

## ***Ficus tikoua*, a new record for Thailand, with associated lectotypifications**

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### ABSTRACT

*Ficus tikoua*, a member of the subgenus *Sycomosus*, is reported for the first time in Thailand. This creeping shrub was discovered in a dwarf forest at high elevation (1,400–1,500 m asl) on the top of Phu Luang Mountain in Phu Luang Wildlife Sanctuary, Loei Province. A key to the Thailand species of *Ficus* subgenus *Sycomorus* is given. A description based on Thai collections is provided. We lectotypify the names *F. tikoua* and *F. nigrescens*.

KEYWORDS: creeping shrub, *Ficus*, new record, Phu Luang Wildlife Sanctuary.

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### INTRODUCTION

The genus *Ficus* L. is pantropical, rarely warm temperate, and comprises nearly 900 species (POWO, 2023), with centers of diversity on the islands of Borneo and New Guinea (Berg & Corner, 2005; Clement *et al.*, 2020). In the account of the family Moraceae for the Flora of Thailand, 108 native species of the genus *Ficus* were reported and another seven species have been introduced: *F. benghalensis* L., *F. carica* L., *F. cyathistipula* Warb., *F. elastica* Roxb. ex Hornem., *F. lyrata* Warb., *F. natalensis* Hochst. subsp. *lepturiae* (Miq.) C.C.Berg, *F. pumila* L., and probably also *F. religiosa* L. (Berg *et al.*, 2011). Subsequently, a new record (Tanming *et al.*, 2015) and a new species were reported (Chantarasanwan *et al.*, 2019) for Thailand. Moreover, Chantarasanwan *et al.* (2016) discovered a natural population of *F. elastica* in the Western part of Thailand and, therefore, the total of native species is up to 111 species.

Berg (Berg, 2003; Berg & Corner, 2005) classified the genus *Ficus* into six subgenera, i.e. *Ficus* subgenus *Ficus*, *Ficus* subgenus *Pharmacosycea*

(Miq.) Miq., *Ficus* subgenus *Sycidium* (Miq.) Mildbr. & Burret (updated to subgenus *Terega* Raf.; Pederneiras *et al.*, 2015), *Ficus* subgenus *Sycomorus* (Gasp.) Miq., *Ficus* subgenus *Synoecia* (Miq.) Miq., and *Ficus* subgenus *Urostigma* (Gasp.) Miq. (updated to subgenus *Spherosuke* Raf.; Pederneiras *et al.*, 2015). All are represented in Thailand (Berg *et al.*, 2011). The subgenus *Sycomorus* comprises ca 130–155 species distributed from Africa to Fiji (Berg *et al.*, 2011; Harrison *et al.*, 2012), of which 16 occur in Thailand (Berg *et al.*, 2011). It is a species rich, phenotypically diverse, widely distributed and ecologically important Old World lineage (Harrison *et al.*, 2012). The subgenus has subdivided into seven sections (Berg, 2004), of which three are represented in Thailand, i.e., Section *Sycomorus* (three species), Section *Hemicardia* C.C.Berg (one species) and Section *Sycocarpus* Miq. (12 species) (Berg *et al.*, 2011). In 2015, Tanming and his colleagues reported another new record of a species in the subgenus for Thailand, i.e., *Ficus beipeiensis* S.S.Chang (Tanming *et al.*, 2015). However, this species was later treated as synonym of *Ficus auriculata* Lour. (Zhang *et al.*, 2018; 2019).

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During field work on the top of Phu Luang mountain at an altitude around 1,400–1,500 m asl of Phu Luang Wildlife Sanctuary, Loei Province, Northeastern Thailand, the first and second authors found a peculiar *Ficus* species on the ground beside the walkway. The first sign of the fig was similar to a climber, but it was not until after being carefully examined, that characters similar to a climber were found, but it never climbed onto other trees like other root-climbing figs; it is a creeper rather than a climber. It is quite distinct from the other Thai species of *Ficus*. Based on our subsequent morphological studies and molecular phylogenetic analysis, the species was identified as *Ficus tikoua* Bureau, a species not previously reported to occur in Thailand; the discovery in Loei Province is the first record of this species in Thailand.

