Research paper

Molecular and morphological evidence for a new species of *Isodon* (Lamiaceae) from southern China

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**A B S T R A C T**

*Isodon brevipedunculatus*, a new species from southern China, is described and illustrated. The phylogenetic position of the new species within the genus was analyzed based on two nuclear ribosomal DNA regions and an ingroup sampling of about 80% of Asian species of *Isodon*. The results show that *I. brevipedunculatus* is recovered in a clade that consists of species mainly with glandular mericarps and that are distributed in the Sino-Japanese region. Combining molecular and geographical evidence, our study reveals that *I. brevipedunculatus* is most closely related to *Isodon amethystoides* and *Isodon bifidocalyx*, but differs from the former in lamina shape, number of flowers per cyme, and peduncle length, and from the latter in lamina indumentum, calyx morphology, and corolla length.

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**1. Introduction**

*Isodon* (Schrad. ex Benth.) Spach is one of the largest genera in Lamiaceae with approximately 100 species distributed mainly in tropical and subtropical Asia (Harley et al., 2004; Li, 1988; Li and Hedge, 1994; Mabberley, 2008; Wu and Li, 1977). The Himalaya-Hengduan Mountains (HHM) global biodiversity hotspot, which accommodates ca. 70% of the species of *Isodon*, is considered the distribution and biodiversity center of the genus (Yu et al., 2014; Zhong et al., 2010). *Isodon* is recognized as the only genus in subtribe Isodoninae (Zhong et al., 2010), and it differs from other genera of Ocimeae by its pedunculate and bracteolate cymes, slightly or strongly 2-lipped (3/2) calyces, strongly 2-lipped (4/1) corollas, and free filaments inserted at the base of the corolla tube (Harley et al., 2004; Li, 1988; Paton and Ryding, 1998). Some species of *Isodon* have long been used as traditional folk medicine in China and Japan, and contemporary phytochemical studies of *Isodon* species have so far isolated and identified more than 1200 diterpenoids, some of which have important pharmaceutical functions (Liu et al., 2017; Sun et al., 2006).

Several new species of *Isodon* have been reported from China during the last decade (Chen et al., 2014, 2016b, 2017, 2019; Xiang and Liu, 2012). Recently, we collected a distinct species of *Isodon* from Hunan and Guangdong Provinces in southern China. Critical studies based on specimen and literature examination, as well as molecular phylogenetic analyses revealed it to be an undescribed species. Herein, we describe and illustrate the new species.

**2. Material and methods**

**2.1. Morphological and taxonomic studies**

Comparison of morphological features between the new species and other species of *Isodon* were carried out based on our previous field observations, specimen examination, and unpublished mericarp data (Chen, 2017). Specimens of *Isodon* from 29 herbaria (A, AU, BM, CDBI, CSFI, E, G, GMX1, HIBG, HIB, IBK, IBSC, K, KUN, KYO, L, LBG, LE, MW, NAS, P, PE, S, SYS, SZ, TAI, TI, W, and WUK; abbreviations follow Thiers, 2020) and our field collections were examined. Meanwhile, protologues of all published names and all other taxonomic literature for *Isodon* were reviewed. The terminology used by Li (1988) and Li and Hedge (1994) was adopted for the morphological description of the new species.
2.2. Taxon sampling and DNA amplification

The systematic placement of the new species was explored based on an ingroup sampling comprising 90 accessions of 84 species of *Isodon* from Asia, including two individuals of the new species from the type locality in Guangdong Province and from Hunan Province, respectively (Appendix A). Six genera representing all subtribes of Ocmiaeae except Isodoninae (Harley et al., 2004; Zhong et al., 2010) were selected as outgroups (Appendix A). Previous studies have shown that *Isodon* chloroplast DNA sequences have significantly lower numbers of variable sites than nuclear DNA sequences, and consequently generate poorly resolved phylogenies (Chen et al., 2019; Yu et al., 2014; Zhong et al., 2010). Thus, for phylogenetic analyses, we used two nuclear ribosomal DNA markers: the nuclear ribosomal internal and external transcribed spacers (ITS and ETS). A total of 172 sequences were downloaded from GenBank to complement our dataset, of which 168 sequences were generated from our previous study (Chen et al., 2019). Voucher information and GenBank accession numbers for all sequences are listed in Appendix A.

