roxb.) Alston
1. Leaves digitately 3–5-foliolate; petioles 5–15 cm long; berries glabrous 2
 2. Style 1 mm long; ovary 1.7 mm in diam *C. auriculatum* (Roxb.) P. Singh & B. V. Shetty
 2. Style 3 mm long; ovary 3 mm in diam *C. dehongense* L. M. Lu & V. C. Dang

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LITERATURE CITED

- Alston, A. H. G. 1931. *A handbook to the flora of Ceylon*, part 6, supplement. London: Dulau.
- Cattabriga, A. 2007. Notes on *Cyphostemma*. *Cactus and Company* 2: 94–107.
- Chen, I. and S. R. Manchester. 2007. Seed morphology of modern and fossil *Ampelocissus* (Vitaceae) and implications for phylogeography. *American Journal of Botany* 94: 1534–1553.
- Chen, Z.-D., H. Ren, and J. Wen. 2007. Vitaceae. Pp. 173–222 in *Flora of China* vol. 12, eds. Z.-Y. Wu, D.-Y. Hong, and P. H. Raven. Beijing: Science Press and St. Louis: Missouri Botanical Garden Press.
- Cho, S. H., J. H. Lee, D. H. Kang, B. Y. Kim, K. M. Htwe, A. Trias-Blasi, and Y. D. Kim. 2016. *Cissus erecta* (Vitaceae), a new non-viny herbaceous species from Mt. Popa, Myanmar. *Phytotaxa* 260: 291–295.
- Dang, V. C., L.-M. Lu, V. H. Nguyen, S. Habib, R. L. Barrett, and Z.-D. Chen. 2016. A new record of the genus *Yua* (Vitaceae) from Vietnam. *Phytotaxa* 255: 274–280.
- Descouings, B. 1960. Un genre méconnu de Vitacées: compréhension et distinction des genres *Cissus* L. et *Cyphostemma* (Planch.) Alston. *Notulae Systematicae* 16: 113–125.
- Do, T. V., T. H. Luu, S. Wanke, and C. Neinhuis. 2015. Three new species and three new records of *Aristolochia* subgenus *Siphisia* from Vietnam including a key to the Asian species. *Systematic Botany* 40: 671–691.
- Farjon, A., N. T. Hiep, D. K. Harder, P. K. Loc, and L. V. Averyanov. 2002. A new genus and species in Cupressaceae (Coniferales) from northern Vietnam, *Xanthocyparis vietnamensis*. *Novon* 12: 179–189.
- Hillis, D. M. and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
- Ingrouille, M. J., M. W. Chase, M. F. Fay, and D. Bowman. M. Van der Bank, and A. D. E. Bruijn. 2002. Systematics of Vitaceae from the viewpoint of plastid *rbcL* DNA sequence data. *Botanical Journal of the Linnean Society* 138: 421–432.
- IUCN. 2012. IUCN red list categories and criteria version 3.1. Ed. 2. Gland, Switzerland and Cambridge, U. K.: IUCN.
- Jiménez-Saa, H. 2011. Revised nomenclature of compound leaves as an aid in field identification of tropical trees and other woody plants. *Vulpia* 9: 1–11.
- Kochaiphat, P., A. Trias-Blasi, and P. Pornpongrungruen. 2014. A new combination and new records of *Tetrastigma* (Vitaceae) from Thailand. *Phytotaxa* 183: 272–278.

- Kochaiphat, P., A. Trias-Blasi, and P. Pornpongrungruen. 2016. Two new species of *Tetrastigma* (Miq.) Planch. (Vitaceae) from Thailand. *European Journal of Taxonomy* 201: 1–12.
- Long, D. G. and S. J. Rae. 1991. Vitaceae. Pp. 147–161 in *Flora of Bhutan* vol. 2, eds. A. J. C. Grierson and D. G. Long. Edinburgh: Royal Botanic Garden.
- Liu, X.-Q., S. M. Ickert-Bond, Z.-L. Nie, Z. Zhou, L.-Q. Chen, and J. Wen. 2016. Phylogeny of the *Ampelocissus-Vitis* clade in Vitaceae supports the New World origin of the grape genus. *Molecular Phylogenetics and Evolution* 95: 217–228.
- Lu, L.-M., W. Wang, Z.-D. Chen, and J. Wen. 2013. Phylogeny of the non-monophyletic *Cayratia* Juss. (Vitaceae) and implications for character evolution and biogeography. *Molecular Phylogenetics and Evolution* 68: 502–515.