In this paper, we document the record of *Ficus tikoua* and provide a revised key to species of *Ficus* subgenus *Sycomorus* in Thailand. We also lectotypify the names *F. tikoua* and *F. nigrescens* King. Moreover, we also provide the DNA sequences of the internal transcribed spacers (ITS), external transcribed spacers (ETS) and the single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*) of the specimens from Phu Luang Wildlife Sanctuary, which confirmed their identity and phylogenetic placement.

## MATERIALS AND METHODS

Field excursions were carried out between November–December 2019 in Phu Luang Wildlife Sanctuary. Collected samples were processed according to traditional methods (Victor *et al.*, 2004) and incorporated into the herbarium of the Natural History Museum, National Science Museum, Thailand (THNHM), with duplicates sent to Forest Herbarium, Thailand (BKF). Identifications were based on literature (Zhou & Gilbert, 2003; Berg *et al.*, 2011; Chaudhary *et al.*, 2012), and the synonymy follows Rehder (1936) and Zhou & Gilbert (2003). Morphological comparisons were conducted with type specimens and images available online at JSTOR (<https://plants.jstor.org/>). More herbarium samples (as photos) from Harvard University Herbaria (A), Naturalis Biodiversity Center (L), Paris Herbarium (P), Kew Herbarium (K), New York

Herbarium (NY), University of Oslo (O) and Universität Wien (WU) were studied (Herbarium acronyms according to Index Herbariorum [Thiers, continuously updated]). The collected material was photographed in the field and the floral morphology was studied with dissecting microscopes at the Natural History Museum, National Science Museum, Thailand. The description of the species was based on specimens collected in Thailand and from field observations. The morphological characteristics except the receptacle were measured from herbarium specimens.

Three DNA regions from the nuclear genome i.e., internal transcribed spacers (ITS), external transcribed spacers (ETS) and the single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*) of two individuals of *Ficus tikoua* were sequenced as described in Chantarasuwan *et al.* (2015). For the molecular phylogenetic analyses in this study, we added our sequences to the dataset of Zhang *et al.* (2020) that also included 49 samples of *Ficus* subgenus *Sycomorus* from Harrison *et al.* (2012). These DNA sequences of each region were manually aligned in a nexus file using PAUP (Swofford, 2002) and MacClade 4.0 (Maddison & Maddison, 2000). The newly generated six sequences of *F. tikoua* are deposited in GenBank (<https://www.ncbi.nlm.nih.gov/>). Detailed information on all species sampled and GenBank accession numbers are summarised in the Appendix.

Phylogenetic analyses were performed using both maximum parsimony (MP) and Bayesian Inference (BI). *Antiaropsis decipiens* K. Schum., *Castilla elastica* Sessé ex Cerv., *Poulsenia armata* (Miq.) Standl., and *Sparattosyce dioica* Bureau were chosen as outgroups according to previous study (Zhang *et al.*, 2020).

MP analyses were conducted using PAUP v.4.0b10 (Swofford, 2002) by using a heuristic search, with random addition of 1,000 replicates and tree bisection-reconnection (TBR). Node support was evaluated using 1,000 bootstrap replicates of 1,000 random additions.

The best-fitting model of nucleotide substitutions for the combined plastid data matrix was determined according to the Akaike Information Criterion in MrModeltest v2. (Nylander, 2004). Bayesian inference

(BI) analysis was performed using MrBayes v.3.2.6, under the substitution model of GTR+I+G (Ronquist *et al.*, 2012). Two independent runs of four chains using a Markov chain Monte Carlo algorithm were run for ten million generations, with every 1,000 generations sampled and the first 25% of the trees discarded as burn-in. The remaining trees were imported into PAUP\* v.4.0b10 (Swofford, 2002) and a 50% majority rule consensus tree was produced to obtain posterior probabilities (PP) of the clades.