The modified CTAB method (Doyle and Doyle, 1987) was used to extract genomic DNA from the silica-gel-dried leaf material. For
polymerase chain reaction (PCR) amplification, ITS was amplified using the primer pairs 17SE/26SE (Sun et al., 1994), ETS using ETS-B (Beardsley and Olmstead, 2002) and 18S-IGS (Baldwin and Markos, 1998). The PCR and sequencing protocols for the two markers followed those of Chen et al. (2016a).

2.3. Sequence alignment and phylogenetic analyses

Sequences were assembled and edited using Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan, USA), and then aligned using MUSCLE (Edgar, 2004) and manually adjusted in MEGA v.6.0 (Tamura et al., 2013). Gaps were treated as missing data. Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were conducted to reconstruct the phylogeny of Asian Isodon, using MrBayes v.3.2.6 (Ronquist et al., 2012) and RAxML-HPC2 (Stamatakis, 2014) on the Cyberinfrastructure for Phylogenetic Research Science (CIPRES) Gateway (http://www.phylo.org/; Miller et al., 2010), respectively. Parameters of each category of analysis followed that of Chen et al. (2019). TreeGraph 2 (Stover and Müller, 2010) was used to visualize the topology of phylogenetic trees with posterior probabilities (PP) and Bootstrap support (BS) values.

Fig. 2. Isodon brevipedunculatus. (A) habit; (B) flower; (C) dissected calyx; (D) dissected corolla; (E) pistil; (F) mericarp. Drawn by L. Wang.
3. Results and discussion

Consistent with previous molecular phylogenetic studies (Chen et al., 2019; Yu et al., 2014; Zhong et al., 2010), three well-supported clades (Fig. 1; Clades I–III) are recognized for Asian Isodon. Clade III contains ca. 80% of the species, but relationships within the clade are poorly resolved. Within Clade III, a moderately supported subclade Clade IIIa (Fig. 1; BI-PP = 0.99/ML-BS = 72%) can be recognized, with most of the species having a Sino-Japanese distribution, as opposed to the remaining species of Clade III that are predominantly distributed in the HHM region. The two individuals of the new species group together but with moderate support (Fig. 1; BI-PP = 0.94/ML-BS = 73%), which may partially result from our failure in obtaining the ITS sequence of the individual from Guangdong. The new species is further recovered in Clade IIIa. All species of this clade are perennial herbs, most of which are characterized with glandular or glandular and puberulent mericarps (Chen, 2017). One species that has glandular and
puberulent mericarps but is not recovered in Clade IIIa is *Isodon trichocarpus* (Maxim.) Kudô, a species endemic to Japan.

Although the relationships between *Isodon brevipedunculatus* and other species of Clade IIIa are not resolved, *I. brevipedunculatus* is united morphologically with *Isodon amethystoides* (Benth.) H. Hara, *Isodon excisus* (Maxim.) Kudô, *Isodon inflexus* (Thunb.) Kudô, and *Isodon bifidocalyx* (Dunn) H. Hara by having densely glandular mericarps. Morphologically and geographically, the new species is most closely related to *I. amethystoides* and *I. bifidocalyx* (Figs. 2–4; Appendix B).

Both *Isodon brevipedunculatus* and *I. amethystoides* have slightly 2-lipped flowering calyces with subequal teeth and erect fruiting calyces. The most noteworthy difference between the two species is the length of peduncles, which are 1–2 cm long in *I. brevipedunculatus*, but 1–4 cm long in *I. amethystoides* (Table 1). Meanwhile, laminae of *I. amethystoides* are usually lanceolate, whereas those of *I. brevipedunculatus* are ovate to broadly ovate; cymes are 3–7-flowered in the new species, but generally have more flowers per cyme (7–15-flowered) in *I. amethystoides* (Table 1).