- Lu, L.-M., J. Wen, and Z.-D. Chen. 2016. *Cayratia cheniana* (Vitaceae): An endangered new species endemic to the limestone mountains of Ninh Thuan Province, Vietnam. *Systematic Botany* 41: 49–55.
- Lu, L.-M., C. J. Cox, S. Mathews, W. Wang, J. Wen, and Z.-D. Chen. 2017. Optimal data partitioning, multispecies coalescent and Bayesian concordance analyses resolve early divergences of the grape family (Vitaceae). *Cladistics*, doi: 10.1111/cla.12191.
- Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 in *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans: Gateway Computing.
- Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University.
- Planchon, J. E. 1887. Monographie des Ampélidées vrais. Pp. 305–654 in *Monographiae Phanerogamarum* vol. 5(2), eds. A. F. P. P. de Candolle and C. de Candolle. Paris: Sumptibus G. Masson.
- Ren, H., L.-M. Lu, A. Soejima, Q. Luke, D.-X. Zhang, Z.-D. Chen, and J. Wen. 2011. Phylogenetic analysis of the grape family (Vitaceae) based on the noncoding plastid *trnC-petN*, *trnH-psbA*, and *trnL-F* sequences. *Taxon* 60: 629–637.
- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- SA2000 (Systematics Agenda 2000). 1994. *Charting the biosphere: a global initiative to discover, describe and classify the world's species*. Technical report. New York: American Society of Plant Taxonomists, Society of Systematic Biologists, and the Willi Hennig Society.
- Shetty, B. V. and P. Singh. 2000. Vitaceae. Pp. 246–324 in *Flora of India* vol. 5, eds. N. P. Singh, J. N. Vohra, P. K. Hajra, and D. K. Singh. Calcutta: Botanical Survey of India.
- Singh, P. and B. V. Shetty. 1986. Some nomenclatural notes on the Vitaceae of the Indian subcontinent. *Taxon* 35: 596–597.
- Soejima, A. and J. Wen. 2006. Phylogenetic analysis of the grape family (Vitaceae) based on three chloroplast markers. *American Journal of Botany* 93: 278–287.
- Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Trias-Blasi, A., J. A. N. Parnell, and K. Chayamarit. 2010. *Cissus amplexicaulis* (Vitaceae), a new endemic species from Thailand. *Kew Bulletin* 65: 487–490.
- Trias-Blasi, A., J. A. N. Parnell, K. Chayamarit, and A. Teerawatananon. 2011. *Cayratia emarginata* (Vitaceae), a new species from Thailand and Vietnam. *Blumea* 56: 16–17.
- Trias-Blasi, A., J. A. N. Parnell, and T. R. Hodkinson. 2012. Multi-gene region phylogenetic analysis of the grape family (Vitaceae). *Systematic Botany* 37: 941–950.
- Trias-Blasi, A., K. Chayamarit, A. Teerawatananon, and J. A. N. Parnell. 2014. A taxonomic revision of *Pterisanthes* (Vitaceae) in Thailand and a new Thai record for *Pterisanthes cissioides*. *Phytotaxa* 159: 95–104.
- Trias-Blasi, A., K. Chayamarit, and J. A. N. Parnell. 2015a. *Cyphostemma auriculatum* (Roxb.) P. Singh & B. V. Shetty (Vitaceae): Typification and a new generic record confirmed for Thailand. *Kew Bulletin* 70: 1–7.
- Trias-Blasi, A., M. F. Watson, K. Chayamarit, and J. A. N. Parnell. 2015b. Typification of two newly recorded species of *Ampelocissus* (Vitaceae) for Thailand. *Phytotaxa* 212: 149–156.
- Verdcourt, B. 1993. Vitaceae. Pp. 1–151 in *Flora of Tropical East Africa*, ed. R. M. Polhill. Rotterdam, The Netherlands: A. A. Balkema.
- Wen, J. 2007. Vitaceae. Pp. 467–479 in *The families and genera of vascular plants* vol. 9, ed. K. Kubitzki. Berlin: Springer Verlag.
- Wen, J., P. K. Lôc, N. T. Hiep, J. Regalado Jr., L. V. Averyanov, and C.-H. Lee. 2007a. An unusual new species of *Trevesia* from Vietnam and its implications on generic delimitation in Araliaceae. *Taxon* 56: 1261–1268.