## RESULTS AND DISCUSSION

### Morphological study and identification of the *Ficus* samples

Based on the morphological characters, the samples from Phu Luang Wildlife Sanctuary were identified as *Ficus tikoua* by following the key in Zhou & Gilbert (2003). These specimens also fit morphologically very well with the type of the species. The distribution of the species was previously reported as India, China, Laos, and Vietnam (Zhou & Gilbert, 2003). Now, the distribution of *F. tikoua* is expanded to Northeastern Thailand.

The name *Ficus tikoua* was published by Louis Édouard Bureau and the epithet “*tikoua*” means “ground squash or fruit from soil” (Bureau, 1888), which refers to a syconium of the species on the soil (Zhao *et al.*, 2014). Until a few years ago, the species was placed within *Ficus* subgenus *Ficus* subsection *Fructesciae* Sata (Zhou & Gilbert, 2003; Chaudhary *et al.*, 2012). The phylogeny of *Ficus* subgenus *Ficus* published by Li *et al.* (2012), suggested that the species should be transferred to subgenus *Sycomorus*. Its placement in subgenus *Sycomorus* was confirmed in a phylogenetic study of subgenus *Sycomorus* by Harrison *et al.* (2012).

### Molecular identity and placement of the *Ficus tikoua* samples

The concatenated alignment of the 220-terminal dataset consisted of 2,026 characters (ITS 771; ETS 491; *G3pdh* 764), among which 1,222 were variable and 879 were MP-informative. The MP heuristic search retrieved four equally most parsimonious trees of 3,917 steps (consistency index = 0.4787; retention index = 0.7805).

BI and MP analyses produced similar topologies, but only the BI tree is presented in Fig. 1. The overall phylogenetic relationships and the clades recovered within *Ficus* were congruent with a previous report (Zhang *et al.*, 2020). The phylogenetic analyses showed multiple regions with strong support (PP 1.0) to be monophyletic: *Ficus* and six subgenera i.e., *Synoezia* *sensu* Zhang *et al.* (2020), *Ficus*, *Spherosuke*, *Sycomorus*, *Tegera* and *Urostigma* *sensu* Zhang *et al.* (2020). Only subgenus *Pharmacosycea* is polyphyletic, and members of this subgenus are placed in three different clades, namely *Pharmacosycea* clade I, *Pharmacosycea* clade II and *Pharmacosycea* III. Furthermore, the delimitation of *Ficus* subgenus *Spherosuke* Raf. (Pederneiras *et al.*, 2015) has been changed (Zhang *et al.*, 2020). According to the phylogenetic tree (Fig. 1), this subgenus is paraphyletic concurring with prior work on phylogenetic trees for *Ficus* (Cruaud *et al.*, 2012; Clement *et al.*, 2020; Zhang *et al.*, 2020) and Zhang *et al.* (2020) renamed the second clade to subgenus *Urostigma* (Gasp.) Miq. It is important to note that the sampling of these four subgenera, *Ficus*, *Spherosuke*, *Tegera* and *Urostigma*, in this study was small. In this study, we focus on *Ficus* subgenus *Sycomorus*. All samples of *Ficus* subgenus *Sycomorus* were recovered in a well-supported clade (1.00), which is sister to *Pharmacosycea* clade II with high support (Fig. 1). However, the relationships within this subgenus are poorly resolved.

The phylogenetic results (Fig. 1) showed that the two samples of *Ficus tikoua* grouped together with the two other samples of *F. tikoua* with a strong support (PP = 1.00) in the clade of *Ficus* subgenus *Sycomorus*, thus confirming the identification.

## TAXONOMIC TREATMENT

The additional record, *Ficus tikoua*, increases the number of species of *Ficus* subgenus *Sycomorus* for Thailand to 17 species, and to identify *F. tikoua* with the key to the species of *Ficus* subgenus *Sycomorus* (Berg *et al.*, 2011; page 558) the following adaptations are proposed. Replace the present couplet 4 by the following new one and the old couplet 4 and all subsequent couplets in the key renumbered by adding 1.