Though *Isodon brevipedunculatus* resembles *I. bifidocalyx* in the ovate to broadly ovate laminae and narrow panicles (short peduncles), they can readily be distinguished by lamina indumentum, calyx morphology, and corolla length (Table 1). The new species is characterized by having densely pubescent laminae and inflorescences, while *I. bifidocalyx* has subglabrous laminae and densely glandular puberulent inflorescences. *I. bifidocalyx* also differs from *I. brevipedunculatus* in its strongly 2-lipped flowering calyces with unequal teeth and declinate fruiting calyces. Moreover, corollas of *I. bifidocalyx* are about 8 mm long, nearly twice the length of those of *I. brevipedunculatus*.

### 4. Taxonomic treatment

*Isodon brevipedunculatus* Y.P. Chen & C.L. Xiang, sp. nov.

(Figs. 2 and 3).

**Type:** CHINA. Guangdong, Lianshan County, Taibao Town, Dawu Mountain, in Chinese fir forest, 24°45' 34"N, 112°9' 40"E, alt. 690 m, 25 Oct. 2019, Y.P. Chen & Y. Zhao EM1381 (holotype: KUN!; isotypes: A!, K!, KUN!, P!, PE!, W!).

#### 4.1. Diagnosis

The new species is morphologically similar to *Isodon amethystoides* and *I. bifidocalyx*, but differs from the former in its broadly ovate to ovate laminae (vs. ovate to lanceolate), 3–7-flowered cymes (vs.

### Table 1

Morphological comparisons of *Isodon amethystoides*, *I. bifidocalyx*, and *I. brevipedunculatus*.

| Character          | *I. amethystoides*               | *I. bifidocalyx*               | *I. brevipedunculatus*               |
|--------------------|----------------------------------|--------------------------------|-------------------------------------|
| Lamina             | lanceolate to ovate, densely pubescent to subglabrous | ovate to broadly ovate, subglabrous | ovate to broadly ovate, densely pubescent |
| Cyme               | 7–15-flowered                    | 3–5-flowered                   | 3–7-flowered                       |
| Peduncle           | 1–4 cm long                      | 2–4 mm long                    | 1–2 mm long                        |
| Flowering calyx    | pubescent to subglabrous, and densely glandular outside, slightly 2-lipped to 1/3 its length, teeth subequal | densely glandular puberulent and glandular outside, strongly 2-lipped to 1/2 its length, teeth unequal | densely pubescent and glandular outside, slightly 2-lipped to 1/3 its length, teeth subequal |
| Fruiting calyx     | erect                           | declinate                      | erect                              |
| Corolla            | ca. 6 mm long                    | ca. 8 mm long                  | ca. 4 mm long                      |

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### Fig. 4. Distribution of *Isodon amethystoides*, *I. bifidocalyx*, and *I. brevipedunculatus.*
7–15-flowered), and 1–2 mm long peduncles (vs. 1–4 cm long), and differs from the latter in having densely pubescent laminas (vs. subglabrous), flowering calyces slightly 2-lipped to 1/3 of their length with teeth subequal (vs. strongly 2-lipped to 1/2 their length with teeth unequal), erect fruiting calyces (vs. declinate), and corollas ca. 4 mm long (vs. ca. 8 mm long).

4.2. Description

Perennial herbs 50–150 cm tall. Rhizomes woody, tuberose. Stems erect, leafless at base, 4-angular, densely pubescent; internodes 5–9 cm long. Leaves opposite; lamina ovate to broadly ovate, papery, 5–12 × 3–6 cm, apex acute, margin crenate, base cuneate to rounded, adaxially dark green, pubescent, abaxially light green, densely pubescent, with colorless glands, lateral veins 4–5-paired; petioles 1–2 cm long. Panicles terminal and axillary, to 20 cm long; cymes 3–7-flowered, densely pubescent and glandular; peduncles and pedicels 1–2 mm long; floral leaves ovate, gradually reduced toward apex; bracts broadly ovate, sessile or subsessile, 5–10 mm long; bracteole linear, ca. 1 mm long. Calyx campanulate, ca. 2 mm long, 10-veined, densely pubescent and glandular outside, slightly 2-lipped to 1/3 as long as calyx; teeth 5, subequal, triangular, apex acute, fruiting calyx dilated to ca. 4 mm long, erect, slightly curved, broadly campanulate. Corolla white, ca. 4 mm long, pubescent and glandular outside; tube ca. 2 mm long, ca. 1.5 mm in diameter, saccate abaxially near base; apex 2-lipped, posterior lip 4-cleft, bluish purple at middle, lobes reflexed, ca. 1 mm long, anterior lip entire, suborbicular, concave, ca. 2 mm long. Stamens 4, included, inserted at base of corolla tube, filaments pubescent. Style included, apex slightly 2-cleft, ovaries glandular. Mericarps 4, yellowish brown, broadly ovoid, ca. 1.6 × 1.4 mm, densely glandular.