- Wen, J., Z.-L. Nie, A. Soejima, and Y. Meng. 2007b. Phylogeny of Vitaceae based on the nuclear *GAI1* gene sequences. *Canadian Journal of Botany* 85: 731–745.
- Wen, J., R. Kiapranis, and M. Lovave. 2013. *Ampelocissus asekii* J. Wen, R. Kiapranis & M. Lovave, a new species of Vitaceae from Papua New Guinea. *PhytoKeys* 21: 1–6.
- Wen, J., S. M. Ickert-Bond, M. S. Appelhans, L. J. Dorr, and V. A. Funk. 2015a. Collections-based systematics: Opportunities and outlook for 2050. *Journal of Systematics and Evolution* 53: 477–488.
- Wen, J., L.-M. Lu, Z.-L. Nie, S. R. Manchester, S. M. Ickert-Bond, and Z.-D. Chen. 2015b. Phylogenetics and a revised classification of Vitaceae. Presented at Botany 2015, Edmonton, Alberta.
- Wild, H. and R. B. Drummond. 1966. Vitaceae. Pp. 439–492 in *Flora Zambesiaca* vol. 2, part 2, ed. E. Launert. London: Flora Zambesiaca Managing Committee.

APPENDIX 1. List of species and vouchers used in this study, arranged by: Species Authority. COUNTRY. Collector & Number (Herbarium Code), *atpB-rbcl*, *rps16*, *trnC-petN*, *trnH-psbA*, *trnL-F*, and ITS. Accession numbers of newly generated sequences in this study are indicated with asterisks (*) and other sequences are from Lu et al. (2013, 2017) and Liu et al. (2016).

Ampelocissus elegans Gagnep. MALAYSIA. Wen 11825 (US), KC166289, KC166380, KC166467, KC166544, KC166618, KT344573. *A. elephantina* Planch. MADAGASCAR. Wen 9583 (US), HM585516, HM585792, –, HM585659, HM585932, KT344575. *A. erdwendbergii* Planch. MEXICO. Wen 8697 (US), KC166290, KC166381, KC166468, KC166545, KC166619, KT344577. *A. javalensis* (Seem.) W. D. Stevens & A. Pool. COSTA RICA. Wen 6920 (US), AB234911, AB234943, KC166469, KC166546, AB234984, –. *Ampelopsis arborea* Koehne. USA. Wen 11083 (US), KC166291, KC166382, KC166470, KC166547, KC166620, –. *A. cordata* Michx. USA. Wen 7141 (US), AB234916, AB234949, JF437178, JF437064, AB234997, –. *A. glandulosa* (Wall.) Momi. CHINA. Wen 9380 (US), KC166292, KC166383, JF437181, JF437067, JF437289, KX951343. *Causonis corniculata* (Benth.) J. Wen & L. M. Lu. CHINA. Wen 12049 (US), KC166299, KC166390, KC166477, KC166554, KC166627, –. *C. japonica* (Thunb.) Raf. CHINA. Nie & Meng 453 (US), KC166308, KC166399, KC166485, KC166561, KC166634, KX951347. *Cayratia acris* (F. Muell.) Domin. AUSTRALIA. Wen 12183 (US), KC166293, KC166384, KC166471, KC166621, –. *C. albifolia* C. L. Li var. *glabra* (Gagnep.) C. L. Li. CHINA. Wen 11588 (US), KC166294, KC166385, KC166472, KC166549, KC166622, –. *C. cardiophylla* Jackes. INDONESIA. Chen & Lu 160 (PE), KC428766, KC428777, KC428790, KC428809, KC428824, –. *C. cilifera* (Merr.) Chun. VIETNAM. Wen 10824 (US), KC166296, KC166387, KC166474, KC166551, KC166624, –. *C. clematidea* (F. Muell.) Domin. AUSTRALIA. Wen 12184 (US), KC166297, KC166388, KC166475, KC166552, KC166625, –. *C. cordifolia* C. Y. Wu ex C. L. Li. CHINA. Wen 10548 (US), HM585518, HM585794, JX476668, HM585661, HM585934, –. *C. debilis* (Baker) Suss. EQUATORIAL GUINEA. Carvalho 4136 (MO), KC166302, KC166393, JF437190, JF437076, JF437296, KX951344. *C. emarginata* Trias-Blasi & J. Parn. THAILAND. Trias-Blasi et al. 2012 ATB35, HQ214187, HQ214208, –, –, HQ214229, –. *C. geniculata* Gagnep. INDONESIA. Wen 10275 (US), HM585519, HM585795, KC166480, HM585662, HM585935, –, *C. grandifolia* (Warb.) Merr. & L. M. Perry. PAPUA NEW GUINEA. Wen 12263 (US), –, –, KC428796, KC428815, –, *C. maritima* Jackes. INDONESIA. Wen 10304 (US), KC166315, KC166406, KC166490, KC166565, KC166638, –. *C. melananthera* Gagnep. VIETNAM. Wen 11001 (US), KC166316, KC166407, KC166491, KC166566, KC166639, –. *C. mollissima* Gagnep. MALAYSIA. Wen 8403 (US), HM585522, HM585798, JX476671, HM585665, AB235003, KT344589. *C. oligocarpa* Gagnep. CHINA. Wen 11445 (US), KC166319, KC166410, KC166494, KC166569, KC166642, –. *C. pedata* Gagnep. CHINA. Wen 8469 (US), KC166321, AB234953, KC166497, KC166572, AB235005, –. *C. pseudotrifolia* W. T. Wang. CHINA. Wen 8085 (US), AB234920, KC166412, KC166498, KC166573, AB235006, –. *C. saponaria* (Benth.) Domin. AUSTRALIA. Rossetto et al. 2007, –, –, EF179075, –, *C. schumanniana* (Gilg) Suss. PAPUA NEW GUINEA. Wen 12277 (US), –, –, KC428791, KC428810, –, –, *C. trifolia* (L.) Domin. THAILAND. Wen 7488 (US), KC166323, –, KC166500, KC166574, AB235007, –. *C. triternata* (Baker) Desc. MADAGASCAR. Wen 9643 (US), KC166324, KC166414, KC166501, KC166575, KC166644, KX951346. *C. wrayi* (King) Gagnep. VIETNAM. Wen 10913 (US), KC166326,

- laxum* Merr. PHILIPPINES. *Wen* 8314 (US), HM585602, HM585877, –, HM585740, HM586018, –. *T. obtectum* Planch. Ex Franch. CHINA. *Nie & Meng* 454 (US), HM585614, HM585888, JF437266, JF437154, JF437349, KT344626. *T. pachyphyllum* (Hemsl.) Chun. VIETNAM. *Wen* 10919 (US), HM585615, HM585890, KT344516, HM585752, HM586031, KT344627. *T. papillosum* Planch. MALAYSIA. *Wen* 8401 (US), HM585617, HM585892, –, HM585754, HM586033, –. *T. pedunculare* Planch. INDONESIA. *Wen* 10281 (US), HM585620, HM585895, KX925967, HM585757, HM586036, –. *T. planicaule* Gagnep. VIETNAM. *Wen* 10904 (US), HM585622, HM585897, KC166540, HM585759, HM586037, –. *T. rumicispermum* Planch. CHINA. *Tibet* 2003 (US), HM585626, HM585900, –, HM585763, HM586041, –. *T. serrulatum* Planch. CHINA. *Nie & Meng* 445 (US), HM585627, HM585901, JF437261, JF437149, HM586042, –. *T. sp.* CHINA. *Wen* 11412 (US), KC166376, KC166463, KC166541, KC166615, KC166681, –. *T. strumarum* Gagnep. INDONESIA. *Wen* 10757 (US), HM585641, HM585914, –, HM585778, HM586056, –. *T. trifoliolatum* Merr. MALAYSIA. *Wen* 10758 (US), HM585643, HM585916, –, HM585780, HM586058, –. *T. triphyllum* (Gagnep.) W. T. Wang. CHINA. *Nie & Meng* 342 (US), HM585646, HM585919, JF437263, JF437151, HM586061, KX951355. *Vitis riparia* Michx. USA. *Wen* 8658 (US), HM585645, JQ182608, JF437277, JF437165, JF437357, KT344658. *V. rotundifolia* Michx. USA. *Wen* 11087 (US), HM585646, KC166466, KC166542, JF437166, JF437358, –. *Yua austro-orientalis* (F. P. Metcalf) C. L. Li. CHINA. *S. Ickert-Bond* 1313 (US), HM585648, AB234977, KC166543, JF437170, AB235085, KT344662. *Y. thomsonii* (Laws.) C. L. Li. CHINA. *Nie & Meng* 469 (US), HM585649, HM223335, –, KC166617, HM223277, –.