Majority-rule consensus tree

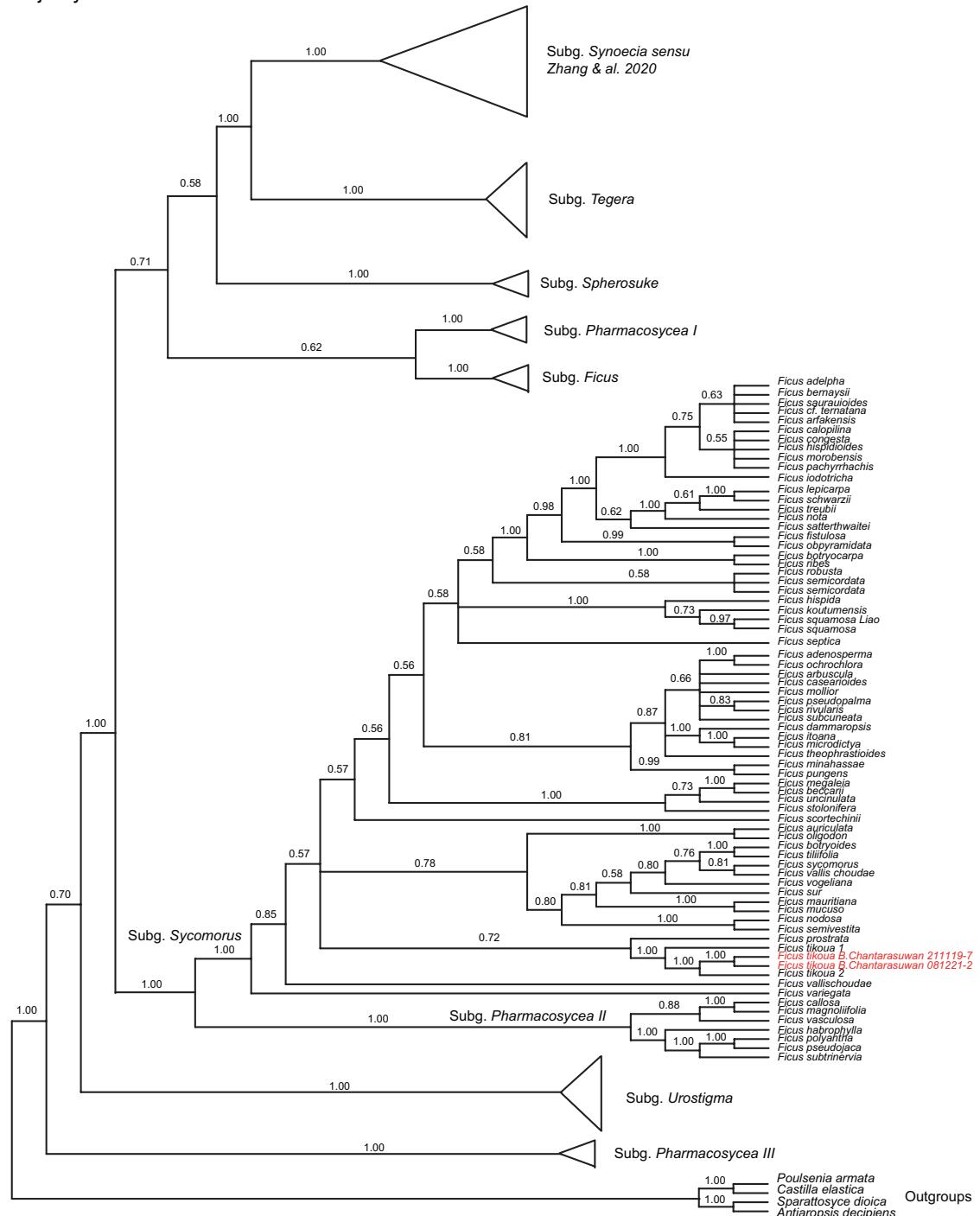


Figure 1. Phylogenetic tree from the Bayesian analysis of the combined data (ITS, ETS and *G3pdh*), showing the phylogenetic position of the samples of *Ficus tikoua* from Phu Luang Wildlife Sanctuary, Loei Province (in red). The numbers above branches indicate Bayesian posterior probabilities.