Phenology

Flowering from August to November, fruiting from September to December.

Distribution and habitat

*Isodon brevipedunculatus* is currently known from Guangdong Province and Hunan Province in southern China (Fig. 4). It can be found in forests or on grassy slopes at altitudes of 600–1250 m.

Etymology

The specific epithet refers to the short peduncles of the new species, as compared to one of the most similar species, *Isodon amethystoides*.

Chinese name

Duan Geng Xiang Cha Cai (短梗香茶叶).

Additional specimens examined

CHINA. Guangdong: Lianshan County, Taibao Town, Dawu Mountain, 25 Oct. 1999, H.G. Ye et al. 2532 (IBSC); Hunan: Guidong County, Zhaiqian Town, Fangcun Village, Zhulongli, in the forest, alt. 1227 m, 1 Oct. 2019, C.Z. Huang GD0074 (CSFI); Xinning County, Ma-Ling-Tung, on the grassy slope, alt. 600 m, 9 Sept. 1935, C.S. Fan & Y.Y. Li 458 (BM, LE, NAS).

Author contributions

YPC, CZH, and YZ first discovered the new species in the field and collected the material. YPC carried out the molecular experiment and analyzed the data. YPC and CLX wrote the manuscript. All authors contributed to the revision of the manuscript.

Declaration of Competing Interest

All the authors declare that there is no conflict of interest.

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Appendix A. Sequence information for all samples used in the present study. Vouchers are only provided for samples newly collected here. Sequences newly generated in this study are marked in bold. “−” indicates missing data. All voucher specimens are deposited in the Herbarium of Kunming Institute of Botany (KUN).

| Taxon | Voucher/Source | ITS | ETS |
|-------|----------------|-----|-----|
| *Isodon albobilusis* (C.Y. Wu & H.W. Li) H. Hara | Chen et al. (2019) | MG232833 | MG232738 |
| *Isodon alborubrus* (C.Y. Wu) H. Hara | Chen et al. (2019) | MG232741 | MG232650 |
| *Isodon amethystoides* (Benth.) H. Hara | Chen et al. (2019) | MG232762 | MG232699 |
| *Isodon angustifolius* (Dunn) Kudo | Chen et al. (2019) | MG232803 | MG232709 |
| *Isodon aniscocilus* (C.Y. Wu) H. Hara | Chen et al. (2019) | MG232788 | MG232695 |
| *Isodon atoruber R.A. Clement* | Chen et al. (2019) | MG232766 | MG232673 |
| *Isodon aurantiacus* Y.P. Chen & C.L. Xiang | Chen et al. (2019) | MG232764 | MG232671 |
| *Isodon barbayanus* (H. Liév.) H.W. Li | Chen et al. (2019) | MG232790 | MG232696 |
| *Isodon bifidocilix* (Dunn) H. Hara | Chen et al. (2019) | MG232744 | MG232653 |
| *Isodon brachythyrsus* (C.Y. Wu & H.W. Li) H. Hara | Chen et al. (2019) | MG232776 | MG232682 |
| *Isodon brevicalcaratus* (C.Y. Wu & H.W. Li) H. Hara | Y.P. Chen et al. EM1363 | MT603971 | MT614332 |
| *Isodon brevipedunculatus sp.* nov. 1 | Y.P. Chen et al. EM1381 | / | MT614331 |
| *Isodon brevipedunculatus sp.* nov. 2 | C.Z. Huang GD0074 | MT603978 | MT614339 |
| *Isodon bulleyanus* (Diels) Kudo | Chen et al. (2019) | MG232798 | MG232704 |
| *Isodon calcitatus* (Hand.-Mazz.) H. Hara | Chen et al. (2019) | MG232795 | MG232701 |
| *Isodon coetra* (Buch.-Ham. ex D. Don) Kudo var. coetra 1 | Chen et al. (2019) | MG232756 | MG232664 |