4. Creeping shrub (or prostrate), stem creeping and erect leafy twigs to 40 cm long **17. F. tikoua**

4. Erect shrub or tree **5**

**Ficus tikoua** Bureau, J. Bot. (Morot) 2: 213. 1888; Wu *et al.*, Fl. China 5: 59. 2003; Chaudhary *et al.*, Taiwania 57 (2): 196. 2012. Type: China, Yunnan, collines rocaillées, côté du nord-est, au dessus du Lac de Lan Kong, 14 May 1887, *J.M. Delavay* 2666 (lectotype **P** [P00756545], designated here; isolectotypes **P** [P00756546, P00756547], **A** [00034605] photo seen). Figs. 2–3.

— *Ficus bonatii* H.Lév., Repert. Spec. Nov. Regni Veg. 6: 112. 1908. Chaudhary *et al.*, *Taiwania* 57 (2: 196. 2012. Type: China, Yunnan, Ravins du mout Tihong Chan, 18 Aug. 1905, *F. Ducloux* 732 (lectotype UC [388257], designated by Rehder, 1936; isolectotype NY [00025346] photo seen).

— *Ficus nigrescens* King, Ann. Roy. Bot. Gard. (Calcutta) 1: 78 t. 95a 1888; King in Hook.f., Fl. Brit. India 5: 520. 1890; Brandis, Indian Trees: 605. 1906; Chaudhary *et al.*, Taiwania 57(2): 196. 2012. Type: India, Kegurina, in the Naga Hills, Assam, 25 Oct. 1885, C.B. Clarke 41174 (lectotype CAL [CAL0000029431], designated here, photo seen).



Figure 2. *Ficus tikoua* Bureau. A. lianescnt habit; B. leaves; C. stem, leaves shoot & fig; D. fig; E. cross-section of fig. Photographed by Bhanumas Chantarasuwan.

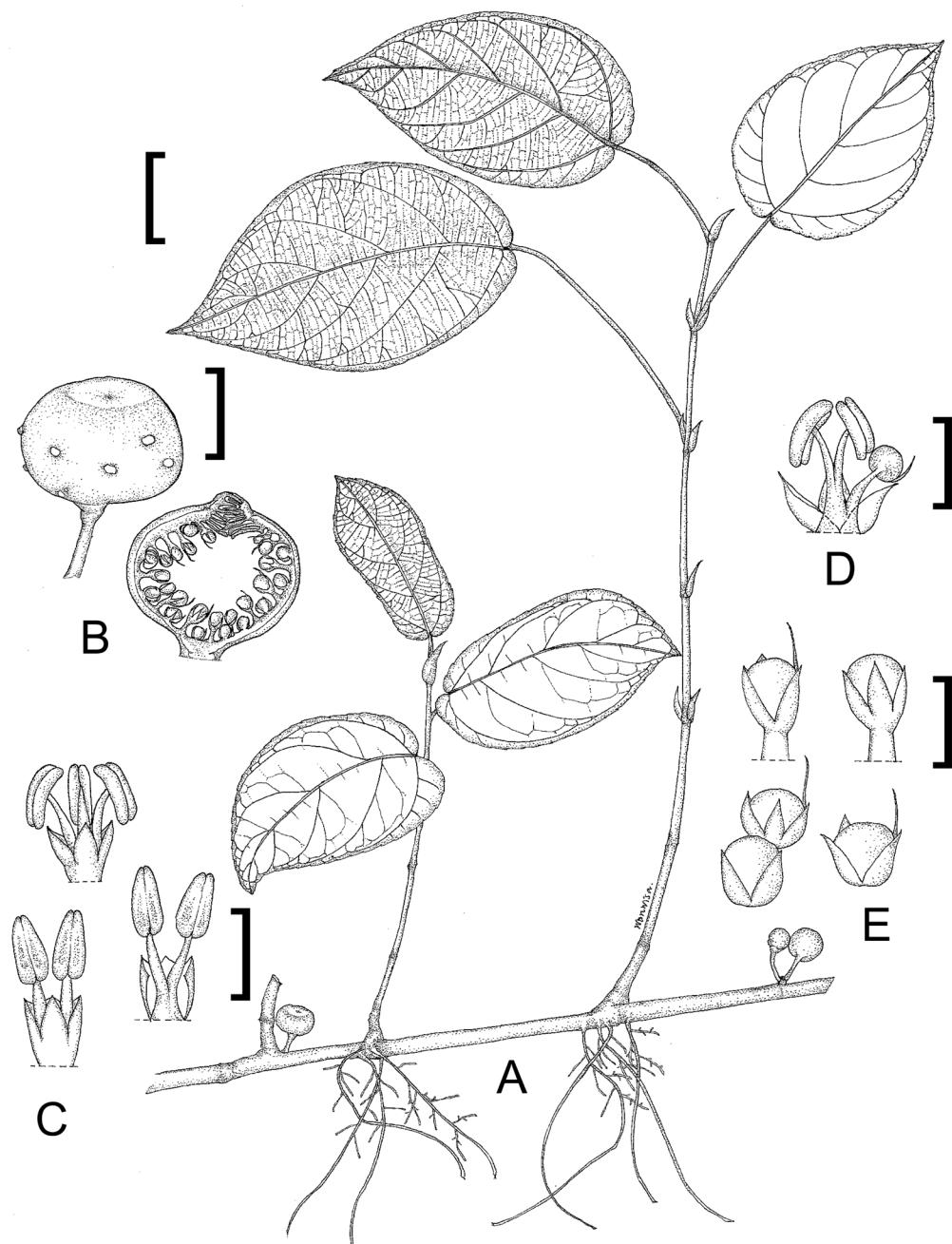


Figure 3. *Ficus tikoua* Bureau. A. fruiting branchlet; B. figs and fig in longitudinal section; C. staminate flowers; D. hermaphrodite flower; E. pistillate flowers. Scale bars: A-B = 2 cm, C-E = 5 mm. Drawn by Wanwisa Bhuchaisri.

Creeping shrub (or prostrate) with adventitious roots on nodes, stem creeping and erect leafy twigs to 40 cm long, (gyno)dioecious; branches drying brown, leafy twigs 1–3 mm thick, solid, minutely brown puberulous or glabrous. *Stipules* in pairs, lanceolate, 3–5 mm long, pubescent, persistent. *Leaves* alternate, lamina cordiform to ovate or obovate, (2–)4–10 by 1.5–6.5 cm, mostly symmetric, coriaceous, apex acute to acuminate, base (sub) cordate to rounded, margin (sub)dentate, upper surface scabrous, lower surface scabrous, with fine hairs on veins, lateral veins 4–6 pairs, the basal pair up to  $\frac{1}{3}$ – $\frac{1}{2}$  the length of the lamina, branched, tertiary venation reticulate, prominent beneath, waxy gland in the axil of the basal lateral veins. *Petiole* (0.6–)1.5–5.5 cm long, brown tomentose when young, glabrous when older, epidermis flaking off. *Figs* solitary or in pairs or up to 4 on a short spur of the older creeping stem; peduncle 1–2 mm long, glabrous; basal bracts 3, verticillate, ca 1 mm long, glabrous, persistent; receptacle subglobose to subpyriform, 1–1.2 cm in diam. when dry, glabrous; ostiole convex, 2–3 mm in diam.; inter floral hairs absent; staminate flowers near the ostiole, (and a few scattered among the pistillate flowers), sessile or pedicellate; tepals 3–4(–5), ovate to lanceolate or sometimes connate at base; stamens 1–3(–4), sometimes with pistil; pistillate flower sessile or pedicellate, tepals 3–4, ovate to lanceolate, ovary red.