(continued on next page)
| Taxon | Voucher/Source | ITS | ETS |
|-------|---------------|-----|-----|
| Isodon coepta (Buch.-Ham. ex D. Don) Kudo var. coepta 2 | Chen et al. (2019) | MH557903 | MH557887 |
| Isodon coepta var. cavalieri (H. Lév.) H.W. Li | Chen et al. (2019) | MG232759 | MG232666 |
| Isodon dawoensis (Hand.-Mazz.) H. Hara | Chen et al. (2019) | MG232804 | MG232710 |
| Isodon delavayi C.L. Chen & Y.P. Chen | Chen et al. (2019) | MG232768 | MG232674 |
| Isodon effusus (Maxim.) H. Hara | Chen et al. (2019) | MH557911 | MH557896 |
| Isodon enanderianus (Hand.-Mazz.) H.W. Li | Chen et al. (2019) | MG232740 | MG232649 |
| Isodon eriocalyx (Dunn) Kudo | Chen et al. (2019) | MG232805 | MG232711 |
| Isodon excisoides (Y.Z. Sun) H. Hara | Chen et al. (2019) | MG232806 | MG232712 |
| Isodon excisus (Maxim.) Kudo | Y.P. Chen et al. EM218 | MT603972 | MT614333 |
| Isodon flaviformis (C.Y. Wu) H. Hara | Chen et al. (2019) | MG232771 | MG232677 |
| Isodon flavius (Hand.-Mazz.) H. Hara | Chen et al. (2019) | MG232780 | MG232686 |
| Isodon flexicaulis (C.Y. Wu & H.W. Li) H. Hara | Y.P. Chen et al. EM673 | MT603977 | MT614338 |
| Isodon forrestii (Diels) Kudo | Chen et al. (2019) | MG232810 | MG232716 |
| Isodon gibbosus (C.Y. Wu & H.W. Li) H. Hara | Chen et al. (2019) | MH557901 | MH557885 |
| Isodon glutinosus (C.Y. Wu & H.W. Li) H. Hara | Chen et al. (2019) | MG232828 | MG232733 |
| Isodon grandifolius (Hand.-Mazz.) H.W. Li | Chen et al. (2019) | MG232801 | MG232707 |
| Isodon hirtellus (Hand.-Mazz.) H. Hara | Chen et al. (2019) | MG232813 | MG232719 |
| Isodon hispidus (Benth.) Murata | Chen et al. (2019) | MG232750 | MG232658 |
| Isodon hiswenii Y.P. Chen & C.L. Xiang | Chen et al. (2019) | MG232770 | MG232676 |
| Isodon inflatus (Thunb.) Kudo | Chen et al. (2019) | MG232814 | MG232720 |
| Isodon interruptus (C.Y. Wu & H.W. Li) H. Hara | Chen et al. (2019) | MG232781 | MG232687 |
| Isodon irroratus (Forrest ex Diels) Kudo | Chen et al. (2019) | MG232779 | MG232685 |
| Isodon japonicus (Burm. f.) H. Hara var. japonicus 1 | Chen et al. (2019) | MG232783 | MG232688 |
| Isodon japonicus (Burm. f.) H. Hara var. japonicus 2 | Chen et al. (2019) | MH557910 | MH557895 |
| Isodon japonicus var. glaucocalyx (Maxim.) H.W. Li | Chen et al. (2019) | MG232818 | MG232723 |
| Isodon kangtingensis (C.Y. Wu & H.W. Li) H. Hara | Chen et al. (2019) | MG232819 | MG232724 |
| Isodon leucophyllus (Dunn) Kudo | Chen et al. (2019) | MG232821 | MG232726 |
| Isodon longistibus (Miq.) Kudo | Chen et al. (2019) | MH557912 | MH557898 |
| Isodon lophanthoides (Buch.-Ham. ex D.Don) H. Hara var. lophanthoides 1 | Chen et al. (2019) | MH557907 | MH557892 |
| Isodon lophanthoides (Buch.-Ham. ex D.Don) H. Hara var. lophanthoides 2 | Chen et al. (2019) | MH557906 | MH557890 |
| Isodon lophanthoides var. graciliformis (Benth.) H. Hara | Chen et al. (2019) | MG232761 | MG232668 |
| Isodon luxurians (Hand.-Mazz.) H. Hara | Chen et al. (2019) | MG232823 | MG232728 |
| Isodon macrophyllus (Migo) H. Hara | Chen et al. (2019) | MG232742 | MG232651 |
| Isodon megathyrsus (Diels) H. Hara | Chen et al. (2019) | MG232800 | MG232706 |
| Isodon mucronatus (C.Y. Wu & H.W. Li) H. Hara | Y.P. Chen et al. EM676 | MT603968 | MT614328 |
| Isodon muiliensis (W.W. Sm.) Kudô | Y.P. Chen et al. EM701 | MT603969 | MT614329 |
| Isodon nervosus (Hems.) Kudô | Y.P. Chen et al. EM444 | MT603973 | MT614334 |
| Isodon ophiius (Diels) Aj. Paton & Ryding | Chen et al. (2019) | MG232825 | MG232730 |
| Isodon oshensis (W.W. Sm.) Kudô | Chen et al. (2019) | MG232827 | MG232732 |
| Isodon parvifolius (Batalin) H. Hara | Chen et al. (2019) | MG232777 | MG232683 |
| Isodon phanthonias (Prain) Murata | Chen et al. (2019) | MG232817 | MG232722 |
| Isodon phylolodopus (Diels) Kudo | Chen et al. (2019) | MG232799 | MG232705 |
| Isodon phyllostachys (Diels) Kudo | Chen et al. (2019) | MG232785 | MG232690 |
| Isodon pleiophyllum (Diels) Kudo | Chen et al. (2019) | MG232830 | MG232735 |
| Isodon politstachys (Y.Z. Sun) H. Hara | Chen et al. (2019) | MG232802 | MG232708 |
| Isodon pseudo-irravatus (C.Y. Wu) H. Hara | Chen et al. (2019) | MG232775 | MG232681 |
| Isodon racemosus (Hems.) Murata | Chen et al. (2019) | MG232745 | MG232654 |
| Isodon remotiflorus (Diels) Kudo | Chen et al. (2019) | MG232749 | MG232657 |
| Isodon rubescens (Hems.) H. Hara | Chen et al. (2019) | MG232760 | MG232667 |
| Isodon rugosiformis (Hand.-Mazz.) H. Hara | Chen et al. (2019) | MG232778 | MG232684 |
| Isodon rugosus (Wall. ex Benth.) Codd | C. Liu et al. IRCS17466 | MT603975 | MT614316 |
| Isodon scoparius (C.Y. Wu & H.W. Li) H. Hara | Chen et al. (2019) | MG232835 | MG232739 |
| Isodon scrophularioides (Wall. ex Benth.) Murata | Y.P. Chen et al. EM1224 | MT603970 | MT614310 |
| Isodon scrophularioides (Wall. ex Benth.) Murata | Y.P. Chen et al. EM1224 | MT603970 | MT614310 |
| Isodon scrophularioides (Wall. ex Benth.) Murata | Y.P. Chen et al. EM1224 | MT603970 | MT614310 |