Thailand.—NORTHEASTERN: Loei [Phu Luang Wildlife Sanctuary, Pha Sadet, 21 Nov. 2019, *Chantarasuwan* 211119-7 (**BKF**, **THNHM**)]; Pha Chang Phan, 8 Dec. 2021, *Chantarasuwan* 081221-2 (**BKF**, **THNHM**)]

Distribution.—India, China, Laos, Vietnam, and Northeastern Thailand.

Habitat and ecology.—Growing on the sandy soil in open area of dwarf forest at altitudes around 1,400–1,500 m asl.

Uses.—It is widely used in traditional folk medicine to treat oedema, diarrhea, rheumatism, dysentery, impetigo, chronic bronchitis, jaundice, amenorrhea and bruises (Wei *et al.*, 2012; Zhou *et al.*, 2018).

Typification notes.—When Bureau (1888) described *Ficus tikoua*, a collection of Mr l'abbé Delavey number 2666 was referenced as type. The specimen, *Delavey* 2666, was collected from China,

Yunnan and is found in **A** and **P**. According to Stafleu & Cowan (1976), the main herbarium of Louis Édouard Bureau is in **P** and **PC**. Therefore, three specimens at **P** [P00756545, P00756546, P00756547] are the one most likely seen by Bureau. These specimens are of similar condition and among them we chose P00756545 as the lectotype.

For *Ficus botanii*, Léveillé (1908) in his protologue cited a collection of *F. Ducloux* number 732, Aug. 18, 1905 without specifying the herbarium. According to Stafleu & Cowan (1979), the main herbarium of Augustin Abel Hector Léveillé is in **E** but some types are not at **E**. Later, Rehder (1936) designated a specimen at **UC** [388257] as holotype [lectotype] and a specimen at **NY** [00025346] as isotype [isolectotype]. According to Article 9.10 of the International Code of Nomenclature for algae, fungi, and plants (Turland *et al.*, 2018), such instances published prior to 2001 and not, therefore, requiring the statement “designated here” (Art. 7.11) are considered effective lectotypifications.

When King (1888) described *Ficus nigrescens*, no types were designated in the protologue but two specimens of Mr. C.B. Clarke, were cited. *Clarke* 41174 is at **CAL** and *Clarke* 41954 is at **K**. Since King was based at **CAL**, *Clarke* 41174 [CAL 0000029431] is designated as lectotype.

Additional specimens examined.—**HAWAIIAN ISLANDS:** Oahu (19 Nov. 1967, *Derral Herbst* 737 [cultivated] [**L**] ). **INDIA:** Manipur (Moa, Muneypoor, 12 Nov. 1885, *Clarke* 41954 [**K**] ). **CHINA:** Hunan (Ad minas Hsikwangshan prope urbem Hsinhwa, in graminosis repens, 1 Sept. 1918, *Handel-Mazzetti* 12588 [**WU**] ); Yunnan (and Kweitschou, Kweitschou, 8 Oct. 1916, *Schoch* 52 [**WU**] ; Yangtse-kiang, 19 Mar. 1914, *Handel-Mazzetti* 736 [**WU**] ); Lac de Lan Kong, 14 May 1887, *Delavay* 2666 [**P**] ); Szechuan (10 Oct. 1891, *Bock* & *v. Rosthorn* 1184 [**O**] ). **LAOS:** (entre N. Het et M. Seng pr, Traninh, 12 Sept. 1929, *Poilane* 16922 [**P**] ). **VIETNAM:** Indochine (N. du Tonkin et du Laos, 3 Oct. 1936, *Poilane* 25646 [**L**] ).

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