(continued)
Appendix B. List of examined specimens of *Isodon* amethystoides and *I. bifidocalyx*.

**Specimens of *I. amethystoides* examined:**

**CHINA. Anhui:** Hefei, Courtois 6058 (NAS), E China Workstation 3771 (NAS, PE); Jingde, Courtois 12529 (NAS); Tongcheng, Courtois 4071 (NAS); Precise locality not known, Courtois 6221 (NAS), R.C. Ching 8968 (NAS). **Fujian:** Dehua, P.C. Tsong 114 (PE); Guangze, Xiamen Univ. Exp. to Wuishian 800874 (AU); Jianyang, Wuishian Exp. 820081 (PY); Jiange, Longxian Exp. 1847, 1909, 3089, 3135 (PE); Liancheng, R. Lin 3257 (PE), 3747 (PE); Nanjing, C.J. Zeng 80 (AU), P. Lin 917, 960 (AU), Xiamen Univ. Exp. 951 (AU); Zhanghang, Xiamen Univ. Exp. to Meihuaishan 547, 626 (AU); Wuishian, Wuishian Exp. 469, 470 (KUN); Precise locality not known, R. Lin 3589 (YS); X.Q. Wang 82216 (NAS). **Guangdong:** Dongguang, S.L. Lau 346 (SYS); Guangzhou, S.B. Guo W217 (IBSC); Huaji, W.T. Tsang 22761 (IBK, IBSC, SYS); Shenzhen, Shenzhen Exp. 356, 405, 1591, 1651 (PE); Wengyuan, X.Q. Liu 24335 (IBK, IBSC); Precise locality not known, W.T. Tsang 31360 (IBSC, SYS). **Guangxi:** Cangwu, Cangwu Exp. 7-098 (GXMI), X.F. Wu 12016 (GXMI, KUN); Cenxi, Cenxi Exp. 7-110, 7-402 (GXMI); Gongcheng, Y.X. Feng 6-5276 (GXMI); Guilping, N.K. Liang 19454 (GXMI); Hezhou, Hexian Exp. 7-185, 7-998 (GXMI), Anonymous 401375 (IBK); Jinxinu, D. Fang et al. 378 (GXMI); Lingui, G.Z. Li 15945 (PE); Mengshan, S.Z. Huang 15611 (GXMI); Pingnan, Y.P. Chen & L. Jiang EM283 (KUN); Xing’an, G.Z. Li & Z.X. Liao 10889 (IBK); Zhaoping, Zhaoping Exp. 7-566 (GXMI). **Henan:** Xinxiang, Anonymous 99 (KUN, PE). **Hubei:** Huangpi, Liu 857 (HIB); Huanggang, Medicine Inspecting Institute s.n. (HIB); Wujiang, C.H. Qian 1675 (WUK), Medicine Inspecting Institute s.n. (HIB); J.W. Wang 2 (PE); Wuhan, Z.E. Zhao 701 (HIB); Xishui, Anonymous s.n. (HIB). **Hjiangsu:** Nanjing, J.S. Yue 600 (NAS); Suzhou, H. Migo s.n. (NAS); Yixing, Courtois 33045 (NAS), Z.L. Ding 39 (NAS), J. Shen 663 (NAS); C.J. Song s.n. (NAS). **Jianguo:** Dayu, Anonymous 1741 (LBG); Guangchang, J.S. Yue et al. 2546 (IBSC, KUN), Huichang, H.M. Ku 3254 (IBSC, KUN, LBG, PE); Longnan, D.C. Wu 78198 (PE); Yanchan, M.X. Nie 4274 (IBSC, KUN, LBG); Ruijin, K.M. Hu 3905 (IBSC, KUN, LBG, PE); Shangrao, M.X. Nie 4781 (KUN, LBG, PE); Shicheng, M.X. Nie 4658 (KUN, LBG, PE); Wuyuan, Courtois 27597, 31475 (NAS); Yihuang, Q.H. Li & C. Chen 1772 (LBG, PE); Zixi, M.X. Nie 3242 (LBG, TAI); Tan, S. Suzuki 8284; Kang & Kao 2574 (TAI); S. Suzuki 849; Kang 6972 (TAI); T.C. Huang 4245, 4275, 4301, 4321 (TAI); Taibei, M. Taio 4730 (TAI), N. Fukuyama 4471 (TAI), S. Tokio 18445 (NAS, PE); TAI, M. Kao 2113, 6298 (TAI), M. Kuo 8993, 9000, 9017 (TAI), C.C. Hsu 13342, 5564, 5565 (TAI); Taidong, S. Sasaki 380480 (TAI); Yilan, N. Taio 4730 (TAI), C.L. Peng 1643 (TAI). **Hong Kong:** S.Y. Lau 3241 (HK), W.J. Tucker 8394 (HK), W.Y. Chun 7847 (SYS). N.Q. Chen 41905 (IBK, IBSC), L. Jiang J00444 (HK). **Hziejian:** Chun’an, S. Chen 2394 (NAS, PE); Hangzhou, H. Migo s.n. (NAS), Oliver 90, 812 (NAS, LL.C. Hu 162 (NAS), S.Y. Zhang 1342 (HBBG, NAS, PE), 1551 (HBBG, NAS, PE), 2720 (HBBG, PE), P. L. Zhu 105 (PE), Anonymous 1032 (HBBG); Jianhe, S. Chen 2094 (PE); Jinyun, K.K. Tsong 833 (PE); Jingning, Anonymous 3641 (NAS); Lishui, S.Y. Zhang 3968 (PE), Anonymous 6015 (NAS); Lin’an, H. Migo s.n. (NAS), Y.P. Chen & Q.R. Zhao EM039 (KUN), Y.Y. Ho 260 (IBSC, WUK), 716 (NAS), 849 (HBBG, IBSC, WUK), 869 (HBBG, IBSC), 25217 (HBBG), 25299 (NAS, PE), 25585 (HBBG, NAS, PE), T.N. Liou 274 (PE), Y.H. Liu 3292 (NAS), Zhejiang Plant Resour. Exp. 29544 (NAS), Anonymous 770 (IBSC, KUN, PE); Linhai, H. Migo s.n. (NAS); Longquan, R.H. Shan 5677 (NAS, PE); Y.Y. Ho 3110 (NAS), 3114 (PE); T.S. Wang et al. 5566 (NAS), S.Y. Zhang 22829 (HBBG, NAS), Zhejiang Exp. 8449 (NAS), D.X. Zuo 21657 (HBBG, NAS), 22121 (NAS), 22904 (NAS, HBBG, Anonymous 5066 (NAS); Ningbo, S. Chen 4301 (PE), Y.Y. Ho 643 (PE), P.C. Tsong 1081 (PE); Pingyang, Anonymous 4444, 44884 (NAS); Qingyuan, K.K. Tsong 833 (PE); Ru’ian, Y.Y. Ho 1504 (PE); Taishun, Zhejiang Exp. 8340 (NAS, D.X. Zuo et al. 23574 (NAS); Tiantai, S. Chen 3802 (PE), Y.L. Keng 1080 (PE, PE); Hangzhou Bot. Garden Herb. 2697 (HBBG), Y.Y. Ho 27863 (NAS), S.Y. Zhang 28327 (NAS), Anonymous 1038 (NAS); Wenling, Hangzhou Bot. Garden Herb. 4083 (PE), Yunhe, S. Chen 672 (PE), Y.Y. Ho 3623 (NAS, PE), Anonymous 4001 (NAS).

**Specimens of *I. bifidocalyx* examined:**

**CHINA. Anhui:** Huangshan, Anonymous 4065 (NAS); Qingyang, E China Workstation 5914 (NAS, PE); Yixian, M. Liu et al. A130122 (PE). **Guangdong:** Renhua, L. Deng 7208 (IBSC, KUN, PE, SE). **Hunan:** Nanxian, Y. Liu 334 (NAS, PE); Wugang, C.L. Xiang et al. 856, 866, 869 (KUN); Xinning, C.S. Fan & Y.Y. Li 458 (NAS); Yizhang, S.Q. Chen 2600 (IBK). **Jiangxi:** Binhai, S.R. Zhang 467 (NAS). **Zhejiang:** Guangchang, Q.M. Hu 5383 (IBSC, KUN); Guizhi, M.X. Nie & S.S. Lai 3802 (PE); Jiujiang, H. Migo s.n. (NAS); H.C. Chen 269 (NAS); K.J. Guan 74562 (PE); Y. Tsing 10728 (NAS, M.X. Nie & S.R. Chen 7631 (LBC), C.M. Tan 95694 (HIB, IBSC, NAS, PE), M.J. Wang 978 (LBG, NAS, PE), 1076 (NAS,